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ALTERNATIVE MICROBIAL INDICATORS OF FAECAL POLLUTION: CURRENT PERSPECTIVE

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ABSTRACT

Worldwide coliform bacteria are used as indicators of fecal contamination and hence, the possible presence of disease causing organisms. Therefore, it is important to understand the potential and limitations of these indicator organisms before realistically implementing guidelines and regulations to safeguard our water resources and public health. This review addresses the limitations of current faecal indicator microorganisms and proposed significant alternative microbial indicators of water and wastewater quality. The relevant literature brings out four such significant microbial water pollution indicators and the study of these indicators will reveal the total spectrum of water borne pathogens. As *E.coli* and enterococci indicates the presence of bacterial pathogens, Coliphages indicate the presence of enteric viruses, and *Clostridium perfringens*, an obligate anaerobe, indicates presence of parasitic protozoan and enteric viruses. Therefore, monitoring a suite of indicator organisms in reclaimed effluent is more likely to be predictive of the presence of certain pathogens in order to protect public health, as no single indicator is most highly predictive of membership in the presence or absence category for pathogens.

Key words: Indicator microorganisms, *E.coli*, fecal coliforms, coliphages, *clostridium perfringens*

INTRODUCTION

During the early history of various countries, epidemics of diseases such as typhoid, shigellosis, cholera and amoebiasis were common threats. It was subsequently determined that the primary source of these pathogens was sewage. The environmental bodies receives a significant amount of treated, partially treated or untreated sewage, which severely depletes the water quality of these water bodies used for drinking, irrigation and other recreational purposes. The wastewater effluents are the major source of fecal contamination of aquatic ecosystems and cause severe disturbance in their functioning. A major goal of wastewater reclamation facilities is to reduce pathogen load in order to decrease public health risks associated with exposure. Despite the fact that raw wastewater also carries large quantities and a wide variety of fecal microorganisms (including pathogens for humans). The reduction of microbiological pollution in wastewater has not been a priority so far in developing country and at present there are no directives regarding the microbiological quality of treated wastewater. However, the direct and indirect exposure of population to sewage is of primary concern (Koivunen *et al.*, 2003). The increasing demography and the growing water demand has lead to a global deterioration of surface waters quality and in areas facing a water shortage, more and more reclaimed water will be used in the future for irrigation of parks and crops. Thus, the need to determine the microbiological safety of these waters by analyzing them for the presence of specific pathogens and the increasing efforts are devoted at present to assessing the treatment efficiency of wastewater treatment facilities for removal of indicator microbes of fecal origin (George *et al.*, 2002; Kazmi *et al.*, 2006). It has been proved that the conventional indicators

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of fecal origin i.e. coliform bacteria (total and fecal coliforms), used to evaluate microbiological quality of waters provide erroneous information. They do not adequately reflect the occurrence of pathogens in disinfected wastewater effluent due to their relatively high susceptibility to chemical disinfection and failure to correlate with protozoan parasites and enteric viruses (Harwood *et al.*, 2005). As well as, coliforms are generally considered unreliable indicators of faecal contamination because many are capable of growth in the environment. Thus, the public health is not protected by using these common indicators (Total coliform and fecal coliform), since methods for the detection of sewage borne pathogen become complex, qualitatively unreliable and do not ensure complete safety of water for consumer. Therefore, the approach is to select some unconventional indicator microbes whose presence presumes that contamination has occurred and suggests the nature and extent of contaminants.

RESULTS

The microorganisms associated with waterborne diseases (typhoid fever, cholera, shigellosis, hepatitis, jaundice fever, diarrhea, amoebiasis etc) found in polluted waters are several members of bacteria, viruses, protozoa, and helminthes (Fecham *et al.*, 1983) (Table 1 and Table 2).

Table 1: Infectious agents potentially present in raw domestic wastewater (*Hurst et al.*, 2002; Metcalf and Eddy, 2003)

Agent	Disease	Clinical Symptoms	Incubation Period	Source
Bacteria				
Ecoli (enteropathogenic)	Gastroenteritis	Diarrhea	2-6 days	Human feces
Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of small intestine	7-28 days	Human feces and urine
Leptospira	Leptospirosis	Jaundice or fever	2-20 days	Urine from infected animals
Shigella	Shigellosis	Bacillary Dysentery	1-7 days	Human feces
Vibrio cholerae	Cholera	Extremely heavy diarrhea, dehydration	9-72 hrs	Human feces
Viruses				
Enteroviruses	Polio, Gastroenteritis, Heart anomalies, Meningitis	Paralytic disease, respiratory illness.	3-14 days	Human feces
Hepatitis A	Infectious Hepatitis	Jaundice, Fever, Anorexia	15-50 days	Human feces
Rota virus	Acute Gastroenteritis	Gastroenteritis with nausea and vomiting	2-3 days	Human feces
Protozoa				
Entamoeba histolytica	Amoebiasis	Abdominal Pain with bloody diarrhea	2-4 weeks	Human feces
Giardia Lamblia	Giardiasis	Diarrhea, nausea, indigestion	5-25 days	Human and animal feces
Cryptosporidium parvum	Cryptosporidiosis	Diarrhea	1-2 weeks	Human and animal feces

Table 2: Pathogens in wastewater (Yates, 1998)

Organisms	Number (L)
Salmonella	23-80000
Shigella	10-10000
E.Coli	Unknown
Vibrio	10-100000
Leptospira	Unknown
Polio Virus	182-492000
Rota virus	400-85000
Hepatitis A	Unknown
Giardia lamblia	530-100000
Entamoeba histolytica	4
Cryptosporidium	5-5180

Public health effects due to microbial pollutants in wastewater

(Epidemiological studies carried out in India)

The existence of large number of microorganisms in raw wastewater is always of great concern to water treatment authorities. Millions of people die every year in different part of the world due to waterborne diseases. Some epidemiological study data of major waterborne disease outbreaks i.e. obtained after extensive literature review is being provided.

(i) Typhoid Fever

The disease is endemic in almost all part of the country with periodic outbreaks of water born or food born disease. In India (1992), about 3, 52,980 cases with 735 deaths were reported. In 1993 the number was 3, 57,452 cases and 888 death, whereas in 1994, about 2, 78,451 cases and 304 death due to typhoid fever reported.

(ii) Malaria

An overall 1.87 million cases of malaria and 1006 deaths were reported from the country in 2003. In 2004, largest number of cases in the country were reported from Orissa, followed by Gujrat, Chattisgarh, West Bengal, Jharkhand, U.P., Rajasthan and the largest number of death were reported from Orissa, followed by West Bengal, Mizoram, Assam, Meghalaya, Karnataka, and Tripura.

(iii) Hepatitis

Out break of hepatitis are more common, with around 60,000 cases reported in the United States each year. These outbreaks will occur due to poor water supply and sanitary facilities.

(iv) Amoebic dysentery

Protozoan infection can be serious nonetheless, as illustrated by an epidemic in Chicago in 1933 in which over 1400 people were affected and 98 deaths resulted when drinking water was contaminated by sewage containing Entamoeba histolytica.

(v) Diarrhea cases

Health services have been badly hit due to flooding of 235 health centers in Orissa. Report of water born disease is being received from Govt. control room, NGOs and National UN Volunteer Doctor. As per the disease Surveillance cell report people from 178 blocks are affected. According to UNICEF, in 1993, 3.8 million developing-world children under age *5* died from diarrhea diseases caused primarily by impure drinking water.

Health Situation	Progressive Cases	Deaths
Diarrhea cases	52707	37
Suspected malarial	24686	12
Acute jaundice	84	2

(vi) Enteric Fever

An out breaks at Maharashtra (India), around 415 individual were affected, and all of them presented with enteric fever. This was attributed to fecal contamination of water. Poor sanitation facilities and waste disposal mechanism can therefore be seen as one of the main contributing factors of almost all water born diseases (Kulkarni *et al.*, 1996).

(vii) Gastroenteritis

Gastroenteritis poses a serious health threat to Indian communities. In the state of Orissa alone, there are approximately 300 infant deaths per day as a result of waterborne gastrointestinal diseases.

(viii) Cholera

In August 1993, an outbreak of cholera was reported in India, Thailand, and Bangladesh. A year later, the cholera epidemic continues to be a problem in India — in the state of Bihar between the months of May and August 1994, approximately 2200 people died from cholera (Times of India, 1994).

(ix) Leptospirosis

Waterborne diseases spreading like epidemic after massive rain fall in Western India – Mumbai. Water supply completely polluted – more than 300 die (Aug.16, 2005). More than 6,000 patients complaining of fever, nausea and breathlessness remain in hospitals across western Maharashtra state and the death toll is expected to rise to 210. Most of the deaths were attributed to Leptospirosis, a bacterial infection. The symptoms of leptospirosis include high fever, body aches and vomiting.

(x). Deaths from water borne diseases each Year (WHO,1992)

Disease	*No. of Infected Persons*	*No. of Deaths*
Diarrhea	2 billion	4 million
Amoebiasis	500million	NA
Typhoid	1million	25,000Cholera
	21000	10,000

Microbiological quality standards for disposal/ reuse/ recycle of urban wastewater in various countries

Increasing demands of water has pushed mankind towards the development of new sources, some of which contain water of a quality inferior to that judged acceptable in the past for water supply purposes. The Government of India envisaged this problem early and accepted urban wastewater as a resource for energy, irrigation water for crops and as a source of pisciculture and aquaculture in its Ganga Action Plan (GAP) in 1986. Therefore, Central Pollution Control Board (CPCB) formulates some microbiological guidelines based on Designated Best Uses (DBU) of water and wastewater (Table 3).

For such uses as landscape irrigation, fodder irrigation, groundwater recharge and certain industrial processes, reclaimed wastewater is widely used in water-short parts of the world and water quality standards have been recommended by WHO (Table 4) and USEPA (Table 5).

The main differences from the 1989 WHO guidelines are new recommendations for a fecal coliform (FC) value for restricted irrigation (<10^5 FC/100mL) and new fecal coliform and nematode egg limits in certain conditions. Worldwide various microbial standards for different uses of waters have been developed (Table 6 and Table 7).

Table 3: Guidelines for surface waters

Sample No.	Parameters	INDIAN standards for various classes* (A, B, C, D, E) of water, 1982)
1	Total count	-
2	Total Coliform	A- 50(MPN/100ml) B- 500 C- 5000 D- - E- -
3	Fecal Coliform	-
4	Fecal streptococci	-
5	*Salmonella*	-
6	*P. aeruginosa*	-
7	*Vibrio cholerae*	-
8	Viruses	-
9	Pathogenic parasites (protozoans and helminths, etc.)	-

* CPCB classification with respect to Designated Best Use (DBU)
A-Surface waters for use as drinking water sources without conventional treatment but after disinfection
B-Surface waters for outdoor bathing
C-Surface waters for use as drinking water sources with conventional treatment followed by disinfection
D- Surface waters used for fish culture and wild life propagation
E- Surface waters for irrigation, industrial cooling or control waste disposal

Table 4: WHO recommended microbiological quality guidelines for wastewater use in agriculture (World Health Organization, 1989)

Category	Reuse conditions	Exposed group	Intestinal nematodes (eggs/litre)	Fecal coliforms (cells/litre)	Treatment to achieve the microbiological quality
A	Unrestricted Irrigation of crops to be eaten, fields, public parks	Workers consumers, public	(<0.1)<1	<1000	Series of stabilization ponds
B	Restricted Irrigation of cereal crops, industrial and fodder crops,	Workers	<1	(10^5) No standard	8-10 day retention in stabilization ponds
C	Localized irrigation of crops in category B if no human exposure	None	Not applicable	Not applicable	At least primary sedimentation

* Values in brackets are the 2000-guideline values.

Table 5: USEPA Typical Guidelines for effluent reuse (United States Environmental Protection Agency, 1992)

Type of reuse	Reclaimed quality
Urban reuse Landscape irrigation Toilet flushing Recreational lakes	pH 6-9, BOD <10 mg/L, Turbidity < 2NTU; No fecal coliforms /100mL; 1 mg/L residual chlorine.
Agricultural reuse Food crops, commercially processed surface irrigation, orchards, and vineyards. Non-food crops.	pH 6-9, BOD <30 mg/L, SS < 30 mg/L; Fecal coliforms < 200 /100mL; 1 mg/L residual chlorine.
Ground water recharge Potable aquifers Indirect reuse	pH 6.5-8.5, Turbidity < 2NTU; No fecal coliforms/100ml; 1mg/L residual chlorine; other parameters as potable standards.

Table 6: Guidelines for potable water (Drinking Water Quality Standards)

Sample No.	Sources	WHO recommendations, 198	EPA, USA (1976)	Middle East	Australian drinking water guidelines, 1996
Parameters					
1.	Total count (per 100 mL)	-	-	10-< 100 (average value)	-
2.	Coliforms	a. 0 b. 0, 3 c. 0, 3 d. 10	not exceed 4/ 100 mL	-	0/100 mL
3.	E.coli	a. 0 b. 0 c. 0 d. 0	-	-	0/100 mL
4.	Total coliforms (per 100 mL)	-	-	Free from this bacteria	0/100 mL
5.	F. streptococci	-	-	Nil	-
6.	Salmonella	-	-	Nil	-
7.	Vibrio cholerae	-	-	Nil	-

a-Treated water fed into mains; b-Untreated water fed into mains; c-mains water
d-Non-mains water supply

Table 7: Water quality standards for recreational water followed in USA and Middle East

Parameters	USA*	Middle East
Total Count	When 10 ml portions of the sample are tested by MPN method –not more than 15% in any month should show agar plate count at 35°C of more than 200 colonies/100ml	Membrane filter method 10 to < 100 bacterial count/100ml
Total coliforms	(i) MPN-any five 10ml portions not more than 2.2 MPN/100ml (ii) Membrane filter-1 coliform/50ml	Nil
Fecal coliforms		Nil
Salmonella		Nil
P. aeruginosa	Free from all kinds of indicator and pathogenic bacteria	Nil
Vibrio cholera		Nil
Pathogenic parasites		Nil

*APHA suggested Ordinances and Regulations Covering Public Swimming Pools, (American Public Health Association, 1984)

DISCUSSION

Worldwide coliforms have been treated as a reliable microbial tool to determine the microbiological quality of waters and to put in orders the water quality guidelines and standards for various modes of use of water and wastewater. The literature revealed that most guidelines and standards put heavy reliance on coliforms as a useful tool to detect the microbiological safety of waters and wastewaters. However, before implementing the water quality standards and guidelines, it should be recognized that either coliforms fulfills the basic criteria of an appropriate indicators or not. Thus, the prevention and control of waterborne diseases requires accurate and rapid methods to measure microbiological water quality and to identify and evaluate risk factors for waterborne disease. Nevertheless, this is not feasible mainly for the reasons

(a) A limited population, suffering from it, excretes pathogens

(b) The enteric pathogens will die out rapidly in the waters due to unsuitable environment

(c) A certain incubation period is usually required for pathogens to give rise to typical symptoms of disease and before this occurs, the polluted water will have already traveled a large distance

(d) Presence of pathogens in treated waters is sparse which renders its isolation difficult and in some cases impossible

(e) Specific organisms of disease may be present only occasionally.

Thus, the detection of waterborne pathogens becomes complex, qualitatively unreliable and does not ensure complete safety of water for consumer. Therefore, microorganisms of fecal origin, whose presence presumes that contamination has occurred and suggests the nature and extent of contaminants, have been chosen as indicator organisms (Metcalf and Eddy, 2003). The basic criterion for making final choice of an appropriate indicator is as follows (WHO, 1996; NHMRC, 2001; and Payment, 1998).

- An indicator should always be present in fecally contaminated water.
- It should be consistently present in fresh fecal waste and should be non-pathogenic.
  - o It should not grow in natural waters.
  - o It must occur in greater numbers than the associated pathogens.
  - o Simple, reliable, and inexpensive methods should exist for the detection, enumeration and identification of indicator organisms.
  - o It should be more resistant to environmental stress or treatment and persist for greater length of time than pathogen.

WHO (2002) has recognized the following three groups in order to elucidate the term microbial indicator:

- General (process) microbial indicators: A group of organisms that demonstrates the efficiency of a process such as total heterotrophic bacteria or total coliforms for chlorine disinfections.
- Fecal indicators: A group of organisms that indicates the presence of fecal contamination, such as the bacterial groups Thermotolerant coliforms or E. coli. Hence, they 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.

Historically, fecal indicator bacteria including total and fecal coliforms 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. However, numerous limitations associated with their application including short survival in water body (Savichtcheva and Okabi, 2006), non-fecal source (Scott *et al.*, 2002; Simpson *et. al.*, 2002), ability to multiply after releasing into water column (Desmarais *et al.,* 2002; Solo-Gabriele *et al.*, 2000), great weakness to the disinfection process (Hurst *et al.*, 2002), inability to identify the source of fecal contamination (point and non-point), low levels of correlation with the presence of pathogens and low sensitivity of detection methods have been widely reported (Horman *et al.*, 2004; Winfield and Groisman,2003). As a result, none of the conventional bacterial indicators currently used to meet all ideal criteria established for water quality. Rely upon coliform bacterial group as indicator for all type of pathogens is not an adequate protection measurement in context with public health significance. Most international water and wastewater quality guidelines and standards include coliforms bacterial indicators as a measurement of microbiological water quality, and for compliance reporting. In response to a growing understanding and acceptance of the limitations of total coliforms, there has been a change of focus worldwide.

1. The European Union (EU) in 1998 removed TC as a mandatory primary indicator and added enterococci (NHMRC, 2001).
2. Volume 2 of 2nd edition (WHO, 1996) however, discussed in detail the inadequacies of total coliforms as an indicator of fecal pollution and debates the merits of alternative indicators such as enterococci and sulphate reducing clostridia.
3. The New Zealand ministry of Health (NZMoH) revised water quality standards, includes only E.coli as a bacterial indicator of fecal pollution, and no longer relies on fecal coliforms or total coliforms. The rational for the move to E.coli

is based on the acknowledgement that both TC and FC can be found in natural waters and their presence does not necessarily indicate the health risk.

4. Australian drinking water guidelines (NHMRC, 1996) recommended that:

i) Total coliform be removed as a health compliance parameter for fecal contamination

ii) E.coli be retained as the primary compliance parameter for fecal contamination.

The indicator organisms presently used for monitoring the efficiency of wastewater treatment facilities and surface water resources in developing countries are TC and FC, although the reliance on indicator organisms as the main source of information about the safety of reclaimed water for public health is under review in many jurisdictions. Total coliform are generally considered unreliable indicators of fecal contamination because many are capable of growth in both the environment and in drinking water distribution systems. It was found that 61% of the total numbers examined over 1000 strains of coliforms were non-fecal in origin (Tallon *et al.*, 2005). Payment (1998) states that total and fecal coliforms probably remains our best tool for establishing basic level knowledge on the fecal pollution level arising from human and animal origin in source waters. However, because the survival of the various types of microorganisms differs considerably, coliforms are not always reliable. Viruses and parasites can survive in the environment for months to years contrary to most pathogens that will die in a matter of a few days to week. Viruses and parasites are obligate pathogens and will not multiply in this environment as can some coliform bacteria such as total and fecal coliform has been reported everywhere in tropical climates. The total coliform and faecal coliform counts can occur from the presence of a variety of bacterial group including *Eschrichia*, *Klebsiella*, *Citrobacter* and *Enterobacter* (not considered in Fecal coliforms gp.). On the contrary, many coliform bacteria originate from soil, vegetation and aquatic environments totally unrelated to fecal pollution. Klebsiella, Enterobacter and Citrobacter have been the predominant environmental coliforms worldwide (Leclerc *et al*., 2001). A disadvantage to the faecal coliform measurement is that faecal coliform occur in both human and animal sources of pollution and detection does not tell whether the water contamination is of human or animal origin. One method to help define the source is to use the faecal streptococcus group, which also occurs in all faecal material. Nevertheless, there are no data available to show a strong relationship between this ratio. Thus using only the faecal coliform indicator assumes a risk (FAO, 2005).

Identification and recommendation for appropriate microbiological indicators (parameters) in treated wastewater for various modes of disposal

Wastewater treatment system and drinking water source system monitoring for bacterial indicators have following major purposes:

1. To identify general fecal contamination of source waters
2. To demonstrate that treatment and /or disinfection process are working effectively
3. To monitor the general system cleanliness
4. To alert for possible cross contamination and contamination from open storages

There is now sufficient evidence that the presence of coliform bacteria (other than *E.coli*) is not putative of the presence of a health risk. The key fecal indicator microorganisms have been proposed including Coliforms, Thermotolerant Coliforms, *Escherichia coli (E. coli)*, fecal streptococci ,enterococci, sulphite-reducing Clostridia (SRC), *Clostridium perfringens*, Bifidobacteria, Bacteriophages (Phages) and Coliphages (Clark *et al.*, 1996). Based on extensive literature review, four most significant indicators of microbial water pollution have been extracted.

Escherichia coli (E.coli) and enterococci- key fecal indicator

E.coli is the best coliform indicator of fecal contamination from human and animal wastes. *E.coli*'s presence is more representative of fecal pollution because it is present in higher numbers in fecal material and generally not elsewhere in the environment. In human and animal feces, 90-100% of coliform organisms isolated are E.coli. (Hurst *et al.*, 2002). As a component of the assessment of public health risk through monitoring of water and treated wastewater, it seems to be most sensitive indicator. The large numbers of *E.coli* present in human gut and the fact that they are not generally present in other environments support their continued use as the most sensitive indicator of fecal pollution available (Edberg *et al.*, 2000).

Percentage distribution of *E.coli* in human faeces (NHMRC-ARMCANZ, 1996; Tallon *et al.,* 2005)

Sample type	*E.coli* (%)	Reference
Human feces	96.8	Dufour (1977)
	94.1	Allen and Edberg (1995)
	>94	Seyfried and Harris (1990)

E.coli retained as primary compliance parameter for fecal contamination by major International bodies such as United States Environmental Protection Agency (USEPA), European Union (EU) and Australian drinking water guidelines. Baudizsova (1997) found that the other thermotolerant and total coliforms were capable of growth in non-polluted river water while *E.coli* was not, and supports a recommendation for *E.coli* to be used as the sole indicator bacteria for recent fecal contamination (Tallon *et al.*, 2005). In temperate climates, fecal coliform organisms are mostly *E.coli*, which are always found in the faeces of humans and animals but rarely found in natural water not subject to pollution. The presence of *E.coli* is regarded as definite proof of faecal pollution. Because *E.coli* is the predominant bacterial species in most human faecal material, its count is often cited as the most reliable indicator of human waste. This indicator, however, has not been used in international or most national standards to assess the usability of irrigation water or for process control on wastewater treatment plants. While the detection methods are easy and widely understood by most university and health ministry laboratories (FAO, 2005).

Criteria for selecting *E.coli* as an indicator (Payment, 1998)

Associated with pathogen	No. in source water	Survival in env.	Resistance to treatment	Pathogenic	Cost	Enumeration
Yes	High	Poor/ growth	Low	Yes/no	low	Easy

The Enterococci are the group of bacteria that has been most often suggested as alternatives of coliform. The enterococci were included in the functional group of bacteria known as "*fecal streptococci*" and now largely belong to the genus Enterococcus that was formed by the splitting of *Streptococcus faecalis* and *Streptococcus faecium* (Schleifer and Klipper-Balz, 1984). Generally, for water examination purposes enterococci can be regarded as indicators of fecal pollution. Enterococci have a number of advantages as indicators over total coliforms including:

1. They generally do not grow in the environment (WHO, 1993) and they have been shown to survive longer (Mcfeters *et al.*, 1974).
2. They are still numerous enough to be detected after significant dilution
3. Rapid and simple methods based on defined substrate technology, are available for the detection and enumeration of enterococci

More recent research on the relevance of faecal streptococci as indicator of pollution showed that the majority of enterococci (84%) isolated from a variety of polluted water sources were true fecal species (Pinto *et al.*, 1999).

The WHO (1996) also recommends the use of fecal streptococci (of which enterococci are a subgroup) as an additional indicator of fecal pollution. When combined with measurement for *E.coli,* the result is increased confidence in the absence or presence of fecal pollution.

Criteria for selecting Enterococci as an indicator (Payment, 1998)

Associated with pathogen	No. in source water	Survival in env.	Resistance to treatment	Pathogenic	Cost	Enumeration
Yes	High	long	Low	Yes/no	low	Easy

Thus, the behavior of *E.coli* and enterococci under environmental conditions is expected to reflect the presence of enteric pathogenic bacteria. *E.coli* and enterococci were more common among the individuals tested, being found in the faeces of 94-100%, while the non-E.coli, thermotolerant coliforms were less common, being found in the faeces of 9-70% of the individual tested (Tallon *et al.*, 2005). As a result, E.coli and enterococci can be used as appropriate model organisms of human bacterial pathogens.

Clostridium perfringens

These are gram +ve, sulphite reducing, spore forming, non- motile, strictly anaerobic rods, enteric micro- organism seriously considered as a possible indicator of the sanitary quality of water (Cabelli, 1978; WHO, 2002). *C. perfringens* is the only reliable indicator of fecal contamination and is being proposed for use in establishing recreational water quality standards. *C. perfringens* spores were identified as the best indicator of fecal pollution and were the only indicator group significantly correlated to any of the pathogen groups in water column (*Giardia sp*. and *Aeromonas sp*.) (Gleeson and Gray, 1997). The spores produced by *C. perfringens* are very resistant to disinfection and the WHO (1996) suggests that their presence in filtered supplies may be an indication of treatment inefficiencies. *C. perfringens* is present in higher concentrations in the feces of animals such as dogs than humans and is generally lower or absent in other warm blooded animals (Leeming *et al.*, 1998). There is evidence to show that *C. perfringens* may be a suitable indicator for viruses and parasitic protozoa when sewage is the suspected cause of contamination (Payment and Franco, 1993). *C. perfringens* rarely multiply in the environment because anaerobic conditions suitable for their growth and spores of anaerobic bacteria are extremely resistant to environmental factors (Payment, 1998). *C. perfringens* can form spores that allow detection but are too enduring to be good indicators of recent faecal contamination (Tallon *et al.*, 2005). Nonetheless, it should be used only in conjunction with *E.coli* and fecal coliforms (used for both wastewaters and fresh waters due to its facultative anaerobic nature) not individually due to its long survival in environment. *Clostridium perfringens* has been shown to be useful indicator for Cryptosporidium oocysts and Giardia cysts. Therefore, it can be used as a model organism for the presence of human pathogenic protozoans.

Criteria for selecting *C. perfringens* as an indicator (Payment, 1998)

Associated with pathogen	No. in source water	Survival in env.	Resistance to treatment	Pathogenic	Cost	Enumeration
yes	low	Very long	high	Y/N	low	Easy

Bacteriophages

Bacteriophages are viruses that infect bacteria and those that infect coliforms are known as coliphages, or more generally phages. Bacteriophages have been proposed as an alternative indicator for enteric viruses, as their morphology and survival characteristics resemble those of human enteric viruses that pose a health risk to water consumers if water has been contaminated with human faeces (NHMRC, 1996; Harwood *et al.*, 2005). Bacteriophages, a wide group of viruses has been previously proposed as fecal and enteric viral indicators. The direct correlations between the presence of certain bacteriophages and intensity of fecal contamination were reported.

Three main groups of bacteriophages have been considered as potential model microorganisms for various aspects of water quality assessment (Bitton, 2002; Hurst *et al.*, 2002).

Somatic coliphages

- Specific viruses of *E.coli*
- Commonly used as indicators of fecal sewage pollution due to their direct correlation with the presence of enteric viruses in various water systems and sewage effluents.
- The methodology to detect them is very simple and results may be obtained in 4 hrs.
- One of the drawbacks of somatic coliphages is their replication potential outside the gut. Considering the difference in origin and ecology

between enteric virus and S. coliphage, it is doubtful that this phage group could successfully be used in all situations as viral indicator (Morinigo *et al*., 1992).

F-Specific RNA Bacteriophages

The use of these phages as fecal pollution indicators has been proposed, because of their inability to replicate in the water ecosystem. A review of literature indicates that the most likely coliphages for use as a water quality indicator is the F-specific RNA coliphages because:

- The group comprises viruses similar in size, shape, and genetic makeup, to human enteric viruses, which are responsible for most of the waterborne diseases.
- It represents viruses, which are more stable than human enteroviruses in environmental waters and more resistant to disinfections.
- Its concentration found in environmental waters has been reported to correlate with sewage contamination (Havelaar and Pot-Hogeboom, 1988).
- A standardized method for the detection and enumeration of this phage is now available; results can be obtained in twelve hours.

Bacteriophages infecting Bacteriodes fragilis (Puig *et al*., 1999; Jofre *et al*., 1986).

Bacteriodes fragilis is a strict anaerobe, found in high concentrations in human intestinal tracts. The phages which infect this group of bacteria have been proposed as a good indicator of water quality because:

- Phages against this strain are human specific and are not isolated from the feces of other warm-blooded animals.
- The levels of phages are related to the pollution degree.
- *B. fragilis* phages always outnumber human enteric viruses.
- In model experiments no replication of these phages has been observed under simulated environmental conditions.
- A relatively simple standardized method is now being discussed; results can be obtained in 18 hours.

Bacteriophages are considered non-pathogenic to humans, and can be readily cultured and enumerated in the laboratory. The methods to recover coliphages from environmental waters is relatively simple and within the capability and resources of most water quality laboratories (Debartolomeis and Cabelli, 1991). Generally, present in faeces of human i.e. human specific and its presence reflect the possible occurrence of human enteric viruses. Consequently, it can be use as an appropriate model organism for human pathogenic viruses. Thus, we can state that study of these cumulative indicators will reveal the possible presence of water borne pathogens and efficacy of treatment processes in order to protect public health. As single indicator will not be sufficient to provide all the answers that we are seeking to evaluate i) source water quality ii) water and wastewater treatment efficiency iii) distribution system contamination iv) possible health effects and v) possible presence or absence of pathogens, it is suggested that the four selected indicators- *E.coli,* enterococci, coliphages and *Clostridium perfringnes* may be used in monitoring source waters, microbial removal efficiency of wastewater treatment plants and monitoring health effects.

Criteria for selecting bacteriophages as an indicator (Payment, 1998)

Organism	Associated with Pathogens	No. in Source water	Survival in Env.	Resistance to treatment	pathogenic	Enumeration	Cost
Somatic Coliphages	Yes/no	High	Long/growth	Intermediate	no	inter	inter
Male specific	yes	Inter	long	Intermediate	no	inter	inter

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