Detection of water-borne pathogens: culture plate to genomics

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Abstract: This paper describes the use of different analytical techniques for detection of water borne pathogens. It discusses the brief information about the development of new diagnostic and monitoring techniques available and explains its important role in risk management of water related diseases. New techniques will aid development of strong early warning systems, reliable field diagnostics, treatments and more effective remediation of impacts from harmful microorganisms. The paper proposes that the future developments in signal detection and miniaturization technologies will provide real-time monitoring and diagnostics for rapid assessment of microbial pathogens.

Keywords: Water-borne pathogens, risk management, microbial indicators, real-time monitoring, health.

Introduction

Water pollution seems to be inevitable consequence of urbanization and industrialization in developing and developed countries. Water resources are overburdened due to contamination from a) sewage and pathogenic agents, b) industrial and trade wastes, c) agricultural pollutants, like fertilizers and pesticides, and d) thermal pollution. Water borne diseases have a major public health and socio-economic impact. WHO/UNICEF Report estimates that over 2 million people die annually due to water related diseases (WHO, 2000).

Water may harbor pathogenic bacteria, viruses, protozoa and parasites responsible for the emerging most widespread infections which are leading cause of death worldwide. These pathogens are diverse in nature placing entire communities at risk (Sarkar et al., 1999). An outbreak of cholera caused by *Vibrio cholerae* was recorded in western Kenya between June1997-March 1998 (Shapiro et al., 1999). In June 1998 hundreds of children playing in water theme park in suburban Atlanta were exposed to *Escherichia coli* due to faecal contamination and insufficient chlorine levels (Rose & Grimes, 2001). Lemus and Weisburg (2000) reported beach closures in 1999, in Huntington Beach, California due to high levels of enterococci and coliform bacteria. Infantile diarrhea due to entropathogenic *Escherichia coli* has been reported in Delhi and Calcutta in 1997-1999 (Prasannan et al., 2001). A study carried out in various countries in Asia identified enteroaggregative and enterotoxigenic *Escherichia coli* as the pathogens responsible for acute/persistent diarrhea in India (Black, 1993). Also concurrent outbreaks of cholera have been reported in Gujarat, Orissa and Kerala in the year 2000 with new locations such as the Kottayam district in Kerala (Samal et al., 2001; Chakraborty et al., 2001). In Japan 1993, enteroaggregative *Escherichia coli* caused massive outbreak of gastrointestinal illness in almost 2,700 school children (Itoh et al., 1997), while enterotoaggregative *Escherichia coli* caused outbreaks of gastroenteritis in U.K. (Smith et al., 1997). In 1995-1996, the largest outbreak of infection caused by SF STEC 0157:H7 resulting in Hemolytic Uremic Syndrome and diarrhea was reported in Bavaria, Germany (Ammon et al., 1999). In 1993, municipal water supply contaminated with *Cryptosporidium* spp. caused largest recognized outbreak in Milwaukee of United States (McDonald et al., 2001). Two *Giardia* outbreaks has been reported in Florida in 1998 caused from untreated ground water (MMWR, 2000). The outbreak of gastroenteritis in May 2000, was predominantly caused by *Escherichia coli* 0157:H7 and *Campylobacter* in Walkerton, Ontario of Canada (Canada Communicable Disease Report (2000).

Regulation and risk assessment

Health based targets provide the basis for the application of the National and International Standards for drinking water quality for community and household supplies. Water quality guidelines and standards, recommended by various authorities, might be similar to the European standards (Table 1), but differ due to economic and technical capabilities and perceptions of acceptable risks of infections in rural and urban environment (Fig.1). Guidelines are intended to support the development and implementation of risk management strategies to ensure the safety of drinking water supplies. To date there are international agreements to legal limits for pathogens as reflected by EU directives and WHO's international quality safety standards (Table 1).

A safe supply of drinking water depends upon use of either protected water sources, or properly selected and operated series of interventions, use of appropriate treatment technologies capable of reducing pathogens and other contaminants and finally prevention of re-contamination in distribution (Fig.1). Characterization of water quality of a resource is carried out, to reduce the health hazards due to water-borne diseases, to acceptable levels. Disinfection is of unquestionable importance in the supply of safe drinking water and is an effective barrier to many pathogens in drinking water. It involves the use of reactive chemical agents such as chlorine which can be easily monitored and controlled as a drinking water disinfectant. Protection of the source and treatment techniques such as chlorination and efficient filtration has been recommended to ensure absence of...
viruses, protozoas and helminths due to lack of routine monitoring techniques (WHO, 2003). Chlorine disinfection has limitations against protozoans, some viruses or pathogens within flocks or particles. It has become necessary to treat sewage water to meet objectives for drinking water in densely populated areas. Soon densely populated cities may have to treat urban storm water and wet water overflows to conserve and overcome water scarcity. Therefore, it is essential that an overall management strategy is implemented, where multiple barriers as well as, protection in distribution are used in conjunction with disinfection, to prevent or remove microbial contamination.

OECD’s expert working group examined approaches for establishing links between drinking water and infectious diseases, and new approaches to enhance current methods for surveillance and outbreak investigation, in particular the development of real-time measurements and predictive models. The World Health Organization, the Pan American Health Organization and UNESCO held an expert consultation in Mexico City in 1997 on Entamoeba histolytica and Entamoeba dispar for developing techniques for differentiating between the two protozoa during diagnosis, identifying virulence factors and developing immunological studies to determine the feasibility of a vaccine (CRC for Water Quality and Treatment, 2000).

The problem of detection and monitoring of microbial pathogens in drinking water is being viewed in a global perspective (Fig. 2). A recent United Nation’s report portrays a grim picture of water quality conditions in some developing countries (WHO, 1997). Yet common surveillance tools for water borne pathogens are needed to reduce the risk by standardizing methodologies and validation on international level. Risk assessment to address the risk of exposure and the potential health impacts has been carried out (WHO, 1997). However, all of the microorganisms currently on EPAs “Contaminant List” lack database resulting in the failure on risk assessment on exposure. Thus, accurate and preemptive monitoring information will provide advances in the microbial risk assessment (Fig.3).

Regulatory framework must be encouraged for the use of new cost-effective technologies with comprehensive efficacy which will assist water utilities for handling data and interpreting sensitive data, thereby protecting the environment as well as human health. e.g.:

- Policy makers to be educated on research, development and availability of new technologies
- Problems requiring risk assessment
- Voluntary monitoring of activities by water utilities
- International collaboration for evaluation of new methods
- Availability of data base for generation of guidelines and recommendation

Thus, the regulatory requirements are demanding and represent a significant challenge for new technologies. New techniques will aid development of strong early warning systems, reliable field diagnostics, symptom treatments and more effective remediation of impacts from harmful microorganisms.

### Table 1. Bacterial contamination regulations and guidelines for drinking water

<table>
<thead>
<tr>
<th>Country</th>
<th>Total coliform</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>India (BIS)</td>
<td>10/100 ml (95% should not contain any coliform organisms in 100 ml)</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>USA</td>
<td>0/100 ml (95%), a consecutive sample from the same site must be coliform free if &gt;5% positive</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>Canada</td>
<td>0/100 ml (90%) none should contain more than 10 CFU/100 ml, a consecutive sample from the same site must be coliform free</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>EEC</td>
<td>0/100 ml, or MPN&lt;1</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>IS 10500</td>
<td>0/100 ml</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>WHO</td>
<td>0/100 ml (95%)</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>OECD</td>
<td>0/100 ml</td>
<td>0/100 ml (100%)</td>
</tr>
<tr>
<td>UK</td>
<td>0/100 ml (95%)</td>
<td>0/100 ml (95%)</td>
</tr>
<tr>
<td>EC</td>
<td>0/100 ml</td>
<td>0/100 ml</td>
</tr>
</tbody>
</table>

(Ref: Parsons, 2000; Balis et al., 1996; Houndt & Ochman, 2000; Bhanumathi et al., 2003; Karlowsky et al., 2003)

Fig. 1. Factors affecting water quality
'Indicator' microorganisms and 'Marker' pathogens

Sources of water pollution must be identified in order to adequate water quality problems and protect public health. Source detection provides direct evidence of the origin of pollution by identifying indicator organisms. Indicator microorganisms are used to predict the potential risk from pathogenic microorganisms and circumvent the need to assess every pathogen. Few criteria have been recognized for the selection of indicator organisms (Payment, 1998): e.g.:

- should be easy to enumerate
- should be associated and present in larger numbers than pathogen
- should survive equally or longer than pathogen in environment
- should have stable characteristics and give consistent reactions in analyses
- should have resistance to treatment equal or greater than pathogen

**Fig. 2. Limitations in detection and monitoring of water-borne pathogens**

**Fig. 3. Role of molecular methods in identification and assessment of water-borne pathogens**
removal and destruction of the indicator versus the target hazard. So differences due to environmental resistance or even ability to multiply in the environment, all influence their usefulness. Indicators have traditionally played a very important role in guidelines and national standards. Increasingly, however, they are being seen as an adjunct to management controls, such as sanitary surveys, and there is a move away from a specified indicator level end product. Hence, there is a need globally to identify ‘marker’ organisms or pathogens in geographically specific water resources. It is necessary to define reference populations of pathogens to focus on specific sensitive groups in deriving national standards. Several indicators (Fig. 4, Table 2) have been studied and recommended for water quality assessment (ISO,1990; Standard Methods,1992; Guyot et al., 2002) which include bacteria (Escherichia coli, Salmonella, Shigella, Vibrio, Pseudomonas), viruses (Enteroviruses, Rotavirus, Hepatitis A and E), protozoa (Entamoeba, Giardia, Cryptosporidium).

Genomics of water-borne pathogens

Bacterial genomes are composed of universally present conserved ‘core’ genes providing backbone of genetic information. The genome also contains a flexible gene pool consisting of an assortment of strain specific genetic information which may provide additional capabilities enabling the adaptation of the species to environmental conditions. This difference in genome size reflects size variations of the flexible gene pool due to the acquisition and loss of genomic DNA (Dobrindt & Hacker, 2001). Bacterial adaptation has been influenced by horizontal gene transfer (HGT). HGT has led to changes in microbial genomes over relatively short time periods and is responsible for ubiquitous occurrence of pathogens. The dissemination of antibiotic resistance genes among human and non-human pathogens is the paradigm for HGT on a global scale (Mazel & Davies, 1999). Studies of antibiotic resistance development are essentially retrospective but less information is available for microbial dynamics of this process. Besides, plasmid and bacteriophages, large genome regions (up to 40 Kb) known as pathogenicity islands (PAIs) are consistently present in pathogenic strains carry many virulence genes. The sequenced Helicobacter pylori strain contains a single contiguous “cag” PAIs (Censini et al., 1996). PAIs has also been reported in Salmonella typhimurium encoding typeII secretion systems during infection (Shea et al., 1996). Pathogenicity islands has been identified in Escherichia coli isolates (PAIs I, II, IV, V, hlyI, hlyII, kps, pap, sfa, prf) (Boyd & Hartl, 1998). Also STEC 0157:H7 strains has been shown to harbour a TAI which encodes for a novel adherence-confering protein and tellurite resistance (Tarr et al., 2000). The deletion of PAIs from the chromosome or the acquisition by other species or genera may lead to new pathogenic variants showing flexibility and evolution of microbial genomes.

Virulence in pathogens is often multifactorial and coordinately regulated and virulence genes tend to be clustered in the genome. Numerous phages carrying virulence determinants in Pseudomonas aeruginosa, Vibrio cholerae, Shigella dysenteriae and Escherichia coli have been identified (Hacker et al.,1997). Salama et al. (2000) and Israel et al. (2001) have reported association of specific genes with Virulence Island in Helicobacter pylori with increase in pathogenicity. A study on the association between known virulence factors of shiga toxin producing Escherichia coli revealed that stx2 positive isolate is approximately five times more likely to be associated with severe disease in humans than a stx2

Fig. 4. A schematic presentation of discrepancy of culture methods to detect evolving pathogenic ‘indicator’ biotypes

<table>
<thead>
<tr>
<th>‘INDICATOR’ Bacteria</th>
<th>Detection by culture methods</th>
<th>Suspect sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal coliforms</td>
<td>None Detected</td>
<td>E. coli 0157 present</td>
</tr>
<tr>
<td>Diarrheagenic E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteroaggregative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteropathogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterohemorrhagic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cause acute and persistent diarrhea in human population</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Water Pollution Monitoring

Cause acute and persistent diarrhea in human population
negative isolate of the same serotype. Genome of *Escherichia coli* 0157:H7 sequenced so far indicates more than 20 potential virulence genes clustered in several mobile genetic elements (Cruz & Davies, 2000). Mobile genetic elements such as plasmids and transposons can effectively circumvent the genetic barriers between bacterial species by conjugation and transductional transfer of DNA molecules. For example, several mobile genetic elements (Cruz & Davies, 2000).

Recently, the existence of a complementary but more than 20 potential virulence genes clustered in certain microorganisms present in water resources.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome* size</th>
<th>Pathogenicity</th>
<th>Antibiotic Resistance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>5.2Mb</td>
<td>Diarrhea, haemorrhagic colitis (HC), hemolytic uraemic syndrome (HUS)</td>
<td>Ampicillin, chloramphenicol, kanamycin, tetracycline, trimethoprim</td>
<td>Balis et al., 1996; Houndt &amp; Ochman, 2000</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>4.0Mb</td>
<td>Cholera</td>
<td>Ampicillin, ciprofloxacin, streptomycin, piperacillin, piperacillin-tazobactam</td>
<td>Bhanumathi et al., 2003</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>6.3Mb</td>
<td>Septicaemia, meningitis, nosocomial pneumonia &amp; UTI, wound infection</td>
<td>Ampicillin, chloramphenicol, streptomycin, tetracycline, trimethoprim, sulfamethoxazole</td>
<td>Karlowsky et al., 2003</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>4.59Mb</td>
<td>Abdominal cramps, fever, watery diarrhea</td>
<td>Ampicillin, chloramphenicol, kanamycin, tetracycline, trimethoprim, sulfamethoxazole</td>
<td>MMWR, 2000</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>4.85Mb</td>
<td>Gastroenteritis, bacteremia, septicaemia, septic fever, intestinal ulcer</td>
<td>Ampicillin, chloramphenicol, kanamycin, tetracycline, Proamine</td>
<td>Balis et al., 1996</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>3.36Mb</td>
<td>Endocarditis, biliary tract infection, septicaemia, peritonitis, intra abdominal abscess</td>
<td>Penicillin, vancomycin, ampicillin, erythromycin, tetracycline</td>
<td>Haque, et al., 2000</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>1.66Mb</td>
<td>Chronic gastritis, peptic and duodenal ulcers, gastric cancer</td>
<td>Vancomycin, trimethoprim, polymixin B, tinidazole, amoxycllin, clarithromycin</td>
<td>Grabow, 2002; Belzer et al., 2009</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>20Mb</td>
<td>Dysentery colitis, amoeboma</td>
<td>Metronidazole</td>
<td>APHA, 1992</td>
</tr>
<tr>
<td>Enteric viruses</td>
<td>7176-7478 bp</td>
<td>Gastroenteritis, respiratory disease, meningitis, paralysis, Hepatitis, conjunctivitis, Diarrhea, Gastroenteritis</td>
<td>Antiviral-interferon α, zidovudine, didanosine</td>
<td>Hijnen et al., 2000</td>
</tr>
</tbody>
</table>

*Source: TIGR microbial database (www.tigr.org)*

Comparative and functional genomics is a powerful approach towards understanding mechanisms of microbial pathogenesis and reveals new insights into bacterial evolution and the diversity of microbial pathogens. Therefore comparative genomic analysis is useful for micro organisms for which traditional genetic techniques are difficult or impractical, genomic sequence can be used to make biological predictions of these pathogens. Pathogen diversity can be used to develop new diagnostic procedures for the detection and typing of pathogens. Pathogen diversity can be used to develop models of the evolution of pathogen and pathogenicity which will help in sophistication of public health interventions.

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microorganisms. Analysis of sequenced genomes of strains of *Escherichia coli* and *Helicobacter pylori* (Groisman et al., 1993; Alm et al., 1999) indicates the differences in the nucleotide sequence in most of the same genes. Comparison of the differences in genomic sequences of pathogenic with non-pathogenic microorganisms forms the basis of different behaviour microorganisms and its isolates. Analysis of sequences which are absent in *Escherichia coli* led to the identification of pathogenicity island in *Salmonella typhimurium* (Karch & Bielaszewska, 2001). A complete sequence analysis of STEC 0157:H7 genome has been carried out by Karch and Bielaszewska to understand the full spectrum of virulence characteristics and comparison of genetic evolutionary and phylogenetic relationship between pathogens. *Escherichia coli* K-12 sequence serves as the index genome, against which sequence of pathogenic enteric bacteria can be compared to determine and predict changes in gene pool and virulence traits in future.

Computation on integral component of genomic analysis has been used to determine the functions of genes based on homology sequence searching in different organisms, using a variety of comparison

### Table 3. Methods used for the detection of waterborne pathogens

<table>
<thead>
<tr>
<th>Technique</th>
<th>Organism</th>
<th>Advantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal coliform/ faecal streptococcus ratio</td>
<td>Coliforms</td>
<td>Rapid results with minimal expertise</td>
<td>Edberg et al., 1988</td>
</tr>
<tr>
<td>Culture Methods</td>
<td><em>E. coli</em>, <em>E. faecalis</em>, Coliform</td>
<td>Determination of concentration of microorganisms</td>
<td>APHA, AWWA, AEF, 1998</td>
</tr>
<tr>
<td>MTF, MF</td>
<td>Coliforms, <em>E. coli</em></td>
<td>Inexpensive, easy to implement and perform</td>
<td>Rompere et al., 2002; Versalovic &amp; Lupskin, 2002</td>
</tr>
<tr>
<td>PA</td>
<td><em>E. coli</em>, <em>Pseudomonas sp</em></td>
<td>Inexpensive, rapid and sensitive quantitative detection</td>
<td>Mandrell &amp; Wachtel, 1999</td>
</tr>
<tr>
<td>MAR</td>
<td><em>E. coli</em>, <em>H. pylori</em>, <em>Enterococcus</em></td>
<td>Detection of genetic determinants conferring resistance for different antibiotics</td>
<td>Osaki et al., 1998.</td>
</tr>
<tr>
<td>Immuno-magnetic separation</td>
<td><em>E. coli</em>, <em>H. pylori</em></td>
<td>Increased assay speed, concentration of target micro organism, Recovery of sub lethally damaged cell</td>
<td>Maurer et al., 1999; Eaton &amp; Gasson, 2001; Fu et al., 2005.</td>
</tr>
<tr>
<td>Serotyping</td>
<td><em>E. coli</em>, <em>V. cholerae</em>, <em>Shigella</em>, <em>Salmonella</em>, <em>P. aeruginosa</em></td>
<td>Differentiation of microorganism from various sources</td>
<td>Vandamme et al., 1995.</td>
</tr>
<tr>
<td>Genotypic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td><em>E. coli</em>, <em>E. faecalis</em>, <em>Shigella</em>, <em>H. pylori</em>, <em>S. Vibrio cholerae</em>, <em>E. histolytica P. aeruginosa</em>, <em>Cryptosporidium</em></td>
<td>Detection of specific infective agents and their virulent genes Identification of functionality of genetic element, Rapid and specific</td>
<td>Standard methods, 1992; Hahma et al., 2003; Fu et al., 2005; Lien et al., 2007.</td>
</tr>
<tr>
<td>RAPD, AFLP, PFGE, RFLP, AP-PCR, DNA fingerprint analyses</td>
<td><em>E. coli</em>, <em>P. aeruginosa</em>, <em>Enterococci</em>, <em>Salmonella</em>, <em>H. pylori</em>, <em>enteric viruses</em>, <em>Cryptosporidium</em></td>
<td>Tracing source of disease-causing infectious agents for distribution and prevalence</td>
<td>Grif et al., 1998; Sebat et al., 2003; Purohit et al., 1996.</td>
</tr>
<tr>
<td>DNA Microarray technology</td>
<td><em>E. coli</em>, <em>V. cholerae</em>, <em>Shigella</em>, <em>Salmonella</em>, <em>P. aeruginosa</em>, <em>Cryptosporidium</em></td>
<td>Efficient and accurate in detection of whole-genome expression and identification of multiplexed PCR products</td>
<td>Chizhikov et al., 2001; Leonard et al., 2003; Wolter et al., 2008; Kim et al., 2008; Li et al., 2008.</td>
</tr>
<tr>
<td>Gene probes</td>
<td><em>E. coli</em></td>
<td>Rapid differentiation of virulent strains from non-virulent</td>
<td>Betts, 1999.</td>
</tr>
<tr>
<td>Bio-sensors</td>
<td><em>E. coli</em>, <em>V. cholerae</em>, <em>Salmonella</em>, <em>Cryptosporidium</em>, <em>Giardia H. pylori</em></td>
<td>Rapid and simple for culturable microorganisms Detection of bacteria bound on beads, membranes, fiber optics probe tips by laser excitation, acoustiogravimetric wave transduction or surface plasmon resonance</td>
<td>Osek, 2002; Rose et al., 2007; Mutharasan, 2007; Zhua et al., 2005.</td>
</tr>
<tr>
<td>Gene-chip Technology</td>
<td><em>E. coli</em>, <em>Shigella</em>, <em>Salmonella</em>, <em>Giardia</em></td>
<td>Specific, less expensive and sensitive to desired level of certain harmful microorganisms</td>
<td>Chizhikov et al., 2001; Lipp et al., 2003.</td>
</tr>
</tbody>
</table>

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algorithms, such as, sequence comparisons, comparing genomic structure and modification searching (Groisman et al., 1993). On the basis of the information obtained, inferences can be made about the evolutionary history and biosynthetic capacity of an organism, for the development of new antimicrobial agents. However, sequence homology searching has limitations of not detecting all genes with similar functions. In addition, bioinformatics provide computational tools needed for detecting all genes with similar functions. In addition, bioinformatics provide computational tools needed for detecting all genes with similar functions.

Box 1. Web resources

<table>
<thead>
<tr>
<th>Resource</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIGR</td>
<td><a href="http://www.tigr.org/tigrdb/mdbcomplete.html">http://www.tigr.org/tigrdb/mdbcomplete.html</a></td>
</tr>
<tr>
<td>IGRMD</td>
<td><a href="http://www.tigr.org/tigrdb/mdb.html">http://www.tigr.org/tigrdb/mdb.html</a></td>
</tr>
<tr>
<td>AceDB</td>
<td><a href="http://www.sanger.ac.uk/software/Acedb/">http://www.sanger.ac.uk/software/Acedb/</a></td>
</tr>
<tr>
<td>EcoGene</td>
<td><a href="http://bmb.med.miami.edu/EcoGene/EcoWeb/">http://bmb.med.miami.edu/EcoGene/EcoWeb/</a></td>
</tr>
<tr>
<td>GOLD</td>
<td><a href="http://igweb.integratedgenomics.com/GOLD/">http://igweb.integratedgenomics.com/GOLD/</a></td>
</tr>
<tr>
<td>EcoCyc</td>
<td><a href="http://ecocyc.pangeasystems.com/ecocyc/">http://ecocyc.pangeasystems.com/ecocyc/</a></td>
</tr>
<tr>
<td>EMBL</td>
<td><a href="http://www.ebi.ac.uk/embbl.html/">http://www.ebi.ac.uk/embbl.html/</a></td>
</tr>
<tr>
<td>KEGG</td>
<td><a href="http://www.ddbj.nig.ac.jp/kegg/docs/rtbl.html">http://www.ddbj.nig.ac.jp/kegg/docs/rtbl.html</a></td>
</tr>
<tr>
<td>WIT</td>
<td><a href="http://www.cme.msu.edu/wit/">http://www.cme.msu.edu/wit/</a></td>
</tr>
<tr>
<td>SCPG</td>
<td>ftp://ftp.sanger.ac.uk/=/pub/pathsens</td>
</tr>
<tr>
<td>OUGC</td>
<td><a href="http://www.genome.uou.edu/strep.html">http://www.genome.uou.edu/strep.html</a></td>
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<tr>
<td>MBGD</td>
<td><a href="http://mbgd.genome.ad.jp/">http://mbgd.genome.ad.jp/</a></td>
</tr>
<tr>
<td>BioCatalog</td>
<td><a href="http://www.ebi.ac.uk/biocat/">http://www.ebi.ac.uk/biocat/</a></td>
</tr>
<tr>
<td>Blast software</td>
<td>ftp://ncbi.nlm.nih.gov/blast/executables</td>
</tr>
</tbody>
</table>

Table 4. Selected water borne pathogens and techniques used for detection

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Survival in water</th>
<th>Relative infective dose</th>
<th>Resist -ance to chlorine</th>
<th>Methods used for detection</th>
<th>Target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>High&gt;1yr</td>
<td>high</td>
<td>low</td>
<td>PCR, Multiplex-PCR, RAPD-PCR, PFGE, RT-PCR, AFLP, Rep-PCR, Ribotyping</td>
<td>uidA, lac, eaeA, hlyA, stx1, stx2, fliC, E-hly, LT1, ST1, STII, rfbE, tir</td>
</tr>
<tr>
<td>V. cholerae</td>
<td>Short</td>
<td>high</td>
<td>low</td>
<td>PCR, Multiplex-PCR, RT-PCR, Ribotyping</td>
<td>ctxA, uidR, rfb, toxR, tcpA, acfB, OmpU</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>May multiply</td>
<td>high</td>
<td>moderate</td>
<td>PCR, Multiplex-PCR, RFLP, Rep-PCR, PFGE, RT-PCR, AFLP</td>
<td>VRE, toxA 16S, 23S Rrna, hba, hybA</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PCR, Rep-PCR, PFGE</td>
<td>efaA, esp, gelE, agg, cymB, VanA, VanB, VanC, ela</td>
</tr>
<tr>
<td>H. pylori</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Rep-PCR, RAPD</td>
<td>23SrRNA</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>moderate</td>
<td>high</td>
<td>low</td>
<td>RFLP, AFLP, RT-PCR, PCR, Multiplex-PCR, RT-PCR, RFLP, PFGE, Ribotyping</td>
<td>VRE, ureC, urea, hpaa, VacA</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>Short</td>
<td>high</td>
<td>low</td>
<td>Multiplex-PCR, nested-PCR, CFLP</td>
<td>ST14, ST11, uidA, rfbE</td>
</tr>
<tr>
<td>Enteric viruses</td>
<td>Long</td>
<td>low</td>
<td>moderate</td>
<td>PCR, RT-PCR, RFLP</td>
<td>udrR, uidA, lamb, SLY-1, SLY-II</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>Moderate</td>
<td>low</td>
<td>high</td>
<td>PCR, nested-PCR</td>
<td>Beg-9, VP-7, EH-1, ED-1, rRNA</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Moderate</td>
<td>low</td>
<td>high</td>
<td>RT-PCR, RFLP, RAPD, nested -PCR</td>
<td>COWP, 18SrDNA, TRAP-C, dhfr</td>
</tr>
</tbody>
</table>

(Ref: Bhanumathi et al., 2003; Haque et al., 2000; Heijnen & Medema, 2006; Fu et al., 2005; Mandrell & Wachtel, 1999; Eaton & Gasson, 2001; Hahma et al., 2003; Purohit et al., 1996; Corbella & Puyet, 2003; Geornaras et al., 1999; Malathum et al., 1998; Frahm & Obst, 2003; He et al., 2002; Johnson & Clabots, 2000; Belzer et al., 2009; Cebula et al., 1995; Dubois et al., 1997; More et al., 1994; Mirelman et al., 1997; Queiroz et al., 2001; APHA, 1992; Kulkarni et al., 1993; Fayer et al., 2000)
An international expert meeting in Interlaken concluded (Karch & Bielaszewska, 2001) that the application of molecular methods has to be considered in a framework of a quality management for drinking water (Fig. 5). In last decade, the development and extensive use of high resolution molecular typing systems based on direct analysis of genomic polymorphism have greatly improved the understanding of epidemiology of infectious diseases (Rose & Grimes, 2001). Methods that index chromosomal DNA polymorphism are the best options for comparative typing. Epidemiologic typing can be used to confirm and delineate the patterns of transmission, evaluation of control measures by documentation of prevalence time and reservoirs of epidemic organisms.

**Real-time polymerase chain reaction**

Recent advances in PCR technology have facilitated the development of real-time PCR with greatly reduced amplification period and improved method for detection of amplified target sequences. In real-time PCR, the target gene is amplified and simultaneously recognized and monitored by the fluorescent probe moiety. As the reaction in this method is homogenous the risks of cross-contamination are minimized and downstream analyses are limited. Recent developments of fluorogenic nucleic acid probes such as Molecular Beacons (MB’s) confer new dimension to PCR, for which results have become quantitative and available in a real time manner, for ecological and epidemiological studies to determine what species are present in the population. MB’s are oligonucleotides probes that possess differential fluorescent properties based on the relative stability between its duplex forms (hairpin and the probe target hybrid). The advantage of MB’s is their extraordinary specificity, as no increase in fluorescence is observed even in the presence of a target strand containing a single nucleotide mismatch (Kim et al., 2008; Li et al., 2008).

Other specific fluorescent oligonucleotide probes for real time monitoring of polymerase chain reaction are TaqMan, Minor Groove Binder (MGB), Fluorescence Resonance Energy Transfer (FRET) and Scorpion. Highly reliable, real-time PCR has the advantages of a regular PCR in addition to the demonstration of a supplementary level of specificity, allelic discrimination (Tyagi et al.,1998; Lee et al., 2009). Reischl et al. (2002) reports amplification and detection of eae, hlyA, stx1 and stx2 in *Escherichia coli* using real-time PCR. Multiplex PCR with

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**Fig. 5. Genomic analysis of flexible gene pool in water-borne pathogenic bacteria to aid geographical profiling and real-time detection**

- Genomic Analysis
  - Genomic Islands & Islets:
    - Pathogenicity islands and islets
    - Resistance islands
    - Virulence factors
    - Physiological traits
  - Geographical Profiles: Ribotyping

**Water-Borne Pathogenic Bacteria**

- Real-Time: PCR, Genechips, Biochips

- Phages, Transposons

- Genomes
  - Chromosome
  - Plasmids

- Genomic Analyses

- Derived (Table 3 & 4). Traditionally source tracking methods have targeted multiple antibiotic resistance (MAR) patterns, cell surface or flagellar antigens or biochemical tests designed to identify variation in the utilization of various substances that may be found within a particular host environment.

Numerous methods currently used show significant drawbacks including inadequate discrimination, limited availability of reagents, poor reproducibility and inability to quantify the genetic relationships between isolates. Culture-based methods are time consuming, tedious, invariably nonspecific and multiple antibiotic resistance profiles have some inherent uncertainties as detection of one gene or specific enzyme product does not produce reliable identification (Table 3). While DNA based identification of microorganisms does not determine viability (Table 4) and no practical approach has been developed to specifically target majority of the uncultivable species in the environment. Thus new approaches are needed for development of full range of pathogen detection techniques that will lead to a strong, integrated detection system.
molecular beacons has been used for detection of pathogenic retroviruses in human blood samples and Shiga-toxin producing bacteria in feces (Vet et al., 1999; Belanger et al., 2002). The speed of detection and the availability of potential subtyping information make RT-PCR a better alternative to block cyclers PCR assays. Quantitative detection of multiple target organisms within a single sample, preferably in real time (Nathalie et al., 2001; Mackay, 2007) could be an ideal system in monitoring drinking water contamination.

Ribotyping
Phylogenetic analysis is necessary for emerging pathogens with partially known virulence factors. Ribotyping, a versatile and widely used strategy of southern blot analysis of bacterial genome polymorphism, has emerged as one of the most powerful tools and 'Gold Standard' of molecular epidemiology (Grimont & Grimont, 1986; Henegariu et al., 1997). Ribotyping uses restriction fragments of ribosomal RNA genes for characterization of organisms (Amann & Ludwig, 1994, 2000). It is a robust method with excellent reproducibility and stability during the course of outbreaks and is commercially available in a fully automated and well-standardized format. Griff et al. (1998) used EcoRI as restriction enzyme for discriminating epidemiologically related and unrelated Escherichia coli 0157:H7 isolates. Ribotype patterns of many pathogens such as Enterobacteriaceae, Pseudomonas sp., Helicobacter alvei have been produced. Ribotyping is being increasingly explored for differentiating fecal Escherichia coli of human origin from pooled fecal Escherichia coli isolated of non-human origin (Parveen et al., 1999; Carson et al., 2001). Ribotyping can generate an electronic riboprint database for rapidly investigating emergence of virulent strains provided precise geographic, socio-economic and medical data is available.

Oligonucleotide microchips
Oligonucleotide microchips are used in determinative and environmental microbiology and provide powerful format for the systematic exploration of natural microbial diversity. In addition to their use in phylogenetic group identification, microchip array can be used to evaluate sequence motifs that have yet to be identified in a habitat with microbial populations of interest. The use of microchips for determinative studies provides several advantages over conventional hybridization formats. Hundreds of different oligonucleotides can be immobilized on a single microchip allowing simultaneous detection of a great variety of different microorganisms in a single sample and can be used 20-30 times without noticeable deterioration of the hybridization signal. It can be used for direct analysis of environmental populations thus, not requiring prior amplification of the target nucleic acids. Oligonucleotide microchips have been used to discriminate Escherichia coli, Salmonella and Shigella sp. (Chizhikov et al., 2001). In addition, direct detection of source-specific genotypic markers in water samples without isolation holds promises. However, for its wider applicability, the possible temporal diversity, geographical limitations and survival of these source-specific genotypic markers in the environment need to be assessed. These microchips technologies are promising for meeting the future demands of biochemical analysis (Table 3).

Conclusion
Little is known about the contribution of chromosomal evolution and genotypic variation in pathogen population that poses a major barrier to disease control. The availability of the complete genomic sequence of a variety of microorganisms, coupled with new technologies for large-scale analysis of gene expression and function, has provided an abundance of new opportunities for understanding complex biological properties of microbial pathogens. Sequence information along with analysis of the sequenced data is opening up new horizons for studying microbial evolution and pathogenesis as well as development of new diagnostic and monitoring techniques. Future developments in signal detection and miniaturization technologies will provide real-time monitoring and diagnostics for rapid assessment of microbial pathogens. Establishment of such highly parallel and specific methods is essential to reduce the health risk from microbial pathogens present in water source. Highly effective and real-time diagnostic systems with a wider coverage of microbial pathogens therefore, play an integral role in facilitating an effective response to be provided against water borne infections.

But to be more useful and usable, the different technologies for assaying microbial water quality must be cost effective fulfilling the needs of public health and environmental regulators as most of the developing countries still do not have the necessary sophisticated laboratory infrastructure to comply with safe drinking water requirements. It is strongly felt that development of a database system at national level on real-time monitoring and diagnostics systems developed at various national laboratories/institutes is essential so as to ease the access of the modern technologies as and when required. It is equally important to create awareness among public, medical practitioners and the entrepreneurs on the available technologies.

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