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7. Climate Change: Monitoring the Pathogens by Traditional and Latest Trends

Dibyarupa Pal

Department of Biosciences,
JIS University,
81, Nilgunj Rd, Agarpara, Kolkata, West Bengal, India.

Pranabi Maji

Department of Physics,
JIS University,
81, Nilgunj Rd, Agarpara, Kolkata, West Bengal, India.

Abstract:

Climate change is undeniably one of the most challenging issues today perplexing the scientific world. Evidence of sporadic outbreak of water related diseases and its frequency highlights the changing interaction and dynamicity of the pathogens. Elevating summer, incessant rainfall, fluctuations in rainfall pattern, drought conditions are the extreme events of climate change. Excessive rainfall is flooding the region leading to deployment of water related pathogens to different locations spreading the risk of gastrointestinal diseases. Stagnant water becomes magical breeding ground of vector borne diseases. Drought conditions evaporate the water and concentrate the pathogen load. Communities depending on these water bodies are inflicted with disease like Amoebiasis, Giardiasis, Cryptosporidiosis caused by water borne protozoan parasites like Entamoeba histolytica, Giardia intestinalis and Cryptosporidium sp. and/or with Coliform bacteria causing Diarrhoea and severe gastrointestinal disorders. Plant pathogens under the influence of climate change are also imposing severity of disease in crop plants. Phenological swings are observed in flowering and fruiting plants compounded with change in soil-microbe plant interaction as well as microbe-microbe-plant interactions. As one of the components of Disease triangle has become unpredictable there is far fetching consequences on long term dynamics of host pathogen relation. Evolutionary mutation is one of the major concerns and monitoring such situation with powerful tool is a prerequisite to prevent any pandemic or endemic disease in future. In this context, the chapter highlights on the various methods and techniques available for monitoring water borne and plant borne pathogens under the influence of climate change.

7.1 Introduction:

Manifestation of the disease in plants or animals depends on three significant parameters: Host (vector), pathogen (agent) and favourable environment (for transmission and survival). This forms the basis of disease triangle concept which states that changes in one of the factors will inevitably affect the other two. Climate change is putting a direct constraint to

environmental conditions which is inducing the most benign factors responsible for disease dissemination and outbreak. Rise in temperature, incessant and uneven distribution of rainfall, unpredictable drought, ocean acidification is influencing an ecosystem to evolve with most complex and biodiverse strains. Volatilization of water from water bodies leads to concentration of the pathogen whereas over flooding results in spread of the pathogens to long distances (Ramírez-Castillo *et al*., 2015; Weber *et al*., 2016).

Water sources contaminated with microbes are mainly faecal in nature due to mixing of human, farm and wild animals. Majority of the waste discharge points are source points of drinking and sewerage treatment plant, industrial outlets, hospital sources etc. Accumulation of raw and untreated sewage and their overflow during rainfall is common scenario. Water borne outbreaks related pathogens predominantly consist of Coliform group like *E. coli* O157:H7, *Salmonella* spp., and *Vibrio cholerae* (Omarova *et al*., 2018), enteric protozoan parasites like *Entamoeba histolytica*, *Cryptosporidium parvum*, and *Giardia lamblia* (Bouzid *et al*., 2008; Omarova *et al*., 2018; Siwila *et al*., 2020) and water borne viruses like hepatitis A (WHO, 2011). Such outbreak systems can originate at various point or non-point sources like the water source itself when cross contaminated with sewerage pipes, recreational water, failure of water treatment plants, water storage and distribution system and at point of use. Diarrhoea, acute gastroenteritis (AGA), amoebiasis, giardiasis, are the direct influence of contaminated water. Moreover, climate change has severe consequences on the evolutionary diversification and predominance of these parasites. Monitoring water bodies, water shed areas, vegetation and soil sediments with current techniques are envisaged as suitable solutions to prevent outbreak and dissemination.

7.2 Water Borne Coliforms:

Coliforms belong to the family Enterobacteriaceae, and constitute about 10% of intestinal microflora of humans and animals are the biological indicators of water potability (Mishra *et al*., 2012). They include genera like *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* and broadly categorised under Faecal coliform. They are the responsible factors for water borne illness and can be detected using Multiple-Tube Fermentation (MTF) test where the result is interpreted in terms of most probable number (MPN) of Coliforms present in the water sample. The MPN results are statistical values based on mean of total coliforms in sample (Seidler *et al.* 1981; Evans *et al.* 1981**)**. Selective media like MacConkey, EMB or brilliant green lactose bile broth are utilized to grow and confirm coliform where lactose is the main fermenting sugar. Colilert test or detection of β-d-galactosidase enzyme is also a good indicator of coliform presence. Here, for *E. coli* detection the chromogenic substrate Indoxyl-*β*-glucuronide (IBDG) and for detection of total coliforms, the fluorogenic substrate, 4-methylumbelliferyl-*β*-galactopyranoside (MUG) needs to be cleaved enzymatically for their identification (Dyer, 1970).

7.3 Monitoring by Optical Methods:

Optical methods are reliable non-destructive means of identifying and enumerating bacteria in the sample. It can measure bacterial growth, metabolism, antibiotic susceptibility and even biofilms. Various modes like UV-Vis spectroscopy, Fluorescence and Raman

spectroscopy, Dynamic Light Scattering, vibrational spectroscopy and scattering works on the basis of optical methods. Spectrophotometric or turbidimetric method is also widely used to check the turbidity of water sample caused due to dissolved and suspended particles. Importance of this parameter lies in the fact that many pathogens adhere to the suspended particles and survive.

7.3.1 Monitoring by Morphology, Selective Media and Biochemical Characterization:

Microscopy helps in classifying the microbe through Gram staining. Selective or differential media helps to grow specific microbes only while preventing others. Criteria like low pH and high salt (Mannitol Salt Agar, MSA) in selective media favours growth of certain species whereas differential media (Mannitol in MSA) prefers growth of many organisms which can utilize mannitol. Cultivable microbes can be biochemically characterized by one of the cheap and easy methods for species identification consisting of battery of tests like IMViC, catalase, urease, sugar fermentation test etc. For example, catalase test helps in differentiating Micrococcaceae from Streptococcaceae genera on the basis of the enzyme catalase present in the bacteria. Similarly, species differentiation between *Campylobacter* is also possible. Indole test screens for the enzyme tryptophanase in the bacteria which breaks down amino acid tryptophan and produce indole. Methyl red screens lactose fermenters from non-fermenters. Citrate test helps to distinguish on the basis of utilization of citrate as sole carbon source. The urease test differentiates urease-positive bacteria from other Enterobacteriaceae by hydrolysing urea to produce ammonia and carbon dioxide.

7.3.2 Monitoring by Polymerase Chain Reaction (PCR) and DNA Sequencing:

Viable but nonculturable (VBNCs) microbes can be analysed through PCR. Primer designing is one of the key aspects for PCR, since it helps in amplification of the target genome. According to CLSI, for prokaryotes, rRNA genes (5S, 16S, and 23S) and intergenic region are chosen for primer designing. 16S rRNA genes are widely accepted for bacterial taxonomy and identification because they are ubiquitous in all organisms, low Horizontal Gene Transfer (HGT) and highly conserved regions interspersed with variable regions. Advanced and more sensitive techniques like Quantitative real-time PCR, employs measurement of amplified product along with DNA synthesis in a single reaction vessel. Tagging the amplified product with fluorescent DNA binding molecules (SYBR-Green I), or using various other probes like TaqMan (Applied Biosystems), fluorescence resonance energy transfer (FRET), and molecular beacon probes helps in real time monitoring. DNA sequencing is one of the gold standard methods for identification of microorganisms at species level. The principle is based on amplification of 16S ribosomal RNA (rRNA) gene for bacteria which is 1542 base pairs in length. For identification purposes at species level, sequencing the first 500 base pairs reveals enough data for species identification, but sometimes whole length sequencing of 16S rRNA gene is required for differentiation. Microbial diversity and prevalence in water bodies can be detected by Next-generation sequencing (NGS). It enables parallel analysis of DNA sequences from huge samples of environmental nucleic acids targeting hypervariable regions of small subunit (SSU) rRNA gene. Several other technologies like Pyrosequencing or Illumina helps in assessing microbial communities from large water bodies. Once the amplicon is sequenced, it is compared with a public or private database for organism identification.

7.3.3 Monitoring by Biosensor:

Biosensors sense the amplified signal developed between a specific biological element and the transducer. Different biosensors like surface plasma resonance (Taylor *et al*., 2005), mass based biosensor (Singh *et al*., 2013), electrochemical biosensors (Mishra *et al*., 2015) have been used successfully for detection of food borne pathogens and *E. coli*. O157:H7 (Zheng *et al*., 2018). Enzyme based biosensors like interaction of the substrate 4-methylumbelliferyl-β-d-glucuronide (MUG) by β-d-glucuronidase enzyme of *E. coli* to yield a fluorogenic product can be correlated to presence of *E. coli* in water samples (Hesari *et al*., 2015). Latest method includes developing synthetic enzyme substrates specific for certain bacterial enzymes conjugated with signalophore molecule for detection purposes (Pala *et al*., 2020) Thus detecting change in optical properties, mass

7.4 Water Borne Protozoan Parasites:

Protozoan parasites like *Entamoeba histolytica*, *Cryptosporidium parvum*, *Giardia lamblia* exist as trophozoites and cyst/oocyst form in their lifecycle. Trophozoites are the vulnerable stage (non-infective) and cyst are resistant (infective) to environmental stress. Faecal-oral route is the major transmission path of these parasites. Normally the trophozoite form dies after certain period of exposure from the body but cyst/oocyst form survives in sources like water, sediments, skin of fruits and vegetables. Cysts of *E. histolytica* are 5–20 µm diameter and oocyst of *Cryptosporidium* and *Giardia* are very small (1–17 µm) and remains viable in water for 6–12 months. Moreover, the cyst/oocyst are resistant to the most common disinfectant like chlorination used in water treatment plants. The surface coating of cysts is electronegative in nature which is the principle behind many techniques designed for their detection and isolation. Initial steps start with concentrating the cyst/oocyst from water sources by centrifugation (5000g for 20 mins) or by membrane filtration techniques. *Entamoeba* cysts are recovered by filtration through 1.2 mm membrane filters followed by simple staining and microscopy.

7.4.1 Monitoring by Simple Staining:

The most prevalent surveillance technique is staining by Lugol's iodine solution and microscopic identification under 10X and 40X magnifications. It stains nucleus of *Entamoeba* and *Giardia* light brown whereas Modified Ziehl-Neelsen (MZN) stain colours the oocyst nucleus of *Cryptosporodium* red. But genetic diversity cannot be ascertained by this method as cyst/oocyst of different species looks similar.

7.4.2 Monitoring by Immunofluorescence and Immunomagnetic Separation (IMS):

Sensitive detection of cyst/oocyst can be done by labelling with fluorescence molecules like 4`,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) which helps to distinguish between viable or non-viable cells. IMS method is reliable and efficient due to the availability of greater surface area available for target binding. Here magnetic particles (micro/nano dimension) are immunomobilized with specific antibody, incubated

simultaneously with the sample water to bind target pathogen. Then magnetic field is applied to separate target pathogen from non-specific ones (Hsu *et al*., 2001).

7.4.3 Monitoring by PCR and Genome Sequence Analysis:

Eukaryotic genomes are large, complex and repetitive for coding and non-coding sequences which makes it difficult to design universal primers targeting 18S rRNA. Though complete genome sequence of the three protozoan parasites are available in the biological database which can be utilized for searching new species. For differentiating *Entamoeba histolytica* cyst (pathogenic) from *E. dispar* (non-pathogenic and more prevalent) cyst, Antigen Specific Assays (ASA) can be applied. For detecting *Cryptosporidium* species, Nested PCR and real-time PCR assays can be employed targeting genes like hsp70, 18S rDNA and Gp60 where sensitivity as low as one oocyst. PCR coupled with RFLP (Restriction Fragment Length Polymorphism) can differentiate between certain species of *Cryptosporidium*. For *Giardia*, target genes normally used for species identification are 18S ribosomal RNA and triose phosphate isomerase gene which can detect up to sub assemblage level. Real time PCR multiplexed with TaqMan probe can do more sensitive detections in real time zone. Moreover, multiplex and reverse transcriptase PCR can use RNA converted to cDNA as target material. Latest trends for high throughput taxonomic identification include next generation sequencing (NGS) applying metagenomics (Peters *et al*., 2018; Moreno *et al*., 2018).

7.4.4 Monitoring by Biosensors:

One of the most sensitive, selective and portable latest technology based on electrochemical, optical and piezoelectric sensing ability of immobilized species specific biological molecule in a compact analytical unit. For detection of parasites, target specific monoclonal antibody is covalently coated over capture electrode and nonspecific binding treated with Bovine Serum Albmin (BSA). Sample water when passes through this biosensor helps in capturing specific parasites and coupled with immunofluorescence helps in early detection at point-of-use (POU). This ensures faster on-site analysis of very low concentration of cyst/oocyst from water bodies or food sources (Connelly and Baeumner., 2012). Latest trends highlights detection of single cell protozoan parasite utilizing electrical impendance properties to identify live and inactive oocyts (McGrath *et al*., 2017).

7.4.5 Monitoring by Flow Cytometry and Mass Spectroscopy:

Online characterization of water borne pathogens can be done using this technique. Passing of single cyst/oocyst/bacteria is ensured through the channel and detected via the Laser light which measures the forward-scatter and sideways-scatter. Signature peptides, protein fingerprints and metabolites secreted by the microbe can be analysed by mass spectroscopy or employing MALDI-TOF MS.

7.5 Plant Borne Pathogens:

Pathogens attacking plants are diverse in nature ranging from intracellular bacteria, virus to extracellular fungi, nematodes, pest which are biotrophic or necrtrophic in nature.

Environmental parameters like change in CO_2, rise in temperature, distribution of water has a heavy toll on the regional distribution of the plant. These changes affect plant morphology, physiology like flowering or fruiting time as well as its immune system. It is anticipated that marginal differences in the environmental parameters might not impose significant changes but extreme conditions have the potential to influence pathogen survival and increase virulence. Plant diseases are mostly affected by high rainfall, humidity and waterlogging conditions like fungus *Sclerotinia* sp. Similarly, drought conditions also favour certain diseases like *Ralstonia* infecting tomato plants. High temperature melts down permafrost areas exposing origin of new species and community.

7.5.1 Monitoring by Precision Agriculture and Plant Phenotyping:

Precision agriculture is based on real time technology driven crop management system functioning on crop and soil factors like spatial and temporal variability within a particular field. With changing environmental parameters, the crop and soil requirement also varies which can be predicted by this system. Plant phenotyping is based on plant breeding under different abiotic stress like high salt tolerance, less water consumption and disease resistance conditions. Traits selected under these conditions might still face challenges from changing climatic conditions which indirectly influences host-pathogen interaction and thus disease manifestation. Application of current technologies like Biosensors helps in early detection of plant diseases along with molecular and serological analysis (Mahlein, 2016).

7.5.2 Monitoring by Biosensors:

These are very sensitive and promising tools for automated detection of non-invasive plant diseases and normally incorporated with traditional monitoring system. Early detection of diseased areas, onset of fungal spores, effector molecules or elicit production of plant defence molecules can be diagnosed by optical sensors, thermography, or chlorophyll fluorescence. Optical sensors coupled with nanostructures can sense various analytes associated in host microbe interactions. Immunosensors like quartz crystal microbalance (QCM) utilizes surface plasmon resonance (SPR) monitoring changes in the refractive index when analyte interacts with an immobilized ligand. Electrochemical Impedance spectroscopy (EIS) sensors are able to monitor changes in the device impedance when target analytes are observed (Regiart *et al.*, 2017; Malecka *et al.*, 2014).

7.6 Summary:

Surveillance devices and latest technologies are being implemented to monitor presence of water borne and plant borne pathogens. Climate change influences animal-microbe or plant-microbe interaction resulting in prevalence and/or dominance of certain pathogenic species. Expression of pathogenic genes under these circumstances is notable as pathogens will modulate their virulence mechanisms to survive. But to predict such conclusive outcomes comprehensive research on the pathogen distribution and their dynamic interaction with host and changing environmental parameters need to be considered. Thus rigorous monitoring systems are needed with both traditional and latest technologies to prevent future disease outbreaks.

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