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Water Composition and its Microbiological Analysis

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Abstract

Water is essential for all forms of life, composed of hydrogen and oxygen (H_2O), with naturally occurring components that vary depending on its source. Beyond its basic molecular structure, water contains various dissolved minerals, organic matter, and a diverse microbiome. Microbiological analysis of water focuses on identifying microbial species, particularly pathogenic bacteria, viruses, protozoa, and fungi that may pose health risks. This analysis is crucial in assessing water quality for drinking, recreational, agricultural, and industrial uses. Key microbial contaminants include *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, and coliform bacteria, which serve as indicators of fecal contamination. The presence of these organisms is typically evaluated through methods such as membrane filtration, most probable number (MPN), and polymerase chain reaction (PCR) techniques. Understanding the microbiological composition of water allows for effective monitoring and treatment processes to ensure safety and compliance with regulatory standards. This paper explores the components of water, focusing on natural and contaminant microbes, and examines standard methods used in microbiological analysis to maintain public health and environmental safety. The insights gathered from such analyses are instrumental in guiding water treatment practices and policy-making to prevent waterborne diseases.

Keywords: Water Component, Microbiological Analysis, *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, and Coliform Bacteria

Introduction

The major sources of drinking water include: Streams, Lakes, Rivers, Ponds, Rainwater and Underground water (spring, wells, and boreholes). Underground water is safer and purer for domestic use than surface water because the ground itself serves as an effective filter medium (European Food Safety Authority, EFSA). Water from deep wells and deep springs usually dissolves a lot of salts and other minerals which is a major problem with underground water and so the water becomes salty, sometime too salty or "hard" for any use unless the salts are removed which is expensive [1].

Sources of Water Pollution

The main sources of boreholes and well water pollutants are industrial, domestic and agricultural waste. Irrigation water and runoff water from rain carrying fertilizers, herbicides, feacal matters mix with natural water bodies and pollute the water [1]. Recycling of treated/inadequately treated waste water by mixing them with natural water bodies adds microorganisms. When septic tanks are built near the water bodies mixing or seeping of excreta may occur and this may act as a source of waterborne pathogens. Waste water from abattoirs and animal processing plants also contribute to the water borne pathogens (Fig 1). Droppings from nearby birds and faecal materials of domestic and wild animals including those of diseased ones are another potential source (European Food Safety Authority, EFSA).

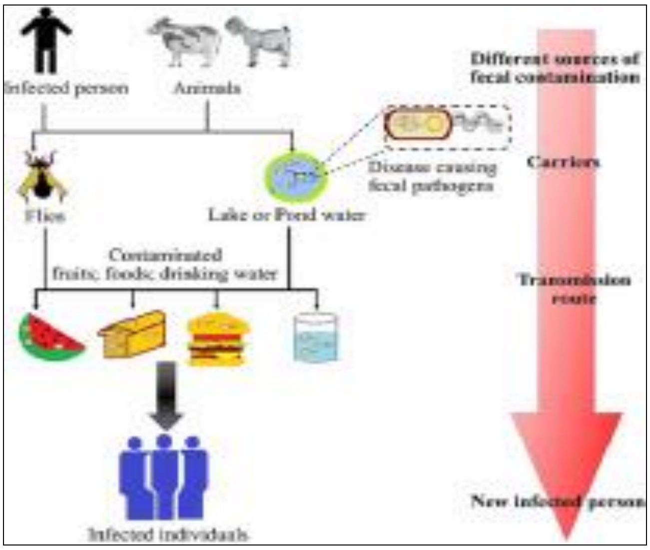

Fig 1: Microbial pollution of water [2]

Bacteriological Water Analysis

Bacteriological water analysis is a method of analysing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is an analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations [1]. The pathogenic organisms which may be present in water are numerous and identifying these organisms individually in practice (bacteria, protozoa, helminths etc.) is difficult. As their presence is always linked to faecal pollution (except for guinea worm), it is preferable to look for organisms which are “indicators” of this pollution [1]. The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern [3]. Indicator organisms are bacteria such as non-specific coliforms, Escherichia coli and Faecal Streptococci such as Enterococcus faecalis that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage [3]. It is therefore reasonable to summarize that if indicator organism levels are low, then pathogen levels will be very much lower or absent. The count of those colonies which develop with a characteristic appearance gives the number of faecal coliforms in the sample of water [4]. Judgments as to suitability of water for use are based on extensive precedents and relate to the probability of any sample population of bacteria being able to be infective at a reasonable statistical level of confidence. Analysis is usually performed using culture, biochemical and sometimes optical methods. When indicator organism’s levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (when suspected) using specific culture methods or molecular biology [4].

Types of water

Portable Water

Portable water is free of pathogens and toxic chemicals. Purification can be done by coagulation, which is by adding alum, Nitrogen aluminates or Ferric chloride to the water. Using the sand bed method, filtration can be carried out or repair sand bed filters can also be used. For the correction of pH of portable water, limestone is added. Portable water is often treated by chlorination. This makes the water free from any coliform organism no matter how polluted the original water may have' been [4]. Water treatment involves the conversion of water taken from the natural sources, the “raw water” into that suitable for domestic use. Ground water and surface water usually require more critical treatment than rain water. Harvested water also requires some form of treatment. Most important is the removal of pathogenic organisms and toxic substances such as heavy metals that can cause health problems [5]. Storage of water may be regarded as a form of treatment. Schistosoma mansoni cercariae are normally unable to survive 48 hours of storage. Also the number of faecal Escherichia coli will be considerably reduced when the raw water is subjected to storages [4].

Non Portable Water

Non- potable water is one contaminated with domestic and industrial waste. There are so many characteristics that make water not potable such as taste, smell, pH, colour/turbidity and mineral salts [4].

Microbiologically Contaminated Water

Water may contain numerous pathogenic organisms and thereby become a means of transmission for many diseases (Fig 2). These includes: Typhoid and paratyphoid fever, Hepatitis A, Cholera, Poliomyelitis, Diarrhoea (caused by Escherichia coli, Salmonellae, Yersinia enterocolitica), Viral gastroenteritis, Bacillary dysentery (caused by various species of Shigella), Campylobacter dysentery, Amoebic dysentery, Giardia (lambliasis), Balantidiasis, Helminthiasis cause by Ascaris and Trichuris [3]. Besides these diseases, water is also involved in the transmission of “water- based” diseases (that is, diseases of which the causative agent passes part of its life cycle in an aquatic plant or animal): The different schistosomiasis or bilharziasis: diseases caused by helminths (worms) which are usually contracted by contact with infected water but sometimes also via the oral route. Dracunculiasis (Guinea worm), transmitted only by drinking infested water [6]. Lastly, water may also transmit: Leptospirosis: a bacterial disease which is contracted primarily by contact with water contaminated with the infected urine of various animals (principally the rat). All the infectious diseases transmitted by water with exception of guinea worm are linked to the pollution of the water by the excreta of humans or other animals infected. One last category of water related diseases is those with an insect vector which develops in or lives near to the water, for example malaria, dengue and yellow fevers, and onchocerciasis [6].

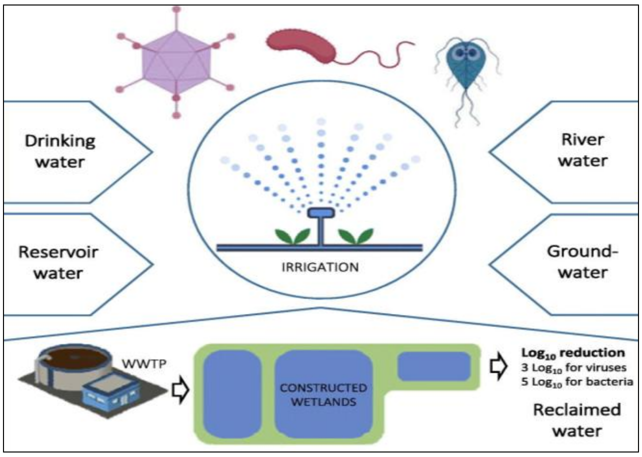

Fig 2: Microbiological contamination of water [7]

Water Borne Diseases

Water-borne diseases are any illness caused by water people drink that is contaminated by animal or human faeces, which contain pathogenic microorganisms. Waterborne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Water should be harmless to health and have an appearance and taste acceptable to the population. Ideally the water supplied should meet the quality standard of the WHO. Quite a number of human pathogens find their way into a susceptible host through contaminated water. These pathogens often called waterborne pathogens, have the ability to survive at least for a short period in water and thus water may act as a route of transmission for them [4]. Waterborne diseases are posing a serious threat to health since the potential of contaminated water to transmit disease is very high. Often they lead to epidemic. According to a WHO survey about 30,000 people die from water-related diseases every day [6]. About 80% of all illness in developing countries is water related [6]. Typhoid fever: this is caused by ingestion of Salmonella typhi bacteria in food or water. Infection causes a sudden high fever, nausea, severe headache, and loss of appetite. It is sometimes accompanied by constipation or diarrhea [4]. Hepatitis (A & E): This is caused by viral infection. Symptoms include yellowing of the skin and eyes (jaundice), dark urine, fatigue, nausea and vomiting. Two forms of the disease, hepatitis A and E, are primarily caused by ingestion of faecally contaminated drinking water [4]. Hepatitis A causes about 1.5 million infections each year (mostly in children), and can occur in epidemics. Hepatitis E is less common than hepatitis A, and occurs mainly in epidemics caused by monsoon rains, heavy flooding, contamination of well water, or massive uptake of untreated sewage into water bodies. No specific treatment exists for hepatitis A or E, but most (>98%) patients recover completely. Hepatitis can have more serious effects on older or immunocompromised people, and pregnant women are particularly vulnerable to hepatitis E, with approximately 20% mortality rates [4]. Haemorrhagic colitis and haemolytic uremic syndrome: This is an infection associated with Escherichia coli 0157:H7.

Water-Washed Diseases: Water-washed diseases are diseases caused by inadequate use of water for domestic and personal hygiene. Control of water-washed diseases depends more on the quantity of water than the quality. Most of the diarrhea diseases should be considered to be water-washed as well as water-borne. Four types of water-washed diseases are; soil-transmitted helminths, acute respiratory infections (ARI), skin and eye diseases, and diseases caused by fleas, lice, mites or tick [6].

Water Quality

The importance of high quality water cannot be over-emphasized as it sustains human life and maintains health. Most waters, before they reach the consumer, have been exposed to greater or lesser amount of contamination, but in the majority of case, they have also undergone a more or less complete purification by natural agencies [6].

The Microbiological Quality of Water

Water supplies in developing countries are devoid of treatment and the communities have to make use of the most convenient supply. Many of these water supplies are unprotected and susceptible to external contamination from surface runoff, windblown debris, human and animal faecal pollution and unsanitary collection methods [6]. Detection of each pathogenic microorganism in water is technically difficult, time consuming and expensive and therefore not used for routine water testing procedures [6]. Instead, indicator organisms are routinely used to assess the microbiological quality of water and provide an easy, rapid and reliable indication of the microbiological quality of water supplies.

In order for a microorganism to be used as an indicator organism of pollution,the following requirements should be fulfilled [8]:

- The concentration of the indicator microorganism should have a quantitative relationship to risk of disease associated with exposure (ingestion/recreational contact) to the water
- The indicator organism should be present when pathogens are present.
- The persistence and growth characteristics of the indicator organism should be similar to that of pathogens.
- The indicator organism should be present in higher numbers than pathogens in contaminated water.
- The indicator organism should be at least as resistant to adverse environmental conditions, disinfection and other water treatment processes as pathogen.
- The indicator organism should be non-pathogenic and easy to quantify.
- The tests for the indicator organism should be easy, rapid, inexpensive, precise, have adequate sensitivity, quantifiable and applicable to all types of water.
- The indicator organism should be specific to a faecal source or Identifiable as to the source of origin of faecal pollution.

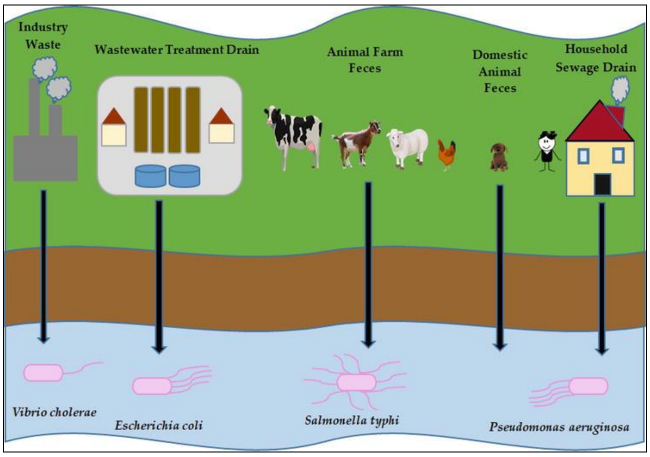

Fig 3: Various factors affecting of water quality and disease-causing some bacteria [7]

In spite of the shortcomings of indicator microorganisms, it is better to use a combination of indicator microorganisms to give a more accurate picture of the microbiological quality of water. In general, every country has its own set of guidelines for drinking water. However, most of these guidelines are similar for different countries and the same indicator microorganisms to indicate the presence of pathogenic microorganisms are used.

Heterotrophic plate counts

Heterotrophic microorganisms or heterotrophs are naturally present in the environment and can be found in soil, sediment, food, water and in human and animal faeces. Broadly defined, heterotrophs include bacteria, yeasts and molds that require organic carbon for growth. Although generally considered harmless, some heterotrophic microorganisms are opportunistic pathogens, which have virulence factors that could affect the health of consumers with suppressed immune systems [6]. Heterotrophic microorganisms can also survive in biofilms inside water distribution systems, water reservoirs and inside household storage containers [6]. Therefore, heterotrophic plate counts can also be used to measure the re-growth of organisms that may or may not be a health risk.

Heterotrophic Plate Count, also known as Total or Standard Plate Count includes simple culture based tests intended to recover a wide range of heterotrophic microorganisms from water environments. Enumeration tests for heterotrophic plate counts are simple and inexpensive giving results within 48 h to 5 days, depending on the method, type of media and the incubation temperature used. The pour plate, membrane filtration or spread plate methods are used routinely in various laboratories, with either Yeast-extract agar, Plate Count Agar (PCA), Tryptone Glucose agar or R2A agar, and incubation periods either at room temperature (25ºC) for 5 to 7 days, or at 35°C to 37°C for 48 h. Heterotrophic plate counts alone cannot indicate a health risk and additional studies on the presence of *E. coli* or other faecal specific indicator microorganisms need to be conducted to establish the potential health risk of the water analysed.

Total coliform bacteria

Total coliform bacteria are defined as aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, which ferments lactose and produce gas at 35°C [1]. Total coliforms include bacteria of known faecal origin such as *E. coli* as well as bacteria that may not be of faecal origin such as *Klebsiella* spp, *Citrobacter* spp, *Serratia* spp and *Enterobacter* spp which are found in nutrient rich water, soil decaying vegetation and drinking water with relatively high levels of nutrients [6]. The recommended test for the enumeration of total coliforms is membrane filtration using mEndo agar and incubation at 35°C to 37°C for 24 h to produce colonies with golden-green metallic shine.

In water quality studies, total coliform bacteria are used as a systems indicator, which provides information on the efficiency of water treatment. The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water environments and pathogenic pathogens such as *Salmonella* spp, *Shigella* spp, *V. cholera, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica* and pathogenic *E. coli* may be present. These pathogens and opportunistic microorganisms could cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis to consumers. In particular, individuals who suffer from HIV/AIDS related complications are more at risk of being infected by these microorganisms [1].

Faecal coliform bacteria

Faecal coliform bacteria are Gram negative bacteria, also known as thermotolerant coliforms or presumptive *E. coli*. The faecal coliform group includes other organisms, such as *Klebsiella* spp, *Enterobacter* spp and *Citrobacter* spp, which are not exclusively of faecal origin. *Escherichia coli* are specifically of faecal origin from birds, humans and other warm blooded animals. Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faeces.

The recommended test for the enumeration of faecal coliforms is membrane filtration using mFC agar and incubation at 44.5°C for 24 h to produce blue colored colonies. Faecal coliforms are generally used to indicate unacceptable microbial water quality and could be used as an indicator in the place of *E. coli*. The presence of faecal coliforms in a water sample indicates the possible presence of other pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, pathogenic *E. coli, V. cholera*, *Klebsiella* spp and *Campylobacter* spp associated with waterborne diseases. Unfortunately faecal coliform bacteria exhibit species to species variations in their respective stability and resistance to disinfection processes; do not distinguish between faeces of human and animals origin; have low survival rates and have been detected in water sources thought to be free of faecal pollution.

***Escherichia coli* bacteria**

Globally *E. coli* is used as the preferred indicator of faecal pollution. It is a Gram negative bacterium and predominantly an inhabitant of the intestines of warm blooded animals and humans, which is used to indicate recent faecal pollution of water samples [1]. Confirmation tests for *E. coli* include testing for the presence of the enzyme β-glucuronidase, Gram staining, absence of urease activity, production of acid and gas from lactose and indole production.

Commercially available growth media containing the fluorogenic substrate 4-methyl-umbelliferyl-β-Dglucuronidase (MUG) is used for the isolation and identification of *E. coli* from water samples (Shadix). The *E. coli* bacteria hydrolyse the MUG in the media, which then fluoresces under ultraviolet light [4]. However, false negative results on this media have been found due to injured cells, lack of expression of the gene which codes for the enzyme β-glucuronidase by the *E. coli* bacterium isolate, and non-utilization of the MUG reagent in the media by some *E. coli* strains.

Faecal enterococci bacteria

Faecal enterococci bacteria are found in the genus *Enterococcus* and include species like *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans* and *Enterococcus hirae*. The genus *Enterococcus* are differentiated from the genus *Streptococcus* by their ability to grow in 6.5% sodium chloride, pH 9.6, temperatures of 45ºC and their tolerance for adverse growth conditions. Faecal enterococci are spherical, Gram positive bacteria, which are highly specific for human and animal faecal pollution. Most of the species in the *Enterococcus* genus are of faecal origin and is regarded as specific indicators of human faecal pollution, although some species are found in the faeces of animals and plant material.

***Clostridium perfringens* bacteria**

Clostridium perfringens is a Gram positive, sulphite reducing anaerobic, rod shaped, spore forming bacteria normally present in faeces of humans and warm blooded animals [1]. However, *C. perfringens* are also found in soil and water environments. The spores can survive much longer than coliform bacteria and are highly resistant to water disinfection and treatment processes. *Clostridium perfringens* are therefore used as an indicator of faecal pollution to indicate the potential presence of enteric viruses, which may include Enteroviruses, Adenoviruses and Hepatitis viruses as well as the cysts and oocysts of protozoan parasites such as *Giardia*, *Entamoeba* and *Cryptosporidium* in treated drinking water. The enumeration test includes membrane filtration using specific medium (e.g. mCP or *Perfringens* selective OPSP medium with supplements) and incubation 35°C to 37°C for 48h at in micro-aerophillic conditions to produce black colonies.

Bacteriophages

Bacteriophages are viruses, which specifically infect bacteria. Bacteriophages have been suggested as useful indicators to predict the potential occurrence of enteric viruses in water [9]. The survival of bacteriophages is affected by the densities of the host and the bacteriophages in the water sample. In addition, the association of the bacteriophage with solids and the presence of organic matter in the water sample could influence the attachment of the bacteriophages to the host bacterium [9]. Several studies have shown that ultra violet light, temperature, pH of the water, and ion concentrations in the water could affect the survival of bacteriophages in water [1]. Bacteriophages show higher resistance to environmental stress compared to bacterial indicators such as total coliforms and faecal coliforms and assays for bacteriophages can be conducted quickly, economically and quantitatively. There are several bacteriophages that can be used as indicator organisms which includes the somatic bacteriophages, *Bacteroides fragilis* HSP40 bacteriophages and male specific F-RNA bacteriophages [9].

Somatic bacteriophages

The somatic bacteriophages are a heterogeneous group of organisms that absorbs to bacterial receptors for infection and replication on the cell wall of the laboratory host strain *E. coli* WG5. Somatic bacteriophages are therefore, used as indicators of the potential presence of enteric viruses in water. These bacteriophages can serve as models for the assessment of the behaviour of enteric viruses in water treatment and disinfection processes [9]. The double layer plaque assay is generally used to detect somatic bacteriophages. However, somatic bacteriophages are not specific to *E. coli*, and may infect and replicate in other species of the Enterobacteriaceae family, which includes the total coliform group [9]. Somatic bacteriophages are therefore, not considered a specific indicator for faecal pollution.

***Bacteroides fragilis* HSP40 bacteriophages**

Bacteroides bacteria are present in high numbers in human faeces. *Bacteroides* is a strict anaerobic, Gram negative, non-spore forming bacterium which is rapidly inactivated by oxygen levels in water, and needs complex growth media with antibiotics to inhibit the interference from other intestinal microorganisms. The *Bacteroides fragilis* HSP40 bacteriophages are a relatively homogeneous group that do not multiply in the environment [6]. In some countries, *Bacteroides fragilis* HSP40 bacteriophages is present in relatively low numbers in human faeces. Although this bacteriophage has been shown to be highly specific for human faeces, tests are complicated and labour intensive.

Male specific F-RNA bacteriophages

The male specific F-RNA bacteriophages have small hexagonal capsomers without tails, are approximately 30 nm long with a single RNA genome. Male specific F-RNA bacteriophages have been recommended as useful models for monitoring the behaviour of human enteric viruses in water treatment processes because of their size and structure, which are similar to those of the Enteroviruses [9]. These bacteriophages are relatively resistant to disinfectants, sunlight, heat- and water treatment processes [6].

Male specific F-RNA bacteriophages specifically attach to the sex pili of the host bacterium [*E. coli* HS(pFamp)R or *Salmonella typhimirium* WG49] in temperatures higher than 30°C. The F-pilli are short tube-like protrusions produced by certain bacteria for the transfer of nucleic acid to other bacteria of the same or closely related species and are only produced by the bacteria in the log growth phase which is usually above 30ºC. These bacteriophages are assayed according to an International Standardization Method. Male specific F-RNA bacteriophages belong to the family *Leviviridae*, which contains two genera, the *Leviviridae* and the *Alloleviviridae*. Both these genera contain distinct subgroups, which is useful in genotyping assays where specific probes are used to distinguish between animal (subgroups I and IV) and human (subgroups II and III) faecal pollution [10].

Human and animal faecal pollution in water

Water polluted with human and animal faeces may contain potentially pathogenic microorganisms that can cause diseases in consumers. The most commonly used faecal indicator microorganisms which include the total coliform bacteria, thermotolerant coliform bacteria, *E. coli* and faecal enterococci bacteria, are found in both human and animal faeces, but do not differentiate between the origins of faecal pollution. Human viral pathogens such as Calicivirus, Hepatitis E virus, Reoviruses, Rotaviruses, somatic bacteriophages and male specific F-RNA bacteriophages also infect other animals which can serve as reservoirs. Consequently, these animals can be important potential sources of contamination of water sources because the release of microorganisms into aquatic environments by animal hosts could lead to human exposure. Poor communities in developing countries share their water sources with cattle and other domestic animals, therefore, the risk of waterborne transmission of zoonotic pathogens to humans, increases [10]. However, water contaminated with human faeces is regarded as a greater risk to human health since it is more likely that it would contain human specific enteric pathogens. Although various microbial and chemical indicators have been described to identify the origin of faecal pollution in water supplies, different levels of success have been obtained [6].

The use of microorganisms to determine the origin of faecal pollution

Several microorganisms have been suggested and tested to distinguish between human and animal faecal pollution in domestic drinking water supplies. Various factors can have an effect on the specificity of microorganisms that can be used as indicators to determine the origin of faecal pollution, such as: (1) specific bacteria, viruses and protozoan parasites can have multiple hosts (not species specific) [10]; (2) different microorganisms can have similar biochemical reactions in the environment, especially within the same species or genus (Sinton) and (3) interspecies gene transfer may occur which include small pieces of DNA (eg. plasmids and integrons) and transposons that are carried from one bacteria to another during sexual and asexual reproduction of bacterial cells.

Microorganisms that have been used in assays to determine the origin of faecal pollution include total coliforms, faecal coliforms, faecal streptococci/enterococci, *Bacteroides* spp, *Bacteroides fragilis* HSP40 bacteriophages, *Pseudonomas aeruginosa*, *Bifidobacterium* spp, *Rhodococcus coprophilus*, male specific F-RNA bacteriophages and specific human enteric viruses [10].

The ratio of faecal coliform bacteria to faecal streptococci bacteria

The ratio between faecal coliform (FC) and faecal streptococci/enterococci (FS) counts in water is an old method used in several earlier studies to determine the origin of faecal pollution [10]. This method is based on the fact that faecal streptococci/enterococci are more abundant in animal faeces than in human faeces while faecal coliforms are more abundant in human faeces than in animal faeces. The test stipulates that a FC:FS ratio greater than 4 is indicative of human faeces and a FC:FS ration of less than 7 is indicative of animal faecal pollution [6].

The limitation of this method is the variable survival rates of some faecal streptococci species, which make this test unreliable.

The ratio of faecal coliform to total coliform bacteria

Faecal coliforms constitute a subset of total coliforms but grow and ferment lactose with the productof gas and acid at 44.5°C within 24 h. The ratio of faecal coliforms to total coliforms is used to show the percentage of total coliforms that comprises of faecal coliforms which comes from the gut of warm blooded animals. If the faecal coliforms to total coliforms ration exceeds 0.1 it may suggests the presence of human faecal contamination. However, this method only shows the possibility of faecal pollution but do not distinguish between human and animal faecal matter [6]. Another disadvantage of this assay is that some faecal coliforms can multiply in soils in tropical regions and give a false positive result for water pollution.

B*acteroides* bacteria and *bacteroides* hsp40 bacteriophages

Bacteroides bacterial species are among the numerous bacteria in human faeces and is also found in low numbers in animal faeces [10]. The bacterium does not survive for long periods outside the human body making the detection of *Bacteroides* difficult.

However, the *Bacteroidis fragilis* HSP40 bacteriophage strain is a highly specific indicator for human faecal pollution but is only present in low numbers in human sewage [6]. The assays used for the *Bacteroides* bacteria and the *Bacteroides fragilis* HSP40 bacteriophages are expensive, complicated, time consuming and require specialised equipment and skilled labour [6].

***Pseudomona aeruginosa* bacteria**

Pseudonoma aeruginosa bacteria are present in 16% of human adults but occur rarely in lower animals. Unfortunately this bacterium is present in water, soil and sewage samples and can rapidly die-off in aquatic environments and is therefore not a suitable candidate to determine the source of faecal pollution [10].

***Bifidobacterium* spp**

Bifidobacteria spp are strickly anaerobic, Gram positive bacteria present in the gut of humans and animals. Species such as *Bifidobacteria adolescentis* are specific to humans while species such as *Bifidobacteria thermophilum* are specific to animal faeces. It is difficult to differentiate between the species based on biochemical and microbiological analysis, which complicates the interpretation of the results [10].

***Rhodococcus coprophilus*bacteria**

Rhodococcus coprophilus is a Gram positive, aerobic nocardioform actinomycete which forms a fungus-like mycelium that breaks up into bacteria-like pieces [6]. The bacteria contaminate grass and when eaten by herbivores these bacteria-like pieces are found in the herbivore dung. *Rhodococcus coprophilus* has never been found in human faeces and is therefore used as an indicator of animal faecal pollution [10]. The disadvantage of this bacterium is the long growth time of 21 days [10]. Saville and co-workers (2001) have designed a PCR protocol to detect this organism in faecal specimens of animals, which showed potential to be used as a routine laboratory test, but more studies are needed to evaluate this detection technique.

Conclusion

Water is a vital resource composed primarily of hydrogen and oxygen, often containing trace minerals and various dissolved substances. Microbiological analysis of water is crucial for assessing its safety and quality, particularly for drinking, agricultural, and industrial use. This analysis identifies microbial contaminants like bacteria, viruses, and protozoa, which can pose health risks. Techniques such as membrane filtration, multiple-tube fermentation, and molecular methods help detect pathogens and ensure water meets health standards. Ensuring safe, clean water is essential for public health, as contaminated water can lead to disease outbreaks and impact ecological systems. Proper water treatment and monitoring are therefore imperative.

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