REVIEW ARTICLE

Persistence of Human Pathogenic Viruses in Water: Use of Viral Indicators for Water Quality Assessment

Mohammed, Jibrin Ndejiko*, and Feroz Mahomed Swalaha

Department of Biotechnology and Food Science, Durban University of Technology, P O Box 1334, Durban, South Africa

ABSTRACT

Human viral pathogens have been persistently detected in water sources and distribution systems, posing substantial health threats to the public. However, the existing microbiological water quality assessment mostly does not predict viral contaminants. This is due to differences in persistence rate, emerging viral pathogens, and climate change-induced alterations in water. This paper examines the occurrence of major human viral pathogens in water systems and the need for their inclusion as indicator organisms for water quality assessment. It also explores recent detection techniques, including molecular procedures, enrichment methods, biosensors, and metagenomics. Emerging research has demonstrated that viral indicators like somatic coliphage and crAssphage correlate with the enteric viral pathogens. The integration of viral indicators into the water quality assessment frame has the capacity to efficiently validate water treatment procedures and advance water-associated risk assessments. This review underscores the need for policy and methodological shifts towards virus-based water quality monitoring for improved public health.
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Corresponding Author:
Mohammed Jibrin Ndejiko
Email: ndejiko@ibbu.edu.ng
Tel: +2347067855089

INTRODUCTION

The globally expanding demand for clean water, poor sanitation facilities, vulnerability of water distribution systems to sewage intrusion and poor access to potable water present a critical challenge to water resource management. Water-related microbial infections have continued to rampage the globe, with about 1.7 billion people estimated to be drinking from contaminated water sources [1]. This kills above two million people annually [2], for example viral hepatitis alone killed about 1.34 million people in 2015 [3]. Waterborne infections are primarily caused by the contamination of water sources and the distribution systems. Generally, water contamination can be attributed to many factors, such as sewage overflow, leaking sewage systems, contaminated irrigation water, inadequately treated wastewater, polluted storm runoffs, human activities and natural disasters, as described in Table I.

Table I: General Sources of water contamination

Contaminant source	Description
Sewage Overflows	Occur when sewage systems are overwhelmed, untreated wastewater released into the environment
Leaking sewage systems	Aging or poorly maintained sewer systems leak, allow viruses from fecal matter to enter groundwater
Polluted storm water runoff	Rain washes contaminants into water bodies from urban areas, farms, and other locations
Inadequately treated Wastewater	Wastewater treatment plants may not completely eliminate viruses, resulting in viral pathogens being discharged into water bodies
Septic systems	Improperly functioning or poorly sited septic systems can lead to the release of viruses into nearby groundwater or surface water
Contaminated irrigation water	Use of water contaminated with fecal matter for irrigation can lead to viruses entering food products and the water supply
Direct human activity	Practices such as open defecation or improper disposal of human waste can directly contaminate water sources
Natural disasters	Events like floods or earthquakes can disrupt infrastructure and lead to increased contamination from sewage systems

The spread of pathogenic viruses via water is globally acknowledged as a public health threat. These viruses are often resilient against disinfection with chlorine and other conventional treatments because even treated waters can retain these viruses . Studies have indicated that up to 20-80% of enteric pathogenic viruses remain viable after the normal wastewater treatment procedures [4, 5]. Thus, viral pathogens can persist in the water for prolonged times to cause epidemics [6]. Incorporation of consistent viral evaluation into wastewater treatment practices is important for protection of public health and efficient water resource management.

The quality of drinking water is regulated by stringent guidelines with universal benchmarks. However, poverty, poor water infrastructure, and diversity of water sources challenge compliance with the laid-down guidelines. It is often impractical to assess water quality by detecting all pathogens present. Thus, the use of specific organisms, mainly *Escherichia coli* and other coliforms, is universally used as indicator organisms to signal contamination of water. Such organisms are used as proxies for the detection of potential microbial contaminants that may be difficult to identify directly [7]. Research reports on waterborne viral disease epidemics have indicated that the nonexistence of bacterial indicators does not automatically signal a lack of viral pathogens that can cause epidemics [8-10]. Through inclusive surveillances that check for possible viral indicators and the formation of an indicator composite that considers both bacterial and viral indicators for the determination of the microbial quality of water, regulatory bodies can effectively manage health threats related to wastewater release and prevent potential outbreaks of viral epidemics. This paper examines the occurrence of major human viral pathogens such as noroviruses, rotaviruses, adenoviruses, enteroviruses, and hepatitis A and E viruses in water systems and the need for their inclusion as part of indicator organisms for a comprehensive water quality assessment. It also explores recent detection techniques, including molecular procedures, enrichment methods, biosensors, metagenomics, and challenges associated with their use for detection of viral pathogens.

The manuscript is presented as a scoping and a narrative review that summarizes recent evidence on the persistence of human pathogenic viruses in water and their application for water quality valuation. Scientific reports and studies were identified mainly from Web of Science, Scopus, and PubMed using combinations of keywords such as “human pathogenic viruses,” “viral persistence,” “water quality,” “viral indicators,” “enteric viruses,” and “wastewater.” The search covered publications from approximately 2010 to 2025, complemented by prior milestone studies frequently cited in the field. We prioritized peer-reviewed articles, relevant reviews, and applicable transnational reports (e.g., WHO, USEPA). Considering the extensive scope and narrative nature of the present review, we applied no formal inclusion or exclusion criteria; instead, selection of papers was based on their relevance to key themes of viral tenacity in water and the utility of viral indicators.

Overview of Indicator Organisms

Indicator organisms are known microorganisms whose existence or nonexistence in food or water offers beneficial information on the overall microbial quality or safety of the food or water. To fulfill the national and global microbial quality criteria established to safeguard water and food products meant for consumption, it is imperative to ascertain the microbiological quality of water and food products. This is also a means of knowing their shelf life and ensuring public health safety. Indicator organisms typically originate from fecal matters and are usually known members of the Enterobacteriaceae family, such as *Escherichia coli*, Streptococci, Enterococcus species, Salmonella, and coliform bacteria.

Due to their fecal origin, their presence, especially *E. coli* in water and food products, can easily signal fecal contamination either from human beings or animals. *E. coli* is a lactose-fermenting Gram-negative bacterium that exists as normal flora in the gastrointestinal tract. This organism can also be found in soil following indiscriminate open defecation. Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC) are mostly responsible for the effects of *E. coli* incursion of the human systems [11]. The presence of the indicator organisms in a particular environment could also show poor sanitation or hygienic conditions of that environment [12, 13].

Generally, indicator microorganisms are used to indicate the extent of microbial impurity or contamination of water and food. This is achieved via microbial enumeration (viable count or total count), most probable number (MPN) tests, membrane filtration , sample culturing on selective media and coliform tests on representative sections of the food and water. Rapid methods for their detection involve the use of chromogens in microbial media, immunological and serological assays and gene-sequence centred methods, including PCR and fluorescence in situ hybridization (FISH) [13].

In each of the microbial enumeration methods, the sample is serially diluted, and an aliquot of the dilution is introduced into a suitable culture broth and agar media such as MacConkey broth, MacConkey agar, salmonella-shigella agar and plate count agar. The inoculated media are placed in an incubator at 37^{o}C for 18-24 hours. The resulting colonies on the agar media are subcultured and subjected to biochemical tests like the indole test for the presumptive identification, the Durham tube test for gas production and the most probable number (MPN) for the estimation of viable organisms. Nevertheless, the type of test(s) needed for determining the existence of indicator organisms in a specific food or water sample depends on a number of factors comprising the physical state of the sample (liquid or solid), the origin of the sample, and the likely indicator organism being sought.

Basic Criteria for Microbial Indicators

An indicator microorganism must fulfil certain criteria to be eligible as an indication of the presence of faecal contamination. According to the Environmental Protection Agency (EPA), an ideal indicator of faecal contamination of the environment including water and food, must meet the following criteria: [12]

1. The microorganism must be a member of intestinal flora of warm-blooded animals, including man.
2. The microorganism must have an extended survival time that is longer than that of the delicate enteric pathogen.
3. The microorganism must exist where enteric pathogens are found.
4. The microorganism must not grow in water to ensure that its presence is exclusively owed to faecal contamination of the sample being analysed.
5. It must be a microorganism that is easily activated during analysis.
6. The testing process for detection of the microorganism must be simple to facilitate consistent monitoring and valuation.
7. Such microorganisms must not be naturally pathogenic.
8. The microorganism must be suitable for all water types, including freshwater, marine, and wastewater.

Limitations of Indicator Bacteria

Although the criteria outlined above help to guarantee the reliability of the information provided by the indicator organisms on the probable health threats related to fecal contaminants, it is rare to find an indicator microorganism that perfectly fulfils these criteria due to pathogen and environmental condition diversity, microbial dynamics, and differences in detection methods. Similarly, there may be an occasional dearth of robust correlation between the existence of indicators and the pathogens [15]. A positive outcome for an indicator may not assure the nonexistence of harmful pathogenic microorganisms or their viability to potentially cause infection (USEPA, 2000). Indicator organisms can exist in nature in certain milieus, leading to false positive results. The presence of non-target organisms, stressed bacteria, the complexity of microbial activities, erratic physio-chemical conditions of different waters and occasional laboratory errors have also been reported to cause false positive results [14-16].

Specifically, indicators such as *E. coli* and enterococci have been found in tropical areas without signifying any fecal pollution [12, 17]. Certain marine bacteria, such as Vibrio cholera and Providencia sp., were reported to cause false positive results in Florida water in an attempt to test for *E. coli* using the Colilert-18 rapid identification test [18]. Peperzak andvan Bleijswijk [19] also reported high enterococci bacteria in Wadden Sea samples without any expected correlation with microbial contamination. Their further analyses showed that this erroneously high bacteria concentration was caused by Bacillus licheniformis, a gram-positive chlorine-resistant bacterium. Even *E. coli*, which is mostly considered a more precise indicator than the fecal coliforms, is not solely restricted to warm-blooded hosts; it has been isolated from reptile faeces [20]. It can also grow on beach sand and plant surfaces, and closely related coliform bacteria can have the same reaction to testing reagents as *E. coli*, thereby giving false positive results [20].

There have been serious concerns about the limitations of existing detection methods, as they are often less sensitive to the samples, necessitating large sample volumes for accurate assessment of low pathogen concentrations. The non-differentiation of viable microorganisms by most of the existing detection procedures can result in an overestimation of indicator organisms [13]. Sample size and statistical value of studies that evaluate the correlation between indicators and pathogenic organisms are also issues of concern, as they mostly rely on little sample size to draw their conclusions. Bigger datasets are better for robust statistical scrutiny to guarantee the reliability of the conclusions.

The complex and technical nature of some testing methods used for detecting indicators makes them labour intensive and necessitates the service of trained personnel as well as specified laboratory facilities and conditions making routine assessment challenging. The detection of indicators in water samples may not necessarily reflect fresh contamination of the water; this is because the indicator organisms can persist in the water for extended periods even after the pathogenic organisms have been eliminated (USEPA, 2000). These limitations underscore the need for cautious consideration when using indicators as proxies for assessment of water quality and possible health threats related to microbial contamination.

Pathogenic Viruses and their Detection in Water

The presence of human pathogenic viruses, notably noroviruses, rotaviruses, adenoviruses, enteroviruses and hepatitis A and E viruses in water [4] and their persistence in even the conventionally treated water (Table III) is exacerbating the challenge of potable water resources [21, 22]. These viruses have been established to cause several ailments such as gastroenteritis (noroviruses and adenoviruses), diarrhea in children (rotaviruses), liver diseases (hepatitis A and E), respiratory diseases (adenoviruses) and neurological disorders and meningitis (coxsackievirus and poliovirus) (detail in Table II). The majority of human viral pathogens are excreted (up to 10^{11} viral particles per gram of stool) by infected symptomatic and asymptomatic persons. This makes water polluted with faecal materials a significant threat for transmission of disease especially when such contaminated water intrudes into water bodies and water supply systems thereby contributing to global waterborne infections. The viral particles may also spread from wastewater to humans via direct contact or through ingestion of contaminated water or food and inhalation of aerosols [21, 23]. This process is further illustrated in Figure 1.

Table II: Common enteric viruses and associated diseases

Enteric Virus	Diseases Caused	Transmission route	Citations
Rotavirus	Severe diarrhea, gastroenteritis, dehydration, especially in children. Responsible for child mortality in developing nations.	Water sources polluted with fecal matters	Dallari et al. [66] Das et al. [67]
Norovirus	Gastroenteritis, vomiting, diarrhea; often outbreaks in closed communities such as schools and hospital settings.	Contaminated food and water	Das et al. [67] Albert et al. [68]
Adenovirus	Gastroenteritis, conjunctivitis, respiratory infections; linked to diarrhea in children	Contaminated food and water	Das et al. [67] Pearce-Walker et al. [69]
Astrovirus	Gastroenteritis, diarrhea; primarily affects young children	Contaminated food and water	Aboubakr, Goyal [70] Aboubakr, Goyal [70]
Sapovirus	Gastroenteritis; less common but can cause outbreaks similar to noroviruses	Contaminated food and water	Daham et al. [71]
Coxsackievirus	Gastroenteritis, myocarditis, respiratory infections, neurological disorder and can lead to serious conditions like diabetes	Contaminated water	Sun et al. [72]
Poliovirus	Poliomyelitis (paralysis), aseptic meningitis; serious complications in unvaccinated individuals	Contaminated water	Wahid et al. [73]
Hepatitis A	Hepatitis, liver disease	Contaminated food and water	Das et al. [67]
Hepatitis E	Hepatitis, liver disease	more rampant in places with poor hygiene	Sayed et al. [74]

Table III: Pathogenic viruses and methods used for their detection

Virus Detected	Detection Method	Citation
SARS-CoV-2	RT-Qpcr TaqPath™ Covid-19 RT–PCR kit Ultrafiltration Electrochemical immunosensors	Kallem et al. [7], Peinado et al. [75], Gogoi et al. [76]
Norovirus	Granular activated carbon (GAC) passive sampling RT-Qpcr	Hayes et al. [58]
Adenovirus	GAC passive sampling Quantitative PCR (qPCR)	Hayes et al. [58]
Rotavirus	GAC passive sampling RT-Qpcr	Hayes et al. [58]
Enterovirus	RT-Qpcr	Corpuz et al. [77]
Hepatitis A Virus	Wastewater-based epidemiology (WBE) PCR techniques	Gogoi et al. [76]
COVID-19 Virus	RT-PCR RT-LAMP Antigen tests	Chantanasaro et al. [30] Love et al. [78]
Hepatitis C Virus	RT-LAMP	Sharma et al. [79]
Hepatitis A Virus	Enzyme-linked magnetic electrochemical assay	D'Agostino et al. [80]

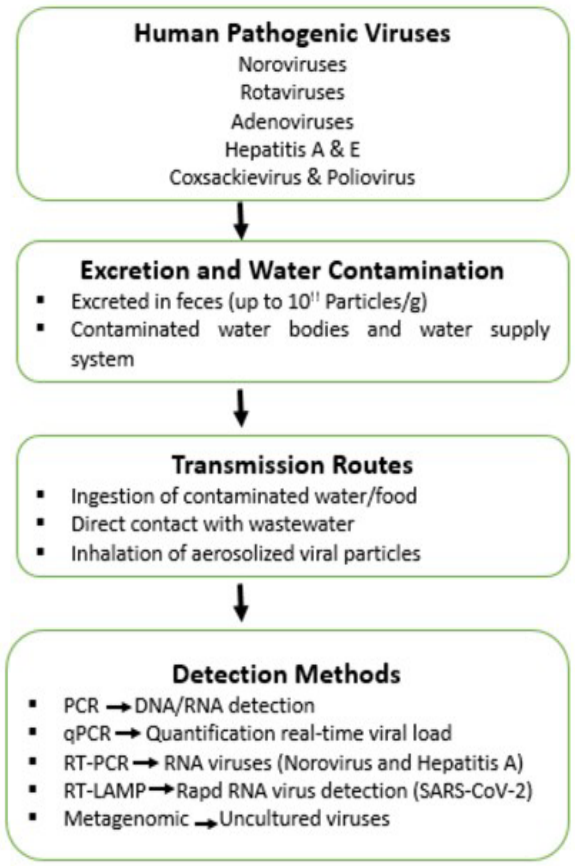

Fig. 1: Human pathogenic viruses, excretion, transmission and their detection methods

The detection of viral pathogens in food and water is essential for assessment of food and water quality and protection of public health. The most popular and recent techniques used for detecting viral pathogens include molecular techniques, enrichment techniques, biosensors, and metagenomics (Table III).

Molecular Methods

In molecular techniques, Polymerase Chain Reaction (PCR), a strong method for amplifying precise DNA or RNA sequences, allows detection of even lower concentrations of viral genome even at low concentrations. It is sensitive and specific and thus has attracted wide application in viral detection from environmental samples.

The PCR variant, quantitative real-time PCR (qPCR), is used for amplification and quantification of a specific viral genome sequence using viral-specific primers. It is a golden standard for viral quantification; it offers enumeration of viral population in water samples and provides real-time information on the specific viral concentration [24]. It uses probes and is thus highly specific and provides a relatively quick result; both primers and probes bind to the target sequence to achieve a signal [25]. The sample preparation may be delayed; rapid isolation kits and qPCR equipment are costly and require trained staff [26]. The reverse transcription PCR (RT-PCR) is employed precisely for

detection of RNA viruses; it has the ability to convert viral RNA to DNA prior to amplification [26-28]. This method is increasingly used for the diagnosis of viral respiratory contagions and for conducting epidemiological studies. It has been specifically applied for the detection of noroviruses and hepatitis A virus [26, 29]. Though it is highly sensitive, the daily screening incurs much cost and has a delayed turnaround time [30].

Another talented molecular technique is the reverse-transcription loop-mediated isothermal amplification (RT-LAMP). It is a variant of the conventional technique. It is used for amplification of RNA sequences for detection of RNA viruses such as SARS-CoV-2 and other viral pathogens. It is reliable, rapid and applicable to field or non-laboratory circumstances. This method is highly sensitive, specific, cost-efficient, and easy to implement in resource-restricted settings [26, 30]. A summary for easier comparison of the major molecular detection methods in terms of cost, sensitivity, specificity and limitations is presented in Table IV.

Enrichment Methods

The enrichment techniques involve concentration of viruses from large volumes of water prior to their detection to increase sensitivity and can be achieved via ultrafiltration, adsorption elution, Modified Diatomaceous Earth (MDE) Systems, immunomagnetic separation, centrifugation, and hydro extraction. Ultrafiltration uses suitable membranes to sieve out large particles and retain viruses to effectively concentrate pathogenic viruses from water samples. In adsorption-elution, viruses from water samples are adsorbed onto suitable adsorbents such as silica and activated carbon and then eluted using a suitable substance. The eluted viral particles are detected using molecular techniques as described above. The MDE system employs reformed diatomaceous earth and ferric hydroxide colloids for efficient concentration of viruses. Researchers [31] used this technique to achieve a substantial decrease in sample size (from 10 L to 4 mL), recovering up to 64% to 180% African swine fever virus (ASFV). This technique is well recognized for its swift processing time and efficiency in several water sources.

Immunomagnetic and Biosensing Methods

Immunomagnetic methods involve the use of antibody-coated magnetic beads to target specific viruses. When suspended in water samples, the antibody-coated magnetic beads bind to the viruses, thus letting the viruses be separated via magnetic power [32]. This technique is highly specific and can be joined to the PCR-based techniques. Description: Immunoassays are generally robust and not expensive and have effective turnaround times. They are however, less sensitive compared to RT-PCR [26, 30].

Biosensors are emerging electrochemical or optical techniques that have rapid detection capacity. The electrochemical biosensing devices are used to identify electrical signalling changes that occur when viral particles bind to the probes found on the surface of sensors [33, 34]. These biosensors offer swift results and can be deployed in field studies. The optical biosensors used light to detect viral particles; they used viral interaction with the light waves to identify viruses. They are highly sensitive and specific. Generally, use of biosensors for viral detection offers real-time monitoring that facilitates incessant monitoring and real-time data collection, enabling instant retorts to the detected pathogens. The portability and simple operating procedures of biosensors have enhanced their extensive use in several settings, including field applications [35]. There is a need to develop specificity and sensitivity of biosensors to enhance their ability to detect low-abundant pathogens [36]. The intricacy of the wastewater

Table IV: A brief comparison of the major molecular detection methods

Method	Cost	Sensitivity	Specificity	Turnaround Time	Limitations	References
PCR	Low to Moderate	High	High	3–5 hours	Knowledge of target sequence is needed; involves contamination risk	Child et al. [24]
qPCR	Moderate to High	Very High	Very High	2–4 hours	Reagents and equipment are costly; Only applicable to specific targets	Kaletta et al. [25] Child et al. [24]
RT-PCR	Moderate to High	Very High (for RNA)	Very High	3–6 hours	Requires high-quality RNA; temperature-sensitive enzymes	Cassedy et al. [26]
RT-LAMP	Low to Moderate	Moderately sensitive	highly specific when primers are well designed	typically 2–4 hours from raw water sample to result	False positives / nonspecific amplification, Lower quantitative accuracy and variable sensitivity in environmental samples	Kaletta et al. [25]
Metagenomics	Very High	Moderate to High	Broad but Variable	Several days (24–72 hrs)	High cost; complex data analysis; lower sensitivity for low-abundance viruses	Bibby et al. [10] Child et al. [24]
Biosensors	Low-cost	Highly sensitive (sub-pM / fg·mL^{-9} or single- to tens-of-copies-equivalent)	Specificity depends on recognition element	1–3 hours (with prep), or near-real-time	Sample pre-treatment often needed, Limited field validation, Specificity challenges.	Cassedy et al. [26] Bibby et al. [10]

environment challenges the use of biosensors for viral detection, as some other composition of wastewater can obstruct the detection capacity of the biosensors. This complication obliges the need for the development of a robust biosensing system that can work in such a complex environmental matrix [37].

Metagenomic Methods

Metagenomics allows comprehensive detection of the viral community of a sample without prerequisite information of any particular genome. This makes it fit for identification of well-known and novel viral pathogens that may constitute health threats and reveals the viral diversity in a given sample [24]. This advanced method is useful for discovering uncultivated viruses such as Bocavirus, CrAssphage, Cosavirus, Klassevirus, and pepper mild mottle viruses detected in water which are often not detectable via conventional culture-based techniques [10]. Metagenomics is also a valuable technique for source tracking of contamination of both freshwater and marine water samples. This is achievable through analysis of viral signatures in wastewaters, to identify specific enteric viruses and their origins, thereby boosting public health surveillance [38]. The incorporation of methods such as dPCR and hybrid-capturing to metagenomics has been shown to boost sensitive and specific detection of low-concentrated viruses in water samples through provision of comprehensive genotypic data of various viral strains existing in the samples [10, 38].

However, researchers [39] have indicated that untargeted shotgun sequencing of the metagenomics method is not sensitive enough to detect certain human viral pathogens, limiting its efficiency in genomic epidemiology. This method has the capacity to detect viral contaminants at higher frequency, especially when elevated sequencing nadirs are used, thereby complicating the data interpretation and possibly obscuring the detection of relevant viral pathogens [10, 39, 40]. Another limitation of this method is the cost and turnaround time, as increasing the sequence depth that is needed for enhanced sensitivity prolongs the processing time and increases cost. For example, to achieve adequate sensitivity needed for low viral loads, Oxford Nanopore Technologies (ONT) involves lengthy sequencing turns and decreases the number of samples that can be handled concurrently; this complicates the practical application of this method [39]. Though the choice of any of these methods depends on a number of factors, including the nature of the water sample, the target virus, the sensitive nature of the method, and the availability of resources, a combination of other methods with real-time PCR or next-generation sequencing can boost the detection of human pathogenic viruses in water thus, promoting public health surveillance and response stratagems.

Use of Viral Pathogens as Indicator Organisms

The need for inclusion of human pathogenic viruses as part of indicator microorganisms for environmental sample assessment, especially water quality has been gaining momentum over the years. Metagenomics sequencing has also uncovered a wide variety of human-associated viruses in water [38]. A deeper study of the limits of existing indicator organisms, benefits of viral indicators, and their implications for public health and water resource management is needed to further establish the need for a robust and inclusive indicator organism.

Current assessments rely heavily on fecal indicator bacteria (FIB), which may not accurately reflect the presence of viruses. A study conducted in South Africa and reported to the South African Water Commission (WRC) reported that norovirus and rotavirus show no correlation with the *E. coli* or total coliforms in the sampled waters, demonstrating a major gap in the traditional monitoring practice [41]. Holcomb andStewart [42] also argued that bacterial indicators are inadequate for water assessment and demonstrated that recreational waters contained significant levels of pathogenic viruses, including adenoviruses and enteric viruses, even when indicator bacteria were within the acceptable values.

Several research reports have also demonstrated the presence of coliphages (virally infected *E. coli*) and other viruses in water samples whose bacterial indicator counts indicated no contamination [7, 12, 17, 43-48]. This inconsistency emphasized the necessity for the incorporation of viral pathogens such as the coliphage that have been demonstrated to be able to signify the presence of both viral pathogens and bacterial pathogens in water [49-51]. Bacteriophages are promising for comprehensive signalling of both bacterial and viral pathogens because they share similar fates and transport mechanisms with both bacteria and viruses. Their similar environmental behavior with enteric viral particles makes them suitable proxies for tracing viral contaminants. Their higher abundance in environmental samples, especially water, provides a very reliable measure for fecal contamination [49, 50].

Further, the difference in the surviving and persisting rates of bacteria compared to viruses presents some challenges for relying on bacterial indicators alone. Enteric viral particles tend to be very resistant to adverse environmental conditions compared to enteric bacteria. The indicative value of bacteria can diminish in response to environmental stresses like UV radiation and predation. This survival and persistency of viruses in adverse conditions often found in polluted waters, coupled with their ability to evade traditional wastewater treatment measures as evident in Table III, increases the risk of human exposure to viruses and underscores the need for incorporation or use of viruses as indicator

organisms [12, 51].

The use of viruses for indicating contamination of water has attracted substantial interest from regulatory bodies such as WHO and USEPA, who have both acknowledged the use of bacteriophages as effective proxies for assessing viral contamination of environmental samples. Integrating viruses into the existing water assessment frameworks can reinforce compliance with water regulations and public health initiatives, thereby ensuring a robust and reliable assessment of water quality [44, 47]. Using viral indicators for assessment of water quality is resource efficient and good for routine surveillance. Detecting individual viruses can be resource-intensive and presents technical challenges; a focus on bacteriophages for water quality assessment programs helps to streamline the monitoring procedures and ensure effective detection of health threats associated with contamination of water [9, 49]. Moving forward, the globe is increasingly witnessing emerging viral infections and climate change impacts on water resources; prioritizing all-inclusive water quality assessment stratagems is getting critical for environmental safety and water resource management.

Emerging Viral indictors - Coliphages and crAssphages

In responding to the threat of the presence and persistence of human pathogenic viruses in water, experts are exploring diverse means to track and detect their presence in water. Though use of noroviruses and adenoviruses has been proposed in a number of studies [8, 52], coliphages and crAssphages are two major bacteriophages that have received much research attention and have been demonstrated in several studies to indicate fecal contamination and the threat of pathogenic viruses in several milieus [49, 50, 53, 54]. Their abundance in drinking water distribution sources and systems, readily detectable at greater densities, justifies their selection as a suitable marker of viral contamination [38, 49, 54]. Somatic and F-specific coliphages show a sturdier association with human viral pathogens than conventional bacterial markers. Researchers demonstrated a statistically significant connection between the existence of human viral pathogens and the somatic coliphages [55].

Generally, coliphages' infection of *E. coli* strain is specific and occurs via attachment to the lipopolysaccharide or protein receptor found on the cell wall of the *E. coli* and then they lyse the host cell after about 20 minutes [53]. Because of this specific host infection dynamic, a model somatic phage such as phiX174, which has specificity for *E. coli* ATCC 13706 and PC 0886 is commonly used for laboratory and pilot-scale investigations [54]. Some somatic phages have been shown to replicate in other hosts like Enterobacteriaceae, mainly Shigella sp. and Klebsiella pneumoniae [49]. Coliphages are classified based on their mechanism of replication in host cells into virulent or temperate coliphages. The virulent phages replicate within the host cell via the lytic cycle to produce new phage DNA or RNA and proteins [54]. These are subsequently incorporated into phage virions, after which the cell wall ruptures to release large quantities of coliphages virions into the environment. While the temperate coliphages goes through a lysogenic cycle to integrate phage DNA into the chromosome of the host cell [53]. This is further demonstrated in Figure 2.

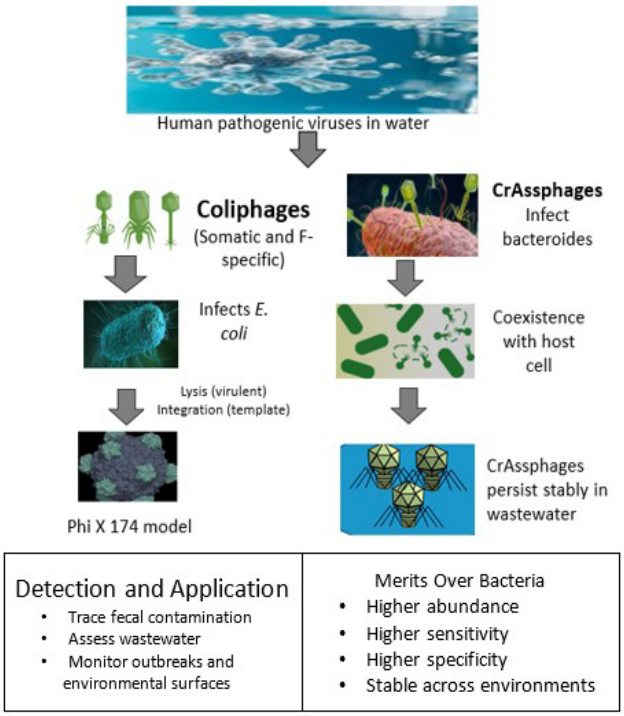

Fig. 2: Coliphages and crAssphages coexistence with bacteria, highlighting their potential as indicators for water assessment.

CrAssphages are a vastly profuse group of bacteriophages specifically found in the human gut. They have been investigated for their ability to indicate human fecal contamination. They infect Bacteroides belonging to the Podoviridae family [9]. The first isolated crAssphage (ΦcrAss001) was similar to Podoviridae viruses. It coexisted in stability with its host (B. intestanalis) devoid of cell lysis, benefiting both bacteria and viruses in the GIT [45]. CrAssBcn phage (a set of 25 recently identified crAssphages) did not also affect the growth of their host cell (*Bacteroides intestinalis*) [56]. The infected cell cultures were found to be viable and even increased in number throughout, demonstrating the indicating potential of the crAssBcn for both bacteria and viruses. Coliphages and crAssphage are both promising as viral indicators considering their prevalence, relationship with human pathogenic viruses, and potential to assess fecal contamination and wastewater treatment efficiency. Table V below summarizes the recent major discoveries on crAssphage and coliphages as efficient viral indicators for tracing human fecal pollution and the occurrence of pathogenic viruses in water. Both viruses were shown to outperform conventional bacterial indicators in sensitivity, abundance, and persistence, making them valued tools for water worth monitoring and public health risk valuation.

Table V: Recent major findings on crAssphage and coliphages as efficient viral indicators for tracing human fecal pollution and the occurrence of pathogenic viruses in water

Viral Indicator	Major Findings	Supporting Evidence
crAssphage	- Vastly numerous human gut bacteriophage that infects Bacteroides. - Co-exist stably with host bacteria without necessarily lysing the host, allowing persistence. - Their detection is at high densities in rivers, drinking water sources and wastewater. - They correlate strongly with viral pathogens such as norovirus and adenovirus - Their abundancy and persistent is higher than bacterial indicators. - They are suitable for monitoring viral contamination and wastewater treatment efficiency	- Consistent detection in raw and treated wastewater with concentrations 1–5 log10 greater than enteric human viruses [81-83] - Coexistence with host bacteria without lysing them, making them stable indicators [84] - Demonstrated as a reliable marker in irrigation water and produce studies [3]
Coliphages	- Bacteriophages that infects *E. coli* through specific receptors on bacterial cell walls - Comprise somatic and F-specific species, categorized as virulent (lytic) or temperate (lysogenic) - Occur in greater concentrations than enteroviruses in water and sludge - More resilient to disinfectants and ecological stressors than bacterial indicators - Has strong correlation with human pathogenic viruses than bacterial indicators - Commonly applied for routine monitoring due to ease of detection and quantification	- Somatic coliphages most times correlate with human viral pathogens in water [49] - alternates for viral contaminants in drinking and recreational waters[49, 50] - Specific phages like phiX174 commonly used in lab studies due to host specificity [50]

Viral indicators have shown inconstant but commonly robust performance across diverse global regions, indicating changes in environmental circumstances and infrastructure. crAssphage has been detected in sewage and water sources across both high-income countries such as the United States, Australia, and Spain, and low- and middle-income countries including South Africa and Nepal. Though their detection rates appear higher in Western and other high-income localities (45–66% in fecal specimens against low detection rates (around 20–40%) recorded in many Asian and African countries [45, 46, 55]. This discrepancy may be attributable to variation in human gut microbiota shaped by diet, lifestyle, and ecological factors, as well as the diversity of crAssphages and bacterial hosts, which may vary more in low and medium-income countries as a result of evolutionary pressures and environmental niches. In spite of the variations, crAssphages and coliphages are sensitive and specific markers of human fecal pollution in different settings, showing stable persistence in wastewater and surface waters [55].

Real-world use of crAssphage as a viral indicator demonstrated its robust potential for detection and tracing of human fecal pollution in many water bodies and surfaces. For example, crAssphage was recorded in over 70% of stool samples collected from the norovirus outbreak community, hands, and environmental surfaces in affected facilities, demonstrating its practicality for monitoring pollution in outbreak environments and managing hygiene interventions. Similarly, long-term studies in a UK river and estuary indicated the presence of crAssphage in both treated and untreated wastewater samples, surface waters, and sediment samples, with steadily 1–5 log10 higher concentrations than those of human enteroviruses like norovirus and adenovirus [67, 82-84]. It is extensively detected across environmental matrices and lacks a robust seasonal pattern. indicate its stability and reliability as an indicator for wastewater-derived contamination and viral contamination risk assessment.

Challenges of Viral Detection in Water

While scientists have achieved milestone progress on detection of pathogenic viruses in water, several challenges persist, including viral infectivity assessment, environmental inhibitors in water, low viral concentration in water, heterogeneous distribution of viruses in water, viable but not culturable viruses, and the threat of emerging viruses [7, 57-60].

Viral Infectivity Assessment

Detection of viral genomic material in water does not automatically indicate that the virus is pathogenic. While there are many sensitive and specific methods for detecting viruses in water, they cannot distinguish between infective and non-infective viruses, making the correlation of the detection with the actual infectivity problematic [6]. The culture-based methods remain a better standard for the assessment of the viral infectivity. However, the culture-based techniques are labor-intensive and may not be applicable to all virus types, especially the non-cultivable ones. Molecular-based methods like PCR and qPCR are commonly employed for detection of viral genetic material in water samples. These methods are highly sensitive and specific to detect non-infectious or viable but non-cultivable viruses but have notable limits. A positive PCR outcome does not essentially mean the detected virus caused infection; this can lead to false or biased interpretations regarding the public health threat of the water [32].

The emerging methods, such as integrated cell-culture PCR, that offer the advantage of molecular detection and viral infectivity assessment are complex and provides no reliable results, especially in diverse viral communities [61]. The 3D cell culture methods are newly developed advanced systems for certain viruses but are yet to be commonly accessible and may be impractical for repetitive water assessment. Their applications also necessitate certain conditions and proficiency that may not be available in several laboratories [60].

Environmental Inhibitors

Environmental Inhibitors: The occurrence of carbon-based matter such as humic acids and suspended solids,

chemical substances, and other pollutants in water can interfere with viral detection in water, especially with the PCR techniques, to give false negative results. Higher content of these matters in the water interferes with nucleic acid extraction during molecular detection, thereby reducing the sensitivity of the method and thus complicating the accuracy of the assessment [10, 58]. Some viral particles can get adsorbed onto particles in water; this makes them less available for detection and compounds the concentration process needed for efficient viral detection [40].

Similarly, temperature exhibits a substantial effect on the persistence of viruses in water and their viability at any given time. At low temperatures, their viability becomes prolonged, while higher temperatures have a tendency to hasten their inactivation. Rotaviruses, for example, have been demonstrated to persist in water for up to 10 days at 20°C and for approximately 32 days when the temperature falls to 4°C [62]. The temperature-facilitated viral inactivation rate varies for many viruses. Researchers have indicated that enteric viral populations, mainly poliovirus and hepatitis A virus, decreased significantly at elevated temperatures, showing a reduction of 1.6 log10 at 4°C over time [49, 62]. These factors are critical for the improvement of monitoring strategies and safeguarding the accuracy of the data generated from detection methods.

Low Viral Concentration in Water

The detection of viral pathogens in water is significantly hindered by the inherently low concentrations at which these viruses typically exist in rivers, lakes, and drinking water. Large volumes of water must be collected for analysis. This complicates routine monitoring needed for water quality assessment. It is also labor-intensive and increases the cost of monitoring [57]. While viruses can exist in measurable numbers in wastewaters and sewages, their concentration in treated water or surface water bodies may be undetectable. Their detection in treated waters may require collection and processing of thousands of liters of water that need to be concentrated to have sufficient virus for analysis [58, 62]. Although many techniques such as ultrafiltration and electromagnetic techniques have been established for the concentration of viral particles from large volumes of water, adopting such techniques can be time- and labor-intensive, not cost-effective, and yield inconsistent results across sample types [60, 63]. Important viral pathogens may also be lost during the concentration process, thereby reducing the integrity of the result obtained [57].

Heterogeneous Distribution of Virus in Water

Generally, pathogenic microorganisms, including viruses that find their way into water bodies, are unevenly distributed within such water bodies. This arises from the dynamics of water flow, currents, sedimentation, the presence of organic matter, the source of the contaminants, and the physical and chemical properties of the water itself [64]; for example, zones close to the origin of the contaminants may harbor more viral concentrations than other parts of the water. This means that samples collected at a single or few points will not precisely exemplify the complete viral population of the water, hypothetically producing variations in their detection rates and giving false negative results. Thus, several sampling points across the water bodies are necessary to correctly ascertain the viral population of any given water. However, this escalates the complexity and cost of the monitoring, making it more challenging for routine assessments.

The viral population of water bodies may fluctuate over time due to influences from rainfall, temperature variations, and recreational use of water. For instance, heavy rain can introduce higher amounts of viruses into surface water bodies via runoff waters. To adequately overcome these temporal variations, a more recurrent sampling is needed, straining the resources and complicating data collection and interpretation.

Threats of Emerging Viruses

The emergence of new viruses occasionally witnessed has an effect on the detection of viruses and the use of viruses for assessment of water quality. As new viruses emerge, principally those linked to waterborne infections, the requirement for updating the monitoring frameworks turns out to be more critical. The advent of SARS-CoV-2 and other respiratory viral strains has stressed the need to widen the scope of water quality assessment above the conventional enteric viruses. Many respiratory viral strains have been reportedly shed in feces and persevered in aquatic milieus; this necessitates their inclusion in routine monitoring [23]. This expansion may lead to complications of the prevailing monitoring frameworks that mainly consider only the enteric viruses.

The frequently used viral indicators, like the phages, may not sufficiently embody the incidence or threats of emerging viral strains. For example, coliphages can serve as indicators for fecal contaminants but do not essentially correlate with the existence of the emerging viruses, which may exhibit varying environmental tenacity and infectivity profiles [9, 45, 65]. This restriction increases anxieties about sole reliance on established viral indicators to assess water safety.

CONCLUSION AND FUTURE PERSPECTIVE

The incorporation of pathogenic viruses into current indicator system can essentially improve water quality monitoring. This will offer a further detection of microbial threats in water, including dogged viruses that are capable of evading traditional treatment practices. By tackling the deficiencies of bacterial indicator system, viral incorporation does not only brace confidence in water supply systems but also serve as a support for the

resilient environmental management amid the challenge of climate change and infectious disease threats.

Rigorous monitoring of viruses in water is crucial for foiling waterborne disease outbreaks and formation of public health policies. While recent techniques like the metagenomics, molecular, and biosensor-based methods have enhanced viral detection, Hurdles of differentiating infectious from non-infectious viruses and in surmounting environmental-based inhibitors like organic matters persist. These challenges underscore the need for consistent, routine viral surveillance to secure human health, mainly in vulnerable regions with insufficient water treatment practices and regions that rely on untreated groundwater.

Though this work advocates use of viral indicators of fecal contamination, it presented limited data from Southeast Asia and underrepresented environments like groundwater. This is concerning, because these contexts are specifically susceptible to fecal contamination as a result of hasty urbanization, poor wastewater treatment system, and reliance on crude groundwater. This gap risks underrating viral prevalence and weaken public health protection. Research should prioritize systematic monitoring of viral indicators in these settings using standardized molecular and biosensing methods. Such efforts will create robust datasets to safeguard water quality and context-specific management strategies

Future studies should lay emphasis on simplification and standardization of detection machineries to enable their routine application in different environmental settings. Combination of molecular and culture-based techniques with in vivo and in vitro models can boost assessment of viral pathogenicity, host interfaces, and zoonotic potentials.

To fully incorporate viral indicators to water quality valuation, regulators should embrace viral indicators along with bacterial indicators, homogenize detection techniques, and employ them to assess treatment performance and contamination events. Funding of capacity buildings, stakeholder's training, and sustained research teamwork is vital for refining assessment frameworks. Overall, there is need for a coordinated global effort to close data gaps, reinforce public confidence in water safety, and protect communities from waterborne viral threats.

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