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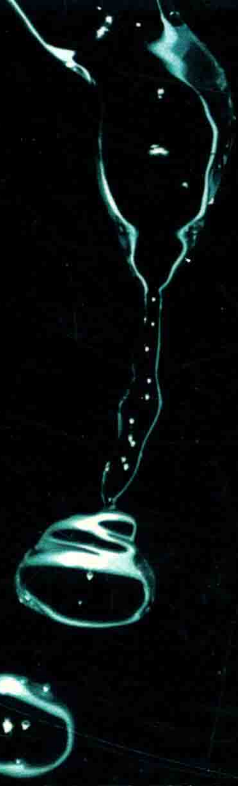
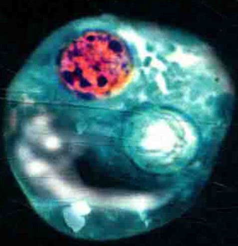


WATER MICROBIOLOGY



Types, Analyses and Disease-Causing Microorganisms

Microbiology Research Advances Series

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MICROBIOLOGY RESEARCH ADVANCES SERIES

WATER MICROBIOLOGY: TYPES, ANALYSES AND DISEASE-CAUSING MICROORGANISMS

ANDRIY LUTSENKO
AND
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Andriy Lutsenko and Vasyl Palahniuk (Editors)
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PREFACE

Water microbiology is concerned with the microorganisms that live in the water, or those that can be transported from one habitat to another by water. The improvement of pathogen detection methodology is an important issue for the efficient prevention of waterborne outbreaks in the populations worldwide. This book describes the direct detection of pathogens by molecular biologic techniques., i.e techniques based on the analysis of the nucleic acid content (DNA and RNA) of pathogens. The biosensor technology is also reviewed, focusing on their potential application for pathogen detection in water environments. Furthermore, this book presents a review on the aerobic and anaerobic processes (nutrients removal) used in the microbiological treatment of domestic wastewater and some new improvements on the bioremediation techniques. Also discussed is the biodiversity of microorganisms involved in these processes and the molecular tools that are applied to these studies. Other chapters examine both the magnitude and variety of microbes and microbial products administered in dental water, a discussion of the probable impacts of global climate change on waterborne parasitic infections, and the effects of the growth of aquaculture production systems on the quality of water and food produced.

Chapter 1 - Water is necessary to continue life so a satisfactory quality must be maintained when supplied as drinking water to consumers. Natural water contaminated with faecal matter, domestic and industrial sewage and agricultural waste may result in health risks of disease transmission to humans who use those waters. Many pathogenic microorganisms of human transmitted by water include bacteria and viruses. Microbial pollution of the aquatic environment induces an increased public health risk where it is used as a source of potable water, for fish and shellfish farming, and for recreational activities. Enteric microorganisms from grazing animals can enter a stream in overspill from the grazing lands, and animals with contact to a stream have been shown to drop a part of their daily fecal material directly therein. The risk of infection to humans due to animal fecal pollution is apparent generally to be lower than the risk due to human fecal water pollution. The polymerase chain reaction (PCR) and other biology-based methods, over the past 15 years, have begun to modernize the determination of pathogenic bacteria and viruses in environmental waters. Traditionally, drinking water treatment plants monitor fecal coliforms and other indicator organisms to provide a probable measure of potential fecal contamination and assess efficiency or removal and or inactivation of pathogenic microorganisms. Standard cell culture assay for specific microbial pathogens are not usually performed because these method are expensive, effort intensive, and time-consuming. Molecular biological techniques enable rapid detection of pathogenic microorganisms in water by providing levels of sensitivity and specificity difficult to accomplish with traditional culture-based assays which take days to achieve. Our understanding of the composition, phylogeny, physiology, and functional of microbial communities in the environment have been updated by molecular techniques. Published applications of molecular methods to drinking water issues include direct detection of pathogens in water, faecal source tracking of either indicator microorganisms or specific pathogens. This Chapter describes the direct detection of pathogens by molecular biologic techniques, i.e. techniques based on the analysis of the nucleic acid content (DNA and RNA) of pathogens. Also, the possibility of genotyping to identify pathogen sources arises, e.g. human versus farm animal.

Chapter 2 - *Legionella pneumophila* was recognized as an important human pathogen after the first discovery during an investigation of a pneumonia outbreak among American Legion convention in 1976 in Philadelphia, USA. *L. pneumophila* is a gram-negative, mesophilic, facultative intracellular parasitic and nonspore-forming rod-shaped bacterium belonging to the gamma-subgroup of proteobacteria. *L. pneumophila* inhabits natural freshwater environments at low concentration. Along with the transfer from natural aquatic habitats into man-made water systems such as cooling towers, evaporative condensers, water distribution systems, whirlpool spas and hot water tanks, *L. pneumophila* reaches high cell density and can cause Legionnaires' disease (pneumonic legionellosis) or Pontiac fever (severe influenza-like illness). Infection occurs primarily via the inhalation of *L. pneumophila*-contaminated aerosols. In aquatic habitats, *L. pneumophila* cells are intracellular parasites of freshwater protozoa and use a similar mechanism to multiply within mammalian cells. *L. pneumophila* can also multiply extracellularly within biofilms and can persist within these microbial communities for years. Transmission to human primarily occurs via the inhalation of *L. pneumophila* containing aerosols. The bacterium enters to human phagocytic cells by coiling or conventional phagocytosis then inhibits phagosome-lysosome fusion and multiplies in the phagosome. A number of virulence factors have been described for *L. pneumophila* such as surface proteins, secreted factors and putative virulence factors. *L. pneumophila* can be identified by using cultural, serologic and various molecular techniques such as DNA sequencing and DNA-DNA hybridization. Diagnosis can be made by culture, direct fluorescent antibody staining, serological tests, urinary antigen detection or nucleic acid detection and various subtyping techniques. In order to eradicate *L. pneumophila* from contaminated water systems several methods are available; Thermal or chemical shock disinfection, UV irradiation, ozone treatment, silver-copper ionization, anodic oxidation and chlorine dioxide application.

Chapter 3 - The improvement of pathogen detection methodology is an important issue for the efficient prevention of waterborne outbreaks in the populations worldwide. The development of the sensor biotechnology in the last decades has improved and simplified the detection of multiple analytes in complex matrices by the development of different electrochemical, piezoelectric, and optical biosensors that have been applied to the detection of bacteria. These biosensors can be designed for the detection of the whole bacteria or a part of it (nucleic acids, enzyme activity, or a part of the cell wall, mainly). Depending on the biorecognition element that is linked to the transducer element, biosensors are classified as immunologic, whole-cell, nucleic acid or enzyme-based biosensors. The advantages of these new technologic methods for pathogen detection over the traditional microbiology-based detection strategies rely on their rapid response, cost-effectiveness, easiness of manipulation and the possibility of performing on-site and real-time analysis of the samples. In this chapter, the biosensor technology is reviewed focusing on their potential application for pathogen detection in water environments.

Chapter 4 - Bioremediation is a technology that includes all those processes and actions that take place in the biotransformation of an environment, altered by contaminants, to its original status. Although the bioremediation concept is not new, the recent developments in microbiology and genetic engineering have made possible an unprecedented improvement on its methods, applicability and knowledge of the associated biologic mechanisms. In general these methods vary considerably, but they are based on the same principle: the use of microorganisms (indigenous or not) or their enzymes to accelerate the natural recovering of an impacted environment. The processes may also be stimulated by the optimization of physic-chemical conditions, such as addition of nutrients or oxygenation. Bioremediation methods transform the contaminants into substances that can be absorbed and used by the microorganisms with no toxic effect on them. They may be applied to recover any environmental system: air, soil or water. Concerning to water pollution, pesticides and fertilizers are the major sources of contamination, followed by industrial and domestic discharges, wastewater sludge disposal, and accidental releases. On this chapter we will present a review on the aerobic and anaerobic processes (nutrients removal) used in the microbiological treatment of domestic wastewater and some new improvements on the bioremediation techniques. Also, we discuss the biodiversity of microorganisms involved on these processes and the molecular tools that are applied to these studies.

Chapter 5 - The water emanating from dental waterlines is flush with microbial life. A veritable zoo of bacterial organisms, ranging from the benign to those known to be aggressively pathogenic, have been isolated from dental water. Microbial products such as endotoxin are there, as are eukaryotic microorganisms. This state of affairs has been recognized for decades, and around the globe. The medical consequences of this contamination are not entirely clear – there is some evidence suggestive of clinical significance. Efforts to control the microbial content of dental water have been minimally effective. The proximal source of these organisms is the flourishing biofilms lining the operatory waterlines. This chapter reviews what has been reported in the literature regarding both the magnitude and variety of microbes and microbial products administered in dental water, and provides an overview of the biology of biofilm as they relate to dental waterlines. The potential for medical significance is discussed, and evidence for the reality of these clinical consequences is presented. Past efforts at dental water microbial control are reviewed, and future directions for research in dental water microbiology are proposed.

Chapter 6 - The word "water" and any associated images often elicit a visceral response. To some, it is the essence of life, an enigmatic substance that takes the shape of all forms of matter: gas, liquid or solid. Yet to others, it is a harbinger of death through disease. About the only thing we can be certain of is that our study of water is far from ended. It should be no surprise that tenets of medicine related to water are subject to scrutiny. One example is the axiom that rigorous hand washing is a sound hygienic practice with the intent and consequence of removing pathogens from the skin to prevent disease transmission through touch contamination. This is predicated on the assumption that the water we wash with is free of clinically relevant pathogenic organisms.

Disinfection at municipal treatment facilities leaves water free of unacceptable levels of microbes when it leaves the plant. However, in the course of its passage through the distribution network, myriad opportunities exist for microbes to gain access to areas that can protect them from the microbicidal effect of residual levels of disinfectants. Protection is afforded to microbes by physical means as a consequence of slow water flow in dead-ended pipes, by engulfment within amoebae wherein they live and benefit from the host's natural resistance to microbicides, by formation of biofilm, or by combinations of these factors.

This chapter attempts to provide evidence supporting the view that potable water used for hand washing in hospitals, at work and at home can be contaminated with microorganisms of clinical relevance, with adverse consequences in immunocompromised individuals. Given that the United States' largest healthcare payor, the Centers for Medicare and Medicaid Services (CMS), is no longer reimbursing healthcare providers for costs associated with healthcare-associated infections that may be deemed preventable, this is an opportune time to reevaluate the potential contribution of water to such infections. Facing the consequences of this, healthcare providers should be ready to think "outside of the box" regarding what is often considered good medical practice in the absence of a full understanding of the confounding variables that can render an apparently sensible practice not only ineffective, but also potentially dangerous. Washing with contaminated water makes no sense, and if we are to deem the practice meritorious, we should be certain that the water used is as free of pathogenic microbes as we expect it to be.

Chapter 7 - It is broadly accepted that climate change is going to impact on the epidemiology and distribution of various infectious diseases, with perhaps those transmitted by the waterborne route amongst the most likely to be affected. Evidence has been accumulating for several years that weather is often a factor in triggering outbreaks of waterborne parasitic infections in which a substantial proportion of a community is affected, with vulnerable groups particularly at risk. Thus, there is a complex web of interrelationships between weather parameters, water quality and quantity, pathogen occurrence and survival, and disease incidence. Climate changes will have an important impact on these relationships, of which we are only now becoming aware. However, the need to prepare for these expected scenarios is self-evident.

Climate change is likely to have different effects and patterns in different world regions. Northern regions can generally expect to experience longer, hotter summers and milder winters and an increase in 'extreme weather events' characterised by excess precipitation and high wind. Those regions that were previously ice-covered may become flood areas due to increased melt.

A study of waterborne disease outbreaks in UK, including those associated with the parasites *Giardia and Cryptosporidium,* has demonstrated not only a significant association between excess cumulative rainfall in the previous 7 days and outbreaks, but also an association between outbreaks and an excess of rainfall *below* 20 mm for the three weeks previous to the outbreak weeks, compared with control weeks. Additionally, cumulative rainfall exceedances were associated with outbreak years. Thus, there is evidence that both low rainfall and heavy rain precede many drinking water outbreaks, and when assessing the health impacts of climate change both these factors should be considered.

The most northern global regions may perhaps be particularly at risk, not only due to flooding from snow/ice melt, but as the population and range of animal hosts, previously limited by Arctic conditions, expands, then previously pristine areas will become suitable environments for animals and humans, with the increased possibility of transmission of zoonotic infections.

This chapter summarises our current knowledge on the probable impacts of global climate change on waterborne parasitic infections in northern regions, and, where possible, uses recently acquired data to discuss these impacts. Additionally, how our current knowledge can be used for informed decision-making and how particular strategies might be able to mitigate some of the potential impacts of climate change on the transmission of waterborne parasites are further discussed.

Chapter 8 - The growth of aquaculture production systems, mostly the sports-fishing kind, coupled to lack of control, brings about concerns on the quality of water and food produced. Current paper determines which factors may trigger the growth of cyanobacteria with subsequent concentrations of microcystins in collected water samples, at the surface and in the water column, from ten aquaculture systems, during the dry and rainy seasons. The above is undertaken by measurements of biotic (counting of Chlorophyceae, cyanobacteria and MC-LR) and abiotic (total nitrogen and total phosphorus) factors. Since water of ten aquaculture production systems had MC-LR concentrations highly correlated with *M. aeruginosa* biomass, most MC-LR microcystins were produced by this species. MC-LR concentrations and *M. aeruginosa* counting were positively correlated with N:P rates and suggest that parameters may affect not only the *M. aeruginosa* biomass but also MC-LR concentrations.

Chapter 9 - Contaminated water is primary, if not a sole, source of inoculum for *Phytophthora* diseases of numerous nursery, fruit and vegetable crops but also an efficient means of spreading *Phytophthora* from one geographical location even to other countries and continents. Such water used for plant irrigation can results in serious disease epidemics and crop losses even to 100%. For preventive of *Phytophthora* spread and managing of diseases caused by this group of pathogens rapid, simple and universal methods were elaborated. Among at least 20 plant species rhododendron leaf baits are the most successful for recovery of *Phytophthora* from water and may be especially used for qualitative detection of that group of pathogens whereas filtration technique is also available for analysis of population density. Using of baits, filtration and molecular methods at least 17 *Phytophthora* species were recovered from water reservoirs, drainage and irrigation canals and rivers. It was found that *Phytophthora* species may be recovered from water irrespective to season but frequency of isolation was correlated with temperature, source of water and its physical and chemical factors. Filtration, ultraviolet irradiation, chlorination and application of nonionic surfactants and calcium may be used for water disinfection.

Chapter 10 - Natural water always contains contaminants, particularly inorganic contaminants, that arise from the geological strata through which the water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals. The presence of these contaminants in water intended for human consumption, irrigation and recreational activity, is a significant risk for the human health.

Recent epidemiological data demonstrate an increase of the diseases caused by waterborne pathogens, especially of the gastroenteritis infections, in the industrialized countries. Numerous studies have evidenced the contamination of deep and surface water; bacteria within contaminated water represent a highly diversified group, that are usually encountered in wastewater and sewage. The aim of this paper was to estimate the presence of waterborne pathogens in groundwater intended for the irrigation. Well water samples were collected at thirty different agricultural areas located in Calabria and analyzed for the presence of waterborne pathogens.

The results obtained in this work evidence a diffuse pollution of the water, in all monitorated zones.

Twenty-five out of thirty examined pools were positive for total coliforms (TC) and the value of total microbial contamination were higher than 100 CFU/ml; six of these Twenty-five wells were contaminated from fecal coliforms and *Escherichia coli*; twenty-eight were positive for *Salmonella spp.* and *Shigella spp.* (minimal value $2x10^1$ CFU/l; maximum value $1x10^4$ CFU/l) and fifteen for *Enterococcus spp.*

Chapter 11 - Cyanobacteria, although non-pathogenic, are known to produce a wide range of potent low molecular weight toxic compounds which present risks to human health. Although knowledge of the modes of action and toxicity of cyanotoxins is increasing, other factors and areas of research of health-relevance still need to be addressed. These include the proper epidemiological assessment of human and animal exposure to cyanobacteria and their toxins through drinking water, co-exposure to multiple toxicants, including multiple cyanobacterial toxins, and interactions with water-borne pathogenic bacteria, viruses and cyanobacteria. These interactions include the ability of cyanobacteria to provide resources and refuges for disease-causing bacteria which may have important ramifications upon the accessibility of safe drinking water and human disease. This contribution highlights and discusses further needs concerning the influence of cyanobacteria on water microbiology.

Chapter 12 - The aim of this work was to investigate the inter-relationships between *Campylobacter*, waterfowl and environment at three amenity ponds. Campylobacters were isolated from duck faeces, run-off water and pond water and sediment and were subjected to molecular typing. At one pond a single strain of *C. jejuni* was found in duck faeces and pond water and sediment; at the other two ponds a single strain of *C. coli* was found in run-off and pond water but not in faeces. The proportion of birds that were carriers, the strains of *Campylobacter*, and the importance of run-off as a source of campylobacters differed between ponds. Generalizations about the ecology of *Campylobacter* in ponds - potentially related to public health – might be inappropriate.

Chapter 13 - For healthy existence of mankind, safe drinking water is an essential commodity. But now-a-days polluted water cause many health concerns worldwide. Among these acute gastrointestinal and diarrheal illnesses continue to be the major water borne diseases, which may be caused by bacteria, viruses and parasites. Unsafe and contaminated water in the distribution system is responsible for occurrence of many reported diarrhoeal disease outbreaks. Other disease may also be caused by an aquatic host like cyclops and snails.

International standards for drinking water relate various water quality variables, which are not mandatory limits and may be modified according to the needs of any country. Among all variables, microbiological pollutants draw special attention due to their widespread nature, immediate and acute effects. There are a number of acceptable indicators like total and fecal coliform for microbiological monitoring of drinking water, which is in the distribution system after treatment, but they may not be acceptable for determining sanitary quality of rural water supplies, especially in tropical areas like India, where many bacteria of no significance occur almost in all untreated supplies. Under this circumstance midterm targets are set by national surveillance agency to determine the levels of water pollution which permits progressive economic, environmental and social developments without presenting health hazards.

Special attention should be paid to enteric viruses contaminating drinking water. Essentially drinking water must be free from human enteric viruses. Bacteriophages, are used as indicators of water quality for enteric viruses, primarily as an index of sewage contamination. This is an additional indicator of treatment efficiency or ground water protection.

Provision of safe drinking water is the most important step for improvement of the health in a community by preventing the spread of water borne diseases. In rural areas this could be done by monitoring of drinking water quality by detecting the presence of indicator organisms using simple kit test. Water surveillance is another important task of local and national health authority. Good communications between two bodies responsible for monitoring and surveillance are essential. Effective mutual exchange of activity and opinion can keep safety and acceptability of drinking water supply upto the expected level.

CONTENTS

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Chapter 1

MOLECULAR APPROACH FOR DETECTION OF WATERBORNE PATHOGENS

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ABSTRACT

Water is necessary to continue life so a satisfactory quality must be maintained when supplied as drinking water to consumers. Natural water contaminated with faecal matter, domestic and industrial sewage and agricultural waste may result in health risks of disease transmission to humans who use those waters. Many pathogenic microorganisms of human transmitted by water include bacteria and viruses. Microbial pollution of the aquatic environment induces an increased public health risk where it is used as a source of potable water, for fish and shellfish farming, and for recreational activities. Enteric microorganisms from grazing animals can enter a stream in overspill from the grazing lands, and animals with contact to a stream have been shown to drop a part of their daily fecal material directly therein. The risk of infection to humans due to animal fecal pollution is apparent generally to be lower than the risk due to human fecal water pollution. The polymerase chain reaction (PCR) and other biology-based methods, over the past 15 years, have begun to modernize the determination of pathogenic bacteria and viruses in environmental waters. Traditionally, drinking water treatment plants monitor fecal coliforms and other indicator organisms to provide a probable measure of potential fecal contamination and assess efficiency or removal and or inactivation of pathogenic microorganisms. Standard cell culture assay for specific microbial pathogens are not usually performed because these method are expensive, effort intensive, and time-consuming. Molecular biological techniques enable rapid detection of pathogenic microorganisms in water by providing levels of sensitivity and specificity difficult to accomplish with traditional culture-based assays which take days to achieve. Our

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understanding of the composition, phylogeny, physiology, and functional of microbial communities in the environment have been updated by molecular techniques. Published applications of molecular methods to drinking water issues include direct detection of pathogens in water, faecal source tracking of either indicator microorganisms or specific pathogens. This Chapter describes the direct detection of pathogens by molecular biologic techniques, i.e. techniques based on the analysis of the nucleic acid content (DNA and RNA) of pathogens. Also, the possibility of genotyping to identify pathogen sources arises, e.g. human versus farm animal.

Keywords: Waterborne pathogens, molecular detection, PCR, real-time PCR, multiplex-PCR, RT-PCR, T-RFLP, LH-PCR, DNA Fingerprinting, FISH, DNA microarray.

1. INTRODUCTION

Around the world, the spread of infectious disease in human populations, animal and plant is a serious problem. Water is a common source in the environment of many pathogens affecting these populations. Waterborne pathogens can create a vital problem to drinking water supplies, recreational waters, and water sources for agriculture, and aquaculture, as well as to aquatic ecosystems and biodiversity. Major sources of pathogenic organisms are municipal wastewater effluents, urban runoff, agricultural wastes and wildlife, sewage, sewage derived from hospitals, and insufficiently treated sewage specially from large urban areas representing the greatest threat. Moreover, pathogenic contamination of shellfish beds or irrigation water can cause risks to human food supplies. The World Heath Organization (WHO, 1996) has declared that infectious diseases are the world's sole largest source of human death. Many of these infectious diseases are waterborne and have massive bad impacts in developing countries. Although developed countries have been more successful in controlling waterborne pathogens, water quality problems are still widespread in some countries. Based on U.S.A. estimates, consequently of acute waterborne infections it is possible that about 90,000 cases of illness and 90 deaths happen annually in Canada (ASM, 1999). Thus, there is a necessary to study the consequences of environmental releases of microbial pathogens because there is insufficient knowledge of the sources, occurrence, concentrations, survival and transport of specific microorganisms in the environment. Funding and efforts are required to validate newer molecular biological detection tools, understand the ecology of pathogens in aquatic environments, better predict disease outbreaks, and improve emergency responses. Human bacterial pathogens are considered as an increasing threat to drinking water supplies worldwide because of decreasing quality and quantity of available raw water and the increasing demand of high-quality drinking water. Current advances in molecular biological detection technologies for bacterial pathogens in drinking water carry the promise in improving the safety of drinking water supplies by precise detection and identification of the pathogens. More importantly, the array of molecular approaches allows understanding details of infection routes of waterborne diseases, the effects of changes in drinking water treatment, and management of freshwater resources. Public drinking water supplies were one of the great technological advancements in the developed countries during the 20th century with a significant impact on public health. Today, access to safe drinking water is considered a human right and is currently available for about 83% of the human population with a lack of safe supplies mainly in rural areas of developing countries (WHO, 2004; Abo-Amer *et al*., 2008). Whereas lack of safe drinking water supply in developing countries is typically because of economic limit, in developed countries health risks by waterborne pathogens may occur through mismanagement of freshwater resources, technological failure and/or improper detection measures (Organization for Economic Cooperation and Development, OECD, 2003). The study of pathogens in drinking water supplies can be seen under different stages such as monitoring of the hygienic quality of the drinking water, assessment of transmission pattern of outbreaks of waterborne infections, identification of sources of microbial contaminations, and understanding the emergence of new microbial pathogens. There is a rather limited knowledge on the microbiological principles controlling the occurrence and pathogenesis of microbial pathogens in drinking water. The most important reason for this lack of knowledge are attributed to that precise detection, identification, and quantification of microorganisms in water are not available and only possible with a combination of classical and molecular methods (OECD, 2003). Currently, bacteria have been identified as the etiological agent in the majority of the waterborne outbreaks in the USA according to the most recent examination data (Liang *et al*., 2006). Also, in other parts of the developed world the contribution of pathogenic bacteria to waterborne outbreaks is increasing because of changes in life style and the emergence of several new bacterial pathogens (WHO, 2003). Consequently, this chapter concentrated on the analysis of bacterial pathogens in drinking water supplies and referred for the other types of waterborne pathogens to several reviews (Szewzyk *et al*., 2000; Quintero-Betancourt *et al*., 2002; Nwachcuku and Gerba, 2004; Fong & Lipp, 2005; Monis *et al*., 2005; Schets *et al*., 2008; Giangaspero *et al*., 2009; Hsu *et al*., 2009). The greatest challenge is the renewed recognition that resurgent and emerging pathogens with a high resistance to treatment are a significant hazard (OECD, 2003). Additional challenges are more intense land use, climate change, and bioterrorism (Jury and Vaux, 2005; Richardson *et al*., 2009). Climate change will increase temperature of all open water bulks, which is expected to enhance growth conditions and the survival for some pathogenic bacteria. Additional effects of global warming are increased frequency of heavy rainfalls, storms, and floods, leading to severe problems with runoff, sewage transport, and treatment and cause increased faecal contamination of freshwater resources. Consequently, this chapter focused on demonstration of molecular biological assessments of bacterial pathogens that can meet some of these challenges for safer drinking water supplies.

2. Waterborne Pathogen Indicators

Indicator microorganisms have traditionally been used to indicate the presence of pathogens (Berg, 1978). Today, however, we realize many of possible reasons for pathogen presence and indicator absence, or vice versa. Therefore, there is no direct correlation between numbers of any enteric pathogens and indicator (Grabow, 1996). The validity of any indicator system is also affected by the relative rates of removal and destruction of the indicator versus the target risk. So differences due to environmental resistance or even ability to multiply in the environment, all influence their utility. Hence, viral, bacterial, parasitic protozoan and helminth pathogens are unlikely to all behave in the same way as a single