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Water and Waterborne Diseases: A Review

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Authors' contributions

This review work was carried out in collaboration between all authors, although the work was masterminded by the first author. All authors read and approved the final manuscript.

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ABSTRACT

Despite numerous efforts by government at various levels and other agencies interested in water and its safety, waterborne diseases are still a major public health and environmental concern. The huge investment towards water research, although worth the spending, has not yielded the much expected result as waterborne diseases continue to plague developing countries with Africa and Asia having the worse hit. The unavailability of pipe-borne water and the dependence of rural dwellers on surface waters which are often contaminated with faecal materials are undoubtedly the major causes of the rising prevalence of waterborne diseases. Water availability and poor hygienic practices amongst these rural dwellers are also of paramount concern as they play significant roles in the spread of water-washed diseases. Also, poor environmental practice which encourages the breeding of insects and other forms of vectors within residential areas contribute to the increasing prevalence of waterborne diseases. This review focuses on waterborne diseases, its classification and the various methods employed in the bacteriological analysis of water.

Keywords: Water; waterborne disease; bacteriological; conventional; molecular.

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1. INTRODUCTION

Countries throughout the world are concerned with the effects of unclean drinking water because water-borne diseases are a major cause of morbidity and mortality [1,2]. Clean drinking water is important for overall health and plays a substantial role in infant and child health and survival [3,4,5,6]. The World Health Organization [7] estimated that globally, about 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters and seafood. Persons with compromised immune systems, such as those with AIDS, are especially vulnerable to water-borne infections, including those infections that are self-limiting and typically not threatening to healthy individuals [8,9]. Throughout the less developed part of the world, the proportion of households that use unclean drinking water source has declined, but it is extremely unlikely that all households will have a clean drinking water source in the foreseeable future [10]. UNICEF [11] reports that 884 million people in the world use unimproved drinking water source, and estimates that in 2015, 672 million people will still use an unimproved drinking water source. In another report, UNDESA [12] put the worldwide estimate for people without access to safe water at nearly 900 million. According to WHO/UNICEF [13], about 2.6 billion, almost half the population of the developing world, do not have access to adequate sanitation. Over 80 per cent of people with unimproved drinking water and 70 per cent of people without improved sanitation live in rural areas [14]. In Nigeria, a vast majority of people living along the course of water bodies still source and drink from rivers, streams and other water bodies irrespective of the state of these water bodies without any form of treatment. These natural waters contain a myriad of microbial species, many of which have not been cultured, much less identified. The number of organisms present varies considerably between different water types, and it is generally accepted that sewage-polluted surface waters contain greater number of bacteria than unpolluted waters [15]. Polluted surface waters can contain a large variety of pathogenic microorganisms including viruses, bacteria and protozoa [16]. These pathogens, often of fecal source, might be from point sources such as municipal wastewater treatment plants [17,18,19,20,21] and drainage from areas where livestock are handled [22] or from non-point sources such as domestic and wild animal defecation, malfunctioning sewage and septic systems, storm water drainage and urban runoff [23,24]. Fecal contamination of water is globally recognized as one of the leading causes of waterborne diseases. The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development. The outbreak of cryptosporidiosis of 1993 in Milwaukee, Wisconsin, in the United States provides a good example. It was estimated that about 400,000 individuals suffered from gastrointestinal symptoms due, in a large proportion of cases, to *Cryptosporidium* [25]. Although subsequent reports suggest that this may be a significant overestimation [26]. More recent outbreaks involving *Escherichia coli* O157:H7, the most serious of which occurred in Walkerton, Ontario Canada in the spring of 2000, resulted in six deaths and over 2,300 cases. The number of outbreaks reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. In Nigeria, cases of water related diseases abound. Agents of these diseases have been found to cut across various classes of organisms. However, most of these cases are not documented since majority of the affected individual subscribes to self-medication rather than seek professional medical attention. The most common waterborne diseases in Nigeria include Cholera, Dracunculiasis, Hepatitis, and Typhoid [27]. Cases of water borne diseases linked to contaminations of drinking water with pathogens have also been reported in several towns [28,29,30]. Waterborne outbreaks of enteric disease occurs either when public drinking water supplies were not adequately treated after contamination with surface water or when surface waters contaminated with enteric pathogens have been used for recreational and or domestic purpose [31]. Instances of disease outbreak due to contaminated drinking water with microbes are also reported [32,33] with the drinking waters sampled from Sokoto, Shuni and Tambuwal towns having *E. coli, Salmonella, Shigella and Vibrio* species far above the WHO [15] allowable limit [32] and are therefore not potable. The role of water as a vehicle for the transmission of all manner of water related illnesses is no longer a subject for debate, even ancient histories and books contain extracts indicative of this fact. Table 1 below shows some of the diseases related to water and sanitation which are endemic in sub Saharan Africa as well as their route of infection.

Table 1. Diseases related to water and sanitation endemic in Sub-Saharan Africa

Group	Disease	Route leaving host	Route of infection
Disease which are often water-borne	Cholera	Faeces	Oral
	Typhoid	Faeces/urine	Oral
	Infectious hepatitis	Faeces	Oral
	Giardiasis	Faeces	Oral
	Amoebiasis	Faeces	Oral
	Dracunculiasis	Cutaneous	Oral
Diseases which are often associated with poor hygiene	Bacillary dysentery	Faeces	Oral
	Enteroviral diarrhea	Faeces	Oral
	Paratyphoid fever	Faeces	Oral
	Pinworm (Enterobius)	Anal	Oral
	Amoebiasis	Faeces	Oral
	Scabies	Cutaneous	Cutaneous
	Skin sepsis	Cutaneous	Cutaneous
	Lice and typhus	Bite	Bite
	Trachoma	Cutaneous	Cutaneous
	Conjunctivitis	Cutaneous	Cutaneous
Diseases which are often related to inadequate sanitation	Ascariasis	Faecal	Oral
	Trichuriasis	Faecal	Oral
	Hookworm (*Ancylostoma/Necator*)	Faecal	Oral/percutaneous
Diseases with part of life cycle of parasite in water	Schistosomiasis	Urine/faeces	Percutaneous
Diseases with vectors passing part of their life cycle in water	Dracunculiasis	Cutaneous	Oral

Adapted from Bradley, D J, London School of Hygiene and Tropical Medicine

2. CLASSIFICATION OF WATERBORNE DISEASES

Waterborne or water related diseases encompass illnesses resulting from both direct and indirect exposure to water, whether by consumption or by skin exposure during bathing or recreational water use. It includes disease due to water-associated pathogens and toxic substances. A broader definition includes illness related to water shortage or water contamination during adverse climate events, such as floods and droughts, and diseases related to vectors with part of their life cycle in water habitats [34]. Basically, waterborne diseases can be transmitted through four main routes: Water-borne route, Water-washed route, Water-based route and Insect vector route or water related route.

3. WATER-BORNE DISEASES

Waterborne diseases are those diseases that are transmitted through the direct drinking of water contaminated with pathogenic microorganisms. Contaminated drinking water when used in the preparation of food can be the source of food borne disease through consumption of the same microorganisms. Most waterborne diseases are characterized by diarrhoea, which involves excessive stooling, often resulting to dehydration and possibly death. According to the World Health Organization, diarrheal disease accounts for an estimated 4.1% of the total daily global burden of disease and is responsible for the deaths of 1.8 million people every year. Further estimates suggest that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene and is mostly concentrated on children in developing countries [13,35,36]. Most waterborne diseases are often transmitted via the fecal-oral route, and this occurs when human faecal material is ingested through drinking contaminated water or eating contaminated food which mainly arises from poor sewage management and improper sanitation. Faecal pollution of drinking-water may be sporadic and the degree of faecal contamination maybe low or fluctuate widely. In communities where contamination levels are low, supplies may not carry life-threatening risks and the population may have used the same source for time

immemorial. However, where contamination levels are high, consumers (especially the visitors, the very young, the old and those suffering from immunodeficiency-related diseases) may be at a significant risk of infection. In rural African regions, faecal contamination of water arises from runoffs from nearby bushes and forest which serve as defecation sites for rural dwellers. Waterborne disease can be caused by protozoa, viruses, bacteria, and intestinal parasites. Some of the organisms remarkable for their role in the outbreak of waterborne disease include Cholera, Amoebic dysentery, Bacillary dysentery (shigellosis), Cryptosporidiosis, Typhoid, Giardiasis, Paratyphoid, Balantidiasis, Salmonellosis, *Campylobacter* enteritis, Rotavirus diarrhoea, *E. coli* diarrhea, Hepatitis A, Leptospirosis and Poliomyelitis [37].

4. WATER-WASHED DISEASES

Water washed or water scarce diseases are those diseases which thrive in conditions with freshwater scarcity and poor sanitation. Control of water-washed diseases depends more on the quantity of water than the quality [38]. Examples of water washed diseases includes; Scabies, Typhus, Yaws, Relapsing fever, Impetigo, Trachoma, Conjunctivitis and Skin ulcers. Four types of water-washed diseases are considered here: soil-transmitted helminthes, acute respiratory infections (ARI), skin and eye diseases, and diseases caused by fleas, lice, mites or ticks. For all of these, washing and improved personal hygiene play an important role in preventing disease transmission [38].

5. SOIL-TRANSMITTED HELMINTHS

Helminths are intestinal worms (nematodes) that are transmitted primarily through contact with contaminated soil. The most prevalent helminths are ascaris (*Ascaris lumbricoides*), hookworm (*Ancylostoma duodenale* and *Necator americanus*) and whipworm (*Trichuris trichiura*). Together, these 'geohelminths' currently infect about one-quarter to one-third of the world's population [38]. Over 130 million children suffer from high intensity geohelminthic infections; helminths cause about 12,000 deaths each year [39]. These diseases can be considered water washed. Improved hygiene and sanitation can reduce their incidence. Mass deworming of children is also recognized as an effective control measure [38].

6. ACUTE RESPIRATORY INFECTIONS

Acute respiratory infections (ARI) including pneumonia are responsible for approximately 19% of total child deaths every year [38]. Evidence demonstrating that good hygiene practices, especially hand-washing with soap, can significantly reduce the transmission of ARI abounds. In view of the link between ARI and hygiene, it can now be considered a water-washed disease [40,41,42].

7. SKIN AND EYE DISEASES

United Nations Children's Fund 2008 posits that trachoma is the world's leading cause of preventable blindness. About 6 million people are blind due to trachoma and more than 10% of the world's population is at risk. Globally, the disease results in an estimated $2.9 billion in lost productivity each year [43] in the US, trachoma is caused by the *Chlamydia trachomatis* bacteria which inflame the eye. After years of repeated infections, the inside of the eyelids may be scarred so severely that the eyelid turns inwards with eyelashes rubbing on the eyeball. Flies are implicated in the transmission of trachoma, and are often seen feeding on the discharge from infected eyes. The best control method for trachoma and conjunctivitis is improved access to water for face washing. Ringworm (tinea) is also water washed disease prevalent among children of school age and the aged. This infectious disease affects the skin, scalp and keratinized tissues and is caused by a fungus [38].

8. WATER-BASED DISEASES

Water-based diseases are infections caused by parasitic pathogens found in aquatic host organisms. These host organisms includes; snail, fish, or other aquatic animal. Humans become infected by ingesting the infective forms or through skin penetration. Examples of water based diseases includes Schistosomiasis (cercariae released from snail, penetrate skin), Dracunculiasis (larvae ingested in crustacean), Paragonimiasis (metacercariae ingested in crab or crayfish) and Clonorchiasis (metacercariae ingested in fish). These diseases can be prevented through avoiding contact with contaminated water, or use of protective clothing or barrier creams.

9. INSECT VECTOR-BASED DISEASES OR WATER RELATED DISEASES

These diseases are not directly related to drinking water quality. They are those diseases that are caused by insect vectors which breed in or around water bodies. Humans become infected by being bitten by these insect vectors. However, consideration of vector control during the design, construction and operation of surface water reservoirs and canals (for drinking water or irrigation purposes) can reduce the potential for water related disease transmission. Prevalence of water related diseases are high in tropical Africa as a result of poor environmental management and sanitation. Drainages are often waterlogged, hence constituting breeding sites for these insect vectors. Malaria is one of the water related diseases endemic in 117 countries with about 3.2 billion people living in risk areas all over the world [44]. The report further stated that there are about 350 to 500 million clinical cases of malaria worldwide each year with over 1 million deaths. About 59% of all clinical cases occur in Africa, 38% in Asia, and 3% in the Americas. The most common vector insects are mosquitoes and flies.

Mosquito-borne diseases	Fly-borne diseases
• Malaria	Onchocerciasis (River-blindness)
• Yellow fever	Loiasis
• Dengue fever	
• Filariasis	

10. BACTERIOLOGICAL ANALYSIS OF WATER

Microbial contamination is by far the most serious public health risk associated with drinking-water supplies. It is impractical to analyze water for every individual pathogen, some of which can cause disease at very low doses. Instead, since most diarrhea-causing pathogens are faecal in origin, it is more practical to analyze water for indicator species that are also present in faecal matter. The use of indicator organisms in the bacteriological analysis of water has remained the mainstay of water bacteriology. For many years, total coliforms have been used as indicators in evaluating water quality for several water uses with respect to faecal contamination [45,46]. Not all coliforms are from faecal source. Hence, feacal coliforms and pathogenic forms such as *Escherichia coli* are now used largely as bacteriological indicators [47]. The term "total coliforms" refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant (ferment lactose and produce gas at 45.5℃) coliforms and bacteria of faecal origin as well as some bacteria that may be isolated from environmental sources. Thus the presence of total coliforms may or may not indicate faecal contamination. In extreme cases, a high count for the total coliform group may be associated with a low or even zero count for thermotolerant coliforms. Such a result would not necessarily indicate the presence of faecal contamination. It might be caused by entry of soil or organic matter into the water or by conditions suitable for the growth of other types of coliform. In the laboratory total coliforms are grown in or on a medium containing lactose at a temperature range of 35-37℃. They are provisionally identified by the production of acid and gas from the fermentation of lactose [48]. Unlike coliforms from environmental sources, coliforms that come from faecal matter can tolerate higher temperatures. These are more closely associated with faecal pollution than total coliforms. The most specific indicator of faecal contamination is *E. coli*, which unlike some faecal coliforms never multiplies in the aquatic environment [38]. *E. coli* is now internationally acknowledged as the most appropriate indicator of faecal pollution. In source water, its level of occurrence is correlated with the inputs of fecal pollution (human or animal) [49]. Other organisms used as indicators of faecal pollution of water includes: Faecal Streptococci, Enterococci, *Clostridium perfringens, Pseudomonas aeruginosa*, Hydrogen sulphide (H_2S)-producing bacteria, coliphages and other bacteriophages.

11. CONVENTIONAL METHODS FOR BACTERIOLOGICAL ANALYSIS OF WATER

The testing of waters for pathogens has been undertaken since waterborne diseases were first recognized. In 1884, after discovery of culture media and microscopy, Robert Koch first isolated a pure culture of *Vibrio*, and Georg Gaffky isolated the typhoid bacillus [50], the known major causes of waterborne disease in the nineteenth century: cholera and typhoid, respectively. The analysis of water for the presence of coliform bacteria has for long been carried out using two classic/conventional methods. These are the multiple fermentation tube or most probable number technique (MPN)

and the membrane filtration methods. In recent years, two alternatives: the enzyme substrate (defined substrate method) and H_2S methods, have been gaining increasing popularity [38].

12. MULTIPLE TUBE FERMENTATION (MTF) OR MOST PROBABLE NUMBER TECHNIQUE (MPN)

The MPN technique has been used for the analysis of drinking-water for many years with satisfactory results. It is most suitable in the analysis of very turbid water samples or if semi-solids such as sediments or sludges are to be analysed. The procedure followed is fundamental to bacteriological analyses and the test is used in many countries [48]. It is customary to report the results of the multiple fermentation tube tests for coliforms as a most probable number (MPN) index. This is an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the test. It is not a count of the actual number of indicator bacteria present in the sample [48]. Although this test is simple to perform, it is time-consuming, requiring 48 hours for the presumptive results [51]. Multiple samples of the water being tested are added to a nutrient broth in sterile tubes and incubated at a particular temperature for a fixed time (usually 24 hours). If the water source is unprotected or contamination is suspected, serial dilutions of the water (usually 10, 1, and 0.1 mL) may be made. Three or five tubes per dilution are commonly used, though ten tubes may be used for greater sensitivity. As coliform bacteria grow, they produce acid and gas, changing the broth colour and producing bubbles, which are captured in a small inverted tube. By counting the number of tubes showing a positive result, and comparing with standard tables, a statistical estimate of the MPN of bacteria can be made, with results reported as MPN per 100 mL. Since some non-coliform bacteria can also ferment lactose, this first test is called a “presumptive” test. Bacteria from a positive tube can be inoculated into a medium that selects more specifically for coliforms, leading to “confirmed” results. Finally, the test can be “completed” by subjecting positive samples from the confirmed test to a number of additional identification steps. Each of the three steps (presumptive, confirmed and completed) requires 1-2 days of incubation. Typically only the first two steps are performed in coliform and faecal coliform analysis, while all three phases are done for periodic quality control or for positive identification of *E. coli*. Disadvantages to this method include the large number of tubes needed and the long time requirement for the full test. Accordingly, this test is most conveniently applied in a laboratory setting, though the presumptive test is sometimes made with field kits. Another disadvantage of this method (and other MPN methods) is that the result is a statistical approximation with fairly low precision, and as such should only be considered semi-quantitative [38].

13. MEMBRANE FILTRATION METHODS

Until the 1950s, practical water bacteriology relied almost exclusively for indicator purposes on the enumeration of coliforms and *E. coli* based on the production of gas from lactose in liquid media and estimation of most probable numbers using the statistical approach initially suggested [52]. In Russia and Germany however, workers attempted to culture bacteria on membrane filters and by 1943, Mueller in Germany was using membrane filters in conjunction with Endo-broth for the analysis of potable waters for coliforms [53]. By the 1950s, membrane filtration was a practical alternative to the MPN approach although the inability to demonstrate gas production with membranes was considered a major drawback [53]. The membrane filter technique shows remarkable advantage over the MPN technique in that it could be used to test relatively large numbers of samples and yields results more rapidly than the multiple fermentation tube technique. However, this method is inappropriate for turbid waters, which can clog the membrane or prevent the growth of target bacteria on the filter [38]. The technique is hence unsuitable for natural waters containing very high levels of suspended material, sludges and sediments, all of which could block the filter before an adequate volume of water has passed through. When small quantities of sample (for example, of sewage effluent or of grossly polluted surface water) are to be tested, it is necessary to dilute a portion of the sample in sterile diluent to ensure that there is sufficient volume to filter across the entire surface of the membrane. Another concern with this method is that it may not detect stressed or injured coliforms. It was originally designed for use in the laboratory but portable equipment is now available that permits use of the technique in the field [48]. The membrane filter method gives a direct count of total coliforms and faecal coliforms present in a given sample of water. A measured amount of water is filtered through a membrane with a pore size of about 0.45 µm,

which traps the bacteria on its surface. The membrane is then placed on selective agar or a thin absorbent pad that has been saturated with a medium designed to grow or permit differentiation of the organisms sought [54]. The success of this method depends on using effective differential or selective media that will enable easy identification of colonies.

14. DEFINED SUBSTRATE TECHNOLOGY (DST)

In recent times, more rapid and simple methods for the detection of indicator bacteria in water have long been sought. One of such methods is the DST sometimes referred to as the enzyme substrate method. Defined substrate technology (DST) is a new approach for the simultaneous detection, specific identification, and confirmation of total coliforms and *Escherichia coli* in water. This test uses specific indicator nutrients: ortho-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). Enzymes produced by these indicator organisms react with these specific indicator nutrients in the nutrient medium and generally produce a striking colour change that is easy to identify. These tests are more rapid than conventional methods; some can produce results in 24 hours or less. Furthermore, they are more specific than conventional tests, so confirmatory tests are generally not necessary. Colilert® was the first commercial DST test to receive U.S. Environmental Protection Agency approval for drinking water analysis [54]. Two of the most relevant enzyme tests for drinking water are: Beta-galactosidase and Beta-glucuronidase. Coliform bacteria produce the beta-galactosidase enzyme, hence when a water sample is incubated with the Colilert® reagent for 24 hours, if a coliform is present, indicator nutrient is hydrolyzed by the enzyme β-galactosidase of the organism, thereby releasing the indicator portion, ortho-nitrophenyl (ONPG). The free indicator imparts a yellow color to the solution. On the other hand, over 95% of *E. coli* produces the beta-glucuronidase enzyme, an additional constitutive enzyme that hydrolyzes the second indicator nutrient, MUG. As a result of this hydrolysis, MUG is cleaved into a nutrient portion (glucuronide), which is metabolized, and an indicator portion, methyl umbelliferone, which fluoresces under ultraviolet light. DST can easily be used in a qualitative way to measure the presence (P) or absence (A) of coliforms or *E. coli* (P/A test). A single sample of undiluted water is incubated for the appropriate time, with a positive result indicating contamination, but giving no information regarding the level of contamination. P/A tests are useful for screening, especially in settings where most samples are expected to give negative results (e.g. treated water) [38]. Defined substrate methods typically require an incubation period of 18 to 36 hours. However, recent studies have shown that shorter incubation periods (<12 hours) can give good results, and if water is highly contaminated, coliforms can be detected rapidly, without a growth phase [55].

15. HYDROGEN SULFIDE (H_2S) TEST

Some group of researchers reported a simple method for the detection of faecal contamination in drinking water. They noted that waters containing coliform bacteria also consistently contained organisms producing hydrogen sulfide (H_2S). Since H_2S reacts rapidly with iron to form a black iron sulfide precipitate, the authors developed an iron-rich growth medium. When water samples are incubated in the medium at 30-37℃ for 12 to18 hours, production of a black colour indicates contamination with H_2S producing organisms [56]. The H_2S test does not specifically test for standard indicator species such as total coliforms, faecal coliforms or *E. coli*. Rather, a large number of bacteria can lead to H_2S production (e.g. *Citrobacter, Enterobacter, Salmonella, Clostridium perfringens*). Most of these are faecal in origin. However both human and animal faeces contain H_2S-producing organisms, so the H_2S test, like the total coliform test, is not specific for human faecal contamination. The H_2S method was reviewed in a WHO report [57], which found the test to be reasonably accurate, simple and inexpensive – approximately one fifth the cost of standard coliform tests. More reviews of literatures have indicated that the test detects faecally contaminated water with about the same frequency and magnitude as the conventional methods (MTF and MF). However, the authors caution that some conditions (in particular, the presence of sulfate-reducing bacteria) may lead to false positive results. False positives are of less public health concern than false negatives, and the advantages of the method (speed, simplicity and low cost) still make the H_2S test an attractive option. The H_2S test is not mature enough to replace conventional methods, but can play a valuable role in screening water supplies. Testing water for faecal contamination with the H_2S method is certainly preferable to not testing at all [38]. A better, yet faster and more accurate

method is the molecular method such as the use of multiplex PCR technique [58] to detect toxigenic and pathogenic strains.

16. MOLECULAR METHODS FOR BACTERIOLOGICAL ANALYSIS OF WATER

Although traditional/conventional microbiological methods such as MPN, MF, DST and serological tests are clearly useful in the bacteriological analysis of water, methods based on specific nucleic acids have significant additional advantages for the detection of indicator bacterial species. Traditional methods for the detection and identification of bacteria rely on growing the organism in pure culture and identifying it by a combination of staining methods, biochemical reactions and other tests. This applies equally to detection of environmental organisms (in soil or water), bacteria in food (including milk and drinking water) or pathogens in samples from patients with an infectious disease. However these methods are slow, requiring at least 24 h or several weeks for slow-growing organisms such as *Mycobacterium tuberculosis*. In addition, there are some bacteria such as *Mycobacterium leprae* (the causative agent of leprosy) that still cannot be grown in the laboratory. The use of molecular techniques which rely on the amplification and detection of specific nucleic acids offers a fast and reliable alternative and hence, they are the choice methods. Here we reviewed many of these techniques, the advantages of these methods, and their use in investigating waterborne *E. coli*. Some of these molecular techniques includes; multiplex PCR, reverse transcription PCR, and microarrays.

17. MULTIPLEX PCR (mPCR)

Multiplex PCR is a recent molecular method used in the detection of bacteria in samples. Among DNA based methods, monoplex PCR is imperfect because it does not give concurrent detection of the toxigenic-pathogenic potential and regulating factors [59]. A key advantage of mPCR involves increased specificity because more than one gene is targeted [58,104]. Further, when the method is combined with real-time PCR, target quantification is possible. A particularly useful mPCR method to differentiate between the *E. coli* categories was recently reported. The researchers used seven primer pairs to differentiate EPEC (typical and atypical), EAEC, ETEC, EIEC, and STEC [60]. The method involved targeting *eae* (structural gene for intimin of EPEC and EHEC) and *bfpA* (structural gene for the bilus-forming pili of typical EPEC) for EPEC, *aggR* (transcriptional activator for the AAFs of EAEC) for EAEC, *elt* and *est* for ETEC (heat-labile and heat-stable enterotoxins of ETEC), *ipaH* (invasion plasmid antigen H found in EIEC and *Shigella*) for EIEC, and *stx* (Shiga toxins 1 and 2 and variants) for STEC. The assay was tested for specificity with reference strains and clinical isolates and was used to detect *E. coli* in stool samples of children with and without diarrhea. They found that atypical EPEC was the most commonly isolated category of diarrheagenic *E. coli*. A multiplex technique that targeted the *Vibrio cholera* virulent genes *hlyA, ctxB, tcp1* have been proposed to be a reliable method to detect toxigenic-pathogenic strains of *V. cholera* [61]. Various mPCR methods have also been developed to identify specific groups of *E. coli* [86]. A particularly large number of techniques have been reported for EHEC and STEC *E. coli*, typically targeting two or more of the following: genes encoding for Shiga toxins 1 and 2 (*stx1* and *stx2*), intimin (*eaeA*), enterohemolysin (*ehxA*), a mega plasmid-encoded adhesion, Saa (*saa*), a subunit gene (*subA*) and/or a novel toxin [63,64,65,66,67, 68,69,70]. Other mPCR assays have enabled identification of the *E. coli* serotypes O157, O26, O111, O103, O121, O145 [63,69,71,72] and *E. coli* O157:H7 [73,74,75,76,77,78], enteroaggregative *E. coli* [79,80,81,82], *E. coli* safety and laboratory strain lineages (K-12, B, C, and W) [83], uropathogenic *E. coli* [84], and enterotoxigenic *E. coli* [85].

18. REVERSE TRANSCRIPTION PCR

Although DNA based PCR methods are reliable, the inability of DNA-based molecular methods to distinguish between live and dead cells is a significant limitation toward monitoring possible pathogens in water samples [86]. Another major limitation of the standard PCR methods is it inability to detect the presence of certain viruses involved in waterborne disease outbreaks. To address these limitations, various research have investigated the potentials of mRNA as possible target for detecting viable bacteria cells [87,88]. Early studies targeted *E. coli* mRNA for two genes (*groEL* and *rpoH*) involved in stress response, as well as a gene (*tufA*) for an abundant cellular housekeeping protein, and concluded that mRNA was indeed an effective indicator of viability [89]. In order to obtain a sensitive method for detecting a specific mRNA, a DNA copy is made initially using an

RNA-directed DNA polymerase reverse transcriptase. Following the initial reverse transcription, a standard PCR can be used to amplify the DNA strand produced. This method, reverse transcript PCR or RT-PCR, provides a very sensitive method of detecting the presence of a specific transcript within small sections of a bacterial population. A serious limitation of RT-PCR as described above is that it is not easily quantifiable. It can be used to detect a transcript but not (or at least not easily) to determine how much of that transcript is present [90].The specificity of mRNA detection methods has been further advanced by combining reverse transcription with other molecular techniques such as multiplex PCR (RT mPCR). For example, viable *E. coli* O157:H7 was detected using RT mPCR targeting the lipopolysaccharide gene (*rfbE*) and the H7 flagellin gene (*fliC*) [91]. Reverse transcription has also been combined with real-time multiplex PCR technologies (RT mRT PCR). For example, techniques have been developed to detect mRNA encoded by *rfbE* and *eae* genes of *E. coli* O157:H7 in pure cultures and bovine feces [92].

19. MICROARRAYS

Microarrays probably represent the future for detecting waterborne pathogens because of the tremendous investigative power provided by the parallel detection of many genes [86]. For instance, one microarray was able to detect 189 *E. coli* virulence and virulence-related genes as well as 30 antimicrobial resistance genes [93]. A large number of other studies have developed microarray technology for *E. coli*, targeting virulence genes [94,93,95,96,97], O-antigen gene clusters [98], or antibiotic resistance genes [99,93,96,97]. Microarray technology has also been developed to detect other bacterial pathogens, targeting virulence genes [100,101], 23S rRNA gene [101], 16S rRNA gene [102], *cpn60* gene (GroEL, a chaperonin protein), *wecE*gene (enterobacterial common antigen biosynthesis) [102], and *gyrB* gene (subunit B of bacterial gyrase) [103].

20. CONCLUSION

The importance of water to man cannot be over emphasized, however its role as vehicle in the transmission of pathogenic organisms has become a source of concern and fear to man as he cautiously scrutinizes every drop of water before consumption. Unfortunately, the cost for safe water is that which only a very few can afford while majority still rely on the available water sources irrespective of their physical, chemical and biological state. Occurrence of pathogenic microorganisms in fresh water bodies demands routine assessment as a means of forestalling future outbreaks. However, the detection of water borne diseases and their vectors has become an evolving art which requires some sought of professionalism. Conventional methods such as Membrane filtration and the MPN requires prolonged timing and are in most cases not exact in their conclusions. Molecular techniques on the other hand though efficient and reliable requires sophisticated instrumentation and expertise which are missing in most developing countries where waterborne diseases still constitute a problem. Hence the scourge of waterborne disease remains unabated. There is therefore a need to build on available methods especially fast and reliable methods which will sufficiently address the crisis within the developing countries, not necessarily discarding the existing methods but strengthening them to be able to tackle the need of the time. It is also pertinent that enlightenment campaigns aimed at educating the people of the need for proper handling of water be embarked upon as a means to addressing the existing water crisis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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