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BACTERIAL INDICATORS OF FAECAL POLLUTION OF WATER SUPPLIES AND PUBLIC HEALTH: A REVIEW

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ABSTRACT

Bacterial indicators of faecal pollution of water supplies and their significance to public health are reviewed in this paper, to highlight their levels of general acceptability and suitability as safeguards against health hazards associated with water supplies. Regular bacteriological analysis with the sole aim of detecting faecal coliform as a confirmation of faecal pollution, is shown to constitute a safeguard against health hazards arising from the consumption of faecally-polluted water. It is also shown that available indicators cannot be fully relied upon for total safeguard espericially as *Cryptosporidium* oocysts and *Giardia* cysts have been implicated in some water-borne outbreaks of gastroenteritis. Furthermore, there are still problems of confusing ecology, characterization and taxonomy, growth temperature in both temperate and hot climates, coupled with the fact that several non-classical indicators, e.g., *Bifidobacterium*, *Bacteroides*, *Eubacterium*, etc., have been found to be equally attractive as apparently reliable indicators. This confusing situation appears to have created considerable doubt on the general acceptability of classical indicators (e.g., coliforms, *Streptococcus faecalis* and *Clostridum perfringens)*, as safeguards against water-related infection. In perspective, further research on methods of detecting reliable indicators, in addition to maintaining hygienic principles in homes, are recommended.

KEY WORDS: Water Pollution, Bacterial indicators, General acceptability, Public Health.

INTRODUCTION

The greatest danger associated with water supplies is contamination by human and animal faeces (Lynch and Poole, 1979). Human faeces, human urine and animal excreta are the principal vehicles for the transmission and spread of many water-borne diseases (Cabelli, 1978, Pipes, 1978). For instance, gastroenteritis, dysentery, cholera, typhoid and paratyphoid fever, infective hepatitis and others are traceable to water polluted by human faeces (American Public Health Association, 1985). Water polluted by human urine may carry causative organisms of leptospirosis, typhoid fever, schistosomiasis, etc., while water polluted by animal excreta is, to some extent, a source of salmonellosis. It has been noted that animals and birds, e.g., seagulls, may carry human intestinal organisms pathogenic to man (Reports on Public Health and Medical subjects, 1969, Benoit, *et al.*, 1993).

Some of these infections are the chief causes of death, especially of infants, in societies where poverty, malnutrition and poor hygienic conditions prevail as in developing countries (Feachem *et al.*, 1977). Poor sanitation habit and improper waste disposal are responsible for the entry of sewage or excrement containing pathogens into water supplies. Notably, the problems of waste disposal began with the formation of agricultural communities based on small permanent settlements (Lynch and Poole, 1979). The wastes produced by the communities were disposed by spreading on agricultural land to promote fertility of soil, or discharged into convenient watercourses. As these settlements grew into large villages and towns, waste disposal became a major problem. Suitable land for disposal of waste was limited, and so increasing use was made of the rivers and estuaries. Apparently, hygienic collection, transportation, treatment and disposal of human excreta (including the use of excreta in agriculture and biogas production) are extremely important for the health of a community (Fattal *et al.*, 1981, Fattal, 1983, IRCWD Report No. 05/85, 1985). Although excreta may be economically justified in agricultural use and biogas production, its use requires technologies that exclude unacceptable health risks.

Water has a very significant effect on human health. The relationship between water and health has been recognized from the time of Hippocrates, if not earlier, in the association of marshy places with fevers (Feachem, McGarry and Mara, 1977). Yet, until the second half of the 19^{th} Century, mankind had no true appreciation of the significance of water as a vehicle of disease. In 1855, Snow was the first to show a precise relationship between a disease and water in his

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famous studies of cholera (Lynch and Poole, 1979). He was closely followed by Budd (1873) who demonstrated the spread of typhoid through water supplies. The works of Snow and Budd were crucial in developing the public awareness of the importance of controlling water pollution (Lynch and Poole, 1979).

Cholera and typhoid which are caused by the bacteria *Vibrio cholerae* and *Salmonella typhi* respectively, were pandemic in Europe during the mid-19th century; and this period of British history marked the beginning of great improvements in both the disposal of sewage and the provision of potable water (Lynch and Poole, 1979). These improvements or advances in the control of water pollution, are one of the greatest contributions ever made to the health and the quality of life of the human population.

Today the situation has changed very little in most third world countries. In Africa in particular, the problem is not that of space for waste disposal. It is one of ignorance of the health implication of improper waste disposal, and the appropriate technology to handle waste and control water pollution. The picture is not different in most Asian communities. Some improvements have however been made in Latin America.

It is satisfying that mankind now knows and accepts that faecally polluted water supply is definitely the source of these infections under discussion, but measures already taken by man for the control of the infections are still not adequate. For instance, the detection of a tracer bacterium (or indicator bacterium) still has some problems of general acceptability in terms of ecology, growth conditions in both temperate and hot climates, specificity of characteristics, taxonomy and absolute reliability as indicator. This study aims to review these problems and relate them to public health and control of water related infections.

WATER-RELATED DISEASES

It has been customary to classify water-borne human pathogenic diseases according to the microbes causing the infections. Few examples are salmonellosis caused by salmonellae, shigellosis caused by *Shigella* species, giardiasis caused by *Giardia* species and schistosomiasis caused by *Schistosoma* species. This system of classification has not been useful in the consideration of how to improve water supplies. The mode of spread is important in the consideration of improvement of water supplies. Thus, it has been more useful to reclassify infective diseases associated with water supplies into four categories (White, Bradley and White, 1972, Bradley, 1977, Feachem, 1977). These categories.

1. Infections spread through water supplies (i.e., drinking water) are known as water borne diseases.
2. Infections due to lack of water for personal hygiene are known as water-washed diseases.
3. Infections transmitted through aquatic animals are known as water-based diseases.
4. Infections spread by water-dependent insects are known as diseases with water-related insect vectors.

Diseases transmitted from infected persons through water all belong to category 1, hence infected persons acquire the infections through oral contact with faecally contaminated water. Therefore, microbiological examination besides chemical analysis of drinking water, and the application of hygienic quality standards, are essential for the preservation of public health.

BACTERIOLOGICAL EXAMINATION OF WATER SUPPLY

Because of the health danger associated with faecal contamination of water supplies, there is need for quality monitoring of the water in order to guard against health hazards arising from the consumption of such faecally- contaminated water.

Bacteriological analysis offers the most sensitive test for the detection of recent and therefore potentially dangerous faecal pollution, which is the rationale for the bacteriological examination of water. Sometimes there could be defective treatment of water, and this could result in very serious health hazards. Cross-connections with impure water sources, backsiphonage, leaks in mains and service reservoirs, access of materials via ball-cocks and stop valves, the effects of pressure variations or temperature and cessation of supply, are all possible means of faecal contamination of pipe-borne water (Reports on Public Health and Medical subjects, 1969, Holden, 1970).

In an exhaustive study of factors like assimilable organic carbon (AOC) in water, disinfectant residual and operational parameters related to coliform regrowth in drinking water, Lechevallier. *et al.,* (1996) concluded that the occurrence of coliform bacteria within a distribution system was dependent upon a complex interaction of chemical, physical, operational and engineering parameters, and no single factor could account for all the coliform occurrences.

Traditionally, the presence of coliforms in water supply indicates faecal pollution through cross connection, inadequate treatment, or inability to maintain a disinfectant residual in distributed water. However, indicator microorganisms have since been recognized in fully treated (finished) water supply; for instance, in 1930, the American Waterworks Association Committee on water supply reported on the problem of regrowth of *Bacillus coli* in drinking water systems (Committee on Water Supply, 1930). Adams and Kingsbury (1937) described the finished water at the point of entry into the distribution system as free of indicator bacteria. It is however evident that coliforms may be found in finished drinking water, and may not be related to faecal or pathogen contamination, or to waterborne disease (LeChevallier *et al.*, 1996). The problem of regrowth of coliform bacteria has become of paramount importance with increasing stringent water quality standards. Infact, criteria by which a variance to the Total Coliform Rule could be granted, had been developed by the United States EPA (Federal Register, 1991).

Recent studies (Carmichael *et al.*, 2001) further cast doubt on the use of classical indicator organisms as safeguard against faecal contamination of water supplies. For instance, in the examination of phytoplankton from the dialysis clinic water source, following human fatalities from cyanobacteria in Brazil, 19.5 μg/l of microcystin which was 19.5 times above World health Organization level for safe water supplies, was detected. In fact, Freeman (2001) reports that a Harward research team on marine pollution, having observed that viruses were extremely abundant in marine waters where they often survived longer and more resistant to sewage treatment than bacteria, concluded that bacterial indicators are inadequate surrogates for overall pathogen levels. Rather, the Harward team proposed that surveillance methods that focused only on these indicators were no longer adequate to protect health. Thus the development of surveillance methods using polymerase chain reaction, fluorescent antibody, or monoclonal antibody techniques, in addition to monitoring algal populations, should be preferred (Freeman, 2001).

RATIONALE FOR THE BACTERIOLOGICAL EXAMINATION OF PUBLIC WATER SUPPLIES

The danger associated with faecal contamination of water supply is the possible presence of pathogens. One would have expected that bacteriological analysis of water would have involved principally the detection of pathogens in the water Unfortunately, the direct search for the presence of specific pathogenic bacteria or viruses in water is impracticable for routine control purposes (Reports on Public Health and Medical subjects, 1969). This is because pathogens present in water are usually greatly out-numbered by the normal intestinal organisms, and tend to die out more rapidly (Reports on Public Health and Medical subjects, 1969). Secondly, isolation of, and specific tests on all possible pathogens would require large volumes (several litres) of water, and generally involving complicated procedures besides biochemical and serological tests on pure cultures (Reports on Public Health and Medical subjects, 1969, Holden, 1970, American Public Health Association, 1985). The procedures are even more complicated and lengthy in the isolation of viruses.

Therefore, simple and rapid tests have been developed for the detection of normal intestinal bacteria that, in this way, are used as indicator or tracer bacteria of faecal pollution of water, their presence indicating *only* that pathogens might also be present. Hence, if water is found to contain faecal indicator bacteria, it is considered unsafe for human consumption. This is the rationale for the bacteriological examination of public water supplies developed in Europe and the United States of America around the turn of the 19th century, with the major aim of reducing the incidence of epidemics of water-borne diseases (Holden, 1970, Pipes, 1978, American Public Health Association, 1985).

The absence of indicator organisms does not guarantee a 'pure' sample of water because, no relationship has been claimed between the presence or absence of indicator micro-organisms and polluting materials (Lynch and Poole, 1979). Townsend (1992) has observed that there is spatial variability in indicator/pathogen relationship. Some studies have shown that water may contain pathogenic bacteria without showing well marked corresponding presence of indicator organism, *Escherichia coli*. For example, during sporadic cases of enteritis caused by *Salmonella montevideo* in Galilee, Israel, examination of well waters (Seligmann and Reitler, 1965) revealed the presence of various salmonellas, including *S. montevideo*, accompanied in some cases by low or even zero *E. coli* counts. It was inferred that the waters had become contaminated and that the salmonellae and *S. montevideo* in particular, had survived drying more effectively than *E. coli* (Seligmann and Reitler, 1965). Similarly, *S. typhimurium* epidemic in Riverside, California, was attributed to the water supply, and even though this organism was found throughout the distribution system, coliform counts were correspondingly lower than 2.2 percent per 100ml sample in most cases (Holden, 1970).

The principle of using indicator bacteria to assess bacterial quality of water is , still epidemiologically valid throughout the world, although with some restrictions when applied to waters and waste-waters in hot climates (White, Bradley and White, 1972). Secondly, recent studies using improved detection techniques for pathogens, have shown that certain pathogens (e.g., *Salmonella* species, viruses) may survive longer in natural environments and also be more resistant to chlorination than the conventional faecal indicator bacteria (White, Bradley and White, 1972, Freeman, 2001). Sen and Jacobs (1969) have demonstrated the survival of pathogenic vibrios and Salmonellae in at least 5 per cent of samples in chlorinated, unfiltered water from Hoogly River distributed in Calcutta. Therefore there should be caution in accepting the wholesomeness of water if analysis does not show indicator organisms. Moreover, absence of outbreaks of water-borne diseases when faecal indicators are used as a yardstick for hygienic quality, warrants continuous application of the use of indicator organisms to confirm faecal pollution.

INDICATOR BACTERIA

Indicator bacteria form a large group of obligate and facultative anacrobic bacteria, as well as small populations of aerobic bacteria. They constitute the normal microbial flora of the gut of man and other warm-blooded animals.

Indicator bacteria (or tracers) of faecal contamination ideally should fulfil some requirements (Reports on Public Health and Medical subjects, 1969, Pipes, 1978, Cabelli, 1978).

They must be:

a) normal members of the intestinal flora of healthy people;
b) exclusively intestinal in habitat, hence exclusively faecal in origin when found in the environment;
c) absent in non-human animals, although this condition is not yet met by any of the bacteria used at present;
d) present whenever faecal pathogens are present or might be expected to be present;
e) present in higher numbers than faecal pathogens;
f) unable to multiply outside the intestine, with a die-off rate slightly less than that of faecal pathogens;
g) resistant to natural antagonistic factors in water and waste water treatment processes, to a degree equal to or greater than faecal pathogens;
h) easy to detect and count and detectable in low densities, and
i) non-pathogenic.

No one bacterial species or group at present in use completely fulfills all these requirements, but a few come close to doing so. In conventional water bacteriology, three main groups or species of bacteria are used as faecal indicators. They are rightly described as "classical" indicators (Opara, 1978). they are:

1. coliform bacteria made up of total coliform (faecal coliform and non-faecal coliform).
2. Faecal streptococci
3. *Clostridium perfringens* (*C. welchii*)

A number of bacteria described as non-classical (Opara,1978) have been proposed to be used for the same purpose (Feachem,1977, Cabelli, 1978). They are: *Bifidobacterium* species, *Pseudomonas* aeruginosa, *Bacteroides*, *Pepto(strepto)coccus* and *Eubacterium* species, all of which are relatively abundant in human faeces (Feachem, 1977). Diet and ethnic factors can influence the bacterial flora of the human intestine (Feachem, 1977). In a study of the microflora of the faeces of different human populations (Drasar, 1974), an insight into the effect of diet and socio-economic changes on the intestinal flora has been given. How the effect comes about has not been convincingly explained. In any case, Drasar (1974) observed that, for most groups of organisms, the distribution pattern was remarkably similar in about six groups, and that anaerobic organisms (*Bacteroides*, *Bifidobacterium*, *Eubacterium* and *Clostridium*) greatly outnumbered the facultative bacteria, and the greatest geographical variation in numbers was in *Enterococcus* group.

However, in a recent study to determine, among others, the effect which changes in diet would have on the shedding of *E. coli* 0157:H7 by sheep, besides the effect of changing of location (confinement to pasture), Kudva, *et al.* (1995) observed that the diet change presumably induced selective microbial growth in the intestine of the sheep so that the level of *E. coli* 0157:H7 shed became detectable, or the diet change might have increased animal susceptibility to colonization with *E.coli* 0157:H7, so that horizontal transmission was increased even though the animal was no longer penned. Kudva, *et al.* (1995) therefore hypothesized that diets high in nutrients and low in fibre induce a lower incidence of transmission and/or shedding of fewer *E.coli* 015:H7 cells, but do not induce clearance of the organism from the intestine. They further stated that conversely the diets low in nutrients and high in fibre and briefly withholding feed, can both induce shedding of larger numbers of *E. coli* 0157:H7, and/or increase subsceptibility to new intestinal colonization, besides inducing elimination of the organism. The mechanism of these dietary effects is assumed to result from changes in volatile fatty acid (VFA) concentrations, in which case, diets with increased fibre content and withholding feed are both known to decrease the VFA concentrations in the ruminant gut and affect bacterial colonization pattern (Brownlie and Grau, 1967, Grau *et al.*, 1969, Wallace *et al.*, 1989).

The hypothesis of Kudva, *et al.* (1995) could partially explain the differences in the bacterial flora of the intestine of different human populations. This is of significance to public health. It could be inferred that dietary habit which is influenced by cultural or ethnic disposition can affect the rate of transmission and shedding of *E. coli*, and thus the rate of faecal pollution of water supplies.

CLASSICAL INDICATOR BACTERIA

Coliform Bacteria

Coliform bacteria are present in human and animal faeces. The number could be up to 10^6-10^9/g of human faeces (Holden, 1970). Irrespective of doubts cast from time to time on the validity of faecal coliform, *E. coli,* as an indicator of faecal contamination of water supply (Dutka, 1973, Evison and James, 1974), the opinion that *E. coli*, adequately defined, is the most sensitive and reliable indicator of excretal pollution at our disposal has remained unshaken till recently (Holden, 1970, White, Bradley and White, 1972, Feachem, 1977). It's sensitivity makes it possible to detect at least one cell in 100ml of water (EPA- 600/8 – 78 – 017, 1978).

Two main groups of coliform are known. They are faecal and non-faecal coliform (together forming total coliforms). The former are exclusively faecal in origin. The latter can often be found in faeces, but are also naturally occurring in faecally unpolluted waters and soils and even vegetable matter (Reports on Public Health and Medical subjects, 1969). Hence the presence of the latter is regarded as presumptive evidence of faecal pollution of water supply, and should as well as faecal coliforms be absent from treated water supplies (Reports on Public Health and Medical subjects, 1969). In the bacteriology of surface water and wastewater, they are of much less importance. This is because, under suitable conditions, e.g. in the presence of decaying vegetation in natural waters, and on pump packages, cellulosic pipe-jointing material, leather tap washers, jute yarns used in pipe joints, and natural organic material in water distribution system, especially in hot climates, multiplication of non-faecal coloforms can take place (Reports on Public Health and Medical subjects, 1969, Deaner and Kerri,1969, Dutka, 1973, Holden, 1970, Evison and James, 1974, Opara, 1978). In this regard, the occurrence is not necessarily related to faecal pollution or to the degree of the latter. Several studies cited by Opara (1978) have created some doubts on the reliability of the use of *E. coli* as an indicator of faecal pollution. These doubts have arisen from the fact that the normal flora of human faeces in India, Ceylon, Egypt and Singapore have been shown to contain aerogenes (cloacae group) and *Citrobacter* which have proved positive to Eijkman test at 44°C, a test which characterizes *E. coli* (Reports on Public Health and Medical subjects 1969).

As defined in Reports on Public Health and Medical subjects, (1969), the coliform organisms are "gram-negative, oxidase-negative, non-sporing rods capable of growing aerobically on an agar medium containing bile salts, and able to ferment lactose within 48 hours at 37°C with the production of both acid and gas". Some coliforms are widely spread in certain forms of vegetation. They ferment lactose at 30°C and are not of epidemiological importance.

E. coli are also coliforms as defined above, but are differentiated from others by their ability to ferment lactose with the production of acid and gas at both 37°C and 44°C in less than 48 hours; and can also produce indole in peptone containing tryptophan (Reports on Public Health and Medical subjects, 1969, American Public Health Association, 1985). They cannot produce acetyl methyl carbinol and are methyl-red positive (Carpenter *et al.*, 1966, Reports on Public Health and Medical subjects, 1969, Cowan and Steel, 1974, American Public Health Association, 1985).

Faecal streptococci

Faecal streptococci are gram-positive cocci which have the ability to grow at 45°C in the presence of 40 per cent bile, and in concentration of sodium azide which are inhibitory to coliform organisms and most other gram-negative bacteria (American Public Health Association, 1985). Some species resist heating at 60°C for 30 minutes, and will grow at pH 9.6, and in media containing 6.5 per cent sodium chloride (Reports on Public Health and Medical subjects, 1969). They are present in human and animal faeces with numbers ranging from 10^5 to 10^8/g (Holden, 1970). They are thus smaller in number than total coliform, although the ratio of total coliform to fecal coliform may vary considerably between different communities (Feachem, 1977).

Faecal streptococci comprise Lancefield's serological group D (Cruickshank *et al.*, 1975), mainly the *Enterococcus* groups (*S. faecalis*, *S. faecium*, *S. durans*) plus *S. bovis* and *S. equinus* which may also be found in water (Holden, 1970). Other streptococci, *S. mitis* and *S. salivarius*, although not in the Lancefield's serological group D, can occasionally be present in the mouth. They originate from the mouth and are swallowed in the saliva (Reports on Public Health and Medical subjects, 1969).

As stated in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1985), the main value of faecal streptococci lies in assessing the significance of doubtful results from the coliform tests, especially when larger numbers of coliforms occur without the presence of *E. coli*. Detection of faecal streptococci serves to confirm the faecal origin of pollution. Hence faecal streptococci tests are mostly used as supplementary to coliform tests, and are especially of value in natural waters and samples from repaired mains. The greater resistance of faecal streptococci to marginal chlorination than the coliform bacteria, also increases the value of the former for use for the same purposes (American Public Health Association, 1985). Two subspecies of serological group D streptococci have been identified (Cruickshank *et al.*, 1975). They are atypical *S. faecalis* (*var. zymogenes*) which hydrolyse starch, and *S. faecalis (var. liquefaciens)* which occur in faecally polluted as well as unpolluted environments. These strains are essentially non-faecal in origin, and in routine tests, are not distinguishable from truly faecal streptococci. The predominance of one of these subspecies (or biotypes) at lower densities of faecal streptococci, would render the usefulness of faecal streptococci indicators questionable, especially in drinking water microbiology.

Clostridium perfringens (C. welchii)

This is a gram-positive anaerobic spore-forming rod, exclusively faecal in origin. It can also be pathogonic (but not enteropathogenic) causing gas gangrene and food poisoning. The number in human and animal faeces could range from 10 – 10^7/g. It can persist for a long time outside the intestine and can resist chlorination because of its possession of endospores (Reports on Public Health and Medical subjects, 1969). It's value lies, therefore, in demonstrating remote or intermittent pollution, or confirming the faecal nature of contamination when only coliform organisms other than *E. coli* are present (American Public Health Association, 1985, Reports on Public Health and Medical subjects, 1969). In wastewater bacteriology, persistence for a long time outside the intestine is a disadvantage because of the formation of residual dormant populations, which might not reflect the degree of possible pathogenic contamination. Hence the test is mostly used for the same purpose and additionally to faecal streptococcus test.

Non-classical indicator bacteria

The non-classical indicators are *Bifidobacterium* species, *Pseudomonas aeruginosa, Bacteroides* and *Eubacterium* species.

Bifidobacterium species are gram-positive rods with a tendency to forming rudimentary branches. They are non-sporing, non-acidfast, anaerobic, catalase-negative, and can ferment sugars. In fact, the species are characterized by their crude Y-shape (Cheesbrough, 1984). The best known is *Bifidobacterium bifidum* which is easiest to identify because it has the ability to attack sugars (Reuter, 1971). From ecological point of view, studies on the distribution of these organisms have shown that they are found in the faeces of infants and adults, in human vagina, and in the faeces (or rumen contents) of animals and birds (Reuter, 1971). Zani *et al.* (1974) has demonstrated the presence of Bifidobacteria in the faeces of piglets.

In human faeces Bifidobacteria are present in large numbers, 10^8 – 10^{11}/g; the number may differ among people of different socio-economic groups, races and diets, as already highlighted in this paper. About eleven main species isolated from human faeces have been recorded (Buchanan and Gibbons, 1974). The most common species are *B. adolescentis, B. infantis*, in addition to *B. bifidum, Lactobacillus bifidus*, and *B. brevey* which are found in animal sources (Opara, 1978). They have been recently proposed for use as faecal indicators in tropical waters, as they overcome the principal disadvantage of faecal coliform counts in tropical samples, especially as a significant proportion of coliform strains which are not of faecal origin, can ferment lactose and produce indole at 44°C (Evison and James, 1973, 1974).

Pseudomonas aeruginosa is a gram-negative, actively motile, non-sporing, strictly aerobic bacillus. It is very widely distributed in nature. It is a known pathogen for man and animals, and because it is primarily associated with human (unlike animal) faeces, and has better survival characteristics than coliforms, it has been suspected as a possible indicator of faecal pollution (Ringen and Drake, 1952).

Pseudomonas aeruginosa has a doubtful status as an intestinal indicator organism because of poor reliability and multiplication in surface waters; thus it has rather been recommended as a sensitive indicator of swimming pool hygiene, as it is often incriminated in eye and ear infections, particularly otitis externa (Cabelli *et al.*, 1976, Cabelli, 1978, American Public health Association, 1985).

Bacteroides species (especially *B. fragilis*), *Peptococcus*- and *Peptostreptococcus* species and *Eubacterium* species, are also non-sporulating anaerobes, normally occurring in faeces. They have been proposed as faecal indicators, although there is no sufficient information available as to their usefulness (Evison and James, 1973, 1974, Feachem, 1977, Opara, 1978). Opara (1978) has observed that *B. fragilis* and Bifidobacteria have the potential for being valuable indicators of faecal pollution in the tropics.

Perspectives

From public health point of view, there is need to monitor water quality in order to guard against health hazards arising from the consumption of faecally - polluted water. Bacteriological analysis of water offers the most delicate test for the detection of faecal pollution; and in principle, the detection of faecal coliform confirms faecal pollution. The safeguard is that, where there is faecal coliform in water, there is a probability that enteric pathogenic bacteria could be present.

However, it is now well established that coliform bacteria are a heterogeneous group of bacterial species made up of *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter* and Aeromonas genera (Menon, 2001). Excluding *Escherichia coli* which is exclusively faecal in origin, the other four genera are widely distributed in nature and commonly found in soils, on vegetation and in industrial waste; further, the non-faecal coliform biotypes are frequently associated with surface runoff and have the tendency to multiply in nutritionally rich waters (Reports on Public Health and Medical subjects, 1969, Menon, 2001). Menon (2001) has proposed that there should be more investigation as to the origin of the coliform organisms, because the presence of total coliforms in surface waters does not always imply faecal contamination, while the sanitary significance of these bacteria becomes doubtful if no obvious pollution source is found.

In fact, the use of indicator bacteria as a safeguard in water treatment plants has been proved not to be 100% safe following the waterborne outbreaks of gastroenteritis in the U.K. in which *Cryptosporidium* and *Giardia* have been implicated and traced to water treatment plants which met all current statutory microbiological and chemical standards (Anon; 1990).

Because *Cryptosporidium* oocysts and *Giardia* cysts are commonly found in surface waters contaminated by cattle and sheep faeces, water treatment process could be challenged by a large number of these parasites in water distribution system (Byrne, 2002). *Cryptosporidium* oocyst and *Giardia* cysts can survive in water for several months unlike the indicators, and are highly resistant to chlorination which has no effect at all to particularly *Cryptosporidium* (Korich *et al.*, 1990, Adam, 1991). This implies that the established bacterial indicators for water quality, for example the coliform group, are not appropriate for these *Cryptosporidium* and *Giardia* (Byrne, 2002).

Up till now, faecal coliform is acclaimed the most reliable indicator of faecal pollution of water. Yet there are still problems of general acceptability in terms of ecology, growth conditions in both temperate and hot climates, specificity of characteristics, taxonomy and absolute reliability as indicator, etc. For instance, El-Ghoddi *et al.* (1998) observed that the highly dominant taxons for some water samples were *Alcaligenes* sp. and *Enterobacter* sp., although most dominant taxons were *Xanthobacter* sp., and *Pseudomonas* sp.

It appears that the ecology of coliform is not conclusively understood and this poses a problem in epidemiological investigation. For instance, faecal coliform is known to occur in the intestine of birds and animals other than man (Reports on Public Health and Medical subjects, 1969). A recent investigation of the input of faecal matter to stormwater drains in Rippleside area of Geelong, Victoria with the combined use of sterol biomarker and four subgroups of bacterial indicators, Leeming *et al.* (1998) indicated that the combination approach could greatly assist in distinguishing sources of faecal pollution, but the use of either sterol or other single indicators alone was inadequate to fully discern faecal contamination from human source. Therefore, there is a limitation to the value of demonstrating *E. coli* (faecal coliform) in water supplies, as the source of the excrement, namely, whether from man, beast or bird, cannot be distinguished since the same organism can occur in all. This situation makes the investigation of the ecology of coliform inconclusive.

Burman (1961) has used differences in growth temperatures to differentiate intestinal coliforms from those isolated from natural environment. He observed that intestinal coliforms have an optimum growth temperature near 37°C whereas those isolated from sources more remote from faecal pollution more frequently have an optimum growth temperature near 30°C. Doubt further arises from the use of optimum growth temperature to differentiate coliforms from natural habitats from those of intestinal origin in tropical climate as the natural temperature in hot climate may be even higher than 30°C or 37°C. Regulated temperature in the laboratory has the danger of providing a result not obtainable under natural conditions.

From taxonomic point of view, bacteriologists have previously carried out a differential test of coliforms by IMVic (Indole, Methyl-red, Voges-Proskauer and Citrate utilization) test (Edwards and Ewing, 1962). It has been noted that in using differential test, not all strains taxonomically assigned to coliform group necessarily conform to the coliform definition used earlier in this paper (Reports on Public Health and Medical subjects, 1969), because (i) not all strains ferment lactose, and may not produce gas if at all they do; (ii) not all lactose-fermenting or sheen-producing gram-negative rods found in water are coliforms (they may be *Aeromonas);* (iii) not all strains of a given species will react uniformly in a given substrate; (iv) the traditional "IMVic" tests which are useful for coliform differentiation do not provide for complete identification, and therefore require additional biochemical test; (v) indicator organisms of the family Enterobacteriaceae are known to ferment lactose with the formation of gas within 48°C at the appropriate temperature, on an Endo- type medium containing lactose, but aerogenic (non-gas-producing) lactose-fermenting strains of *E. coli* may also be encountered (American Public Health Association, 1985).

Thus by using other tests for differentiating organisms of the family Enterobacteriaceae, many of the so-called coliform organisms isolated from water may be seen to differ from those isolated from the intestine or from clinical materials, and may therefore be difficult to assign to the recognised genera of the coliform group. The significance of the coliform organisms other than *E. coli* found in water might therefore generate considerable doubt, although with increase in knowledge of methods of differentiation, it may become possible to accurately distinguish those of intestinal origin from those of non-intestinal origin.

Of interest to water bacteriologists is the fact that some variants of *Enterobacter aerogenes* and cloacae also ferment lactose at 44°C with the production of gas (Holden, 1970). This has created doubt on the reliability of the use of *E. coli* as an indicator of faecal pollution. Further doubts have been created by the fact that the normal flora of human faeces in India, Ceylon, Egypt and Singapore have been shown to contain aerogenes (cloacae group) and *Citrobacter* which have proved positive to Eijkman test at 44°C (Opara, 1978), a test which characterizes *E. coli* (Reports on Public Health and Medical subjects, 1969).

Looking at the requirements which indicators must fulfill, as has earlier been noted in this paper, no bacterial species or group in use, completely fulfills all the requirements, at present. Therefore the search for a reliable indictor is for now inconclusive.

CONCLUSION

The use of indicator organisms still remains epidemiologically valid in water bacteriology, but still requires further research to eliminate doubts and establish greater reliability. To a very large extent, however, regular bacteriological examination of water can contribute to safeguarding a population against epidemic outbreaks of water-related diseases. Of primary importance in the elimination of diseases are health education campaigns, teaching the application of basic hygienic principles in daily life, e.g., washing of hands after toilet, ensuring that no privies are sited over rivers whose water is used as drinking water by people downstream, etc.

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