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Viruses as Indicators of Fecal Pollution in Aquatic Environment

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ABSTRACT

This review was presented to suggest if viruses could be used as fecal indicators for drinking water and treated sewage pollution. The failure of bacterial indicators in a lot of cases to indicate viral pollution in drinking water and treated sewage indicates that bacterial indicators may fail to express fecal pollution in drinking water and treated sewage. We could explain this statement as viruses, especially enteric viruses are generally secreted in the feces of infected or carrier persons. Thus, it always expresses the fecal contamination in sewage and consequently water. Hence, the defect of bacterial indicators to indicate the enteric virus's presence in a lot of cases may indicate failure in expressing the fecal pollution, which is the cause of viral contamination of sewage and water. Adding at least one viral indicator besides bacterial indicators can help supply more perfect water quality results and greater assurance of water quality safety. Adenoviruses and bacteriophages may represent suitable candidates as indices of viral and fecal pollution indicators in drinking water and treated sewage samples.

INTRODUCTION

Water is necessary for life; however, till now a huge number of people can't attain safe, clean, and healthy drinking water, and many cases of death are caused by the contamination of water by pathogenic organisms. Over fifty severe illnesses can be caused by contaminated water, including infectious diseases, skin diseases, digestive sickness, respiratory diseases and cancer (**Wen et al., 2020**). Drinking water safety is a significant public health hazard; as of 2022, approximately two billion people live in countries with extreme water deficiency, as the result of population expansion and climate change. The drinking water sources of over two billion people were contaminated with waste, and 829 000 people annually die from diarrheal diseases caused by unhealthy drinking water, deficiency of sanitation, and poor hand hygiene (**WHO, 2022**). Hospitals, industries considered, decontamination stations, outlets of wastewater treatment plants, and storm drains are considered the most common sources of pollution from natural water

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sources. Some studies show the relation between urban activities and pathogens concentrations (Marsalek & Rochfort, 2004; Selvakumar & Borst, 2006). There are two sources of fecal contamination; the first source is related to human activities such as non-collective sewage systems, water sewage treatment plants, and combined sewage overflow. The second source is manure spreading by wild and domestic animals (Jung *et al.*, 2014). Most studies have found that gastroenteritis diseases occur at higher rates than other diseases (EPA, 2012). Enteric viruses and enteric bacteria have been the most causes of waterborne gastroenteritis diseases (Said *et al.*, 2003; Kay *et al.*, 2007). Because of the difficulties of methodology, enumeration of pathogens (viruses, bacteria, parasites), and a low dose of infectivity to occur the infection, the environmental and health authorities have been used as micro-bioindicators to assess the water's quality and the performance of treatment process (Bartram *et al.*, 2001).

Indicator term can be used to refer to the index and the indicator function (**Bartram** *et al.*, **2001**), in which the index is linked to the presence of microorganism surrogate or material (pathogens, age, fecal remnants); on the other hand, the function of indicator includes features such as stability under the environmental conditions and treatment processes resistance (**García- Aljaro** *et al.*, **2019**). Fecal indicators bacteria (FIB) were first introduced in the 1880s to determine the quality of water when bacteriological media were used to show the microbial presence in food and water (**Ashbolt** *et al.*, **2001**). FIB covers all the roles of indicators, but now viruses are the most resistant to treatment operation and more survival in the environment than the FIB (**Grabow**, **2001**; **García- Aljaro** *et al.*, **2019**); in addition, FIB fails to detect fecal contamination sources (**Malakoff, 2002**). There is no common indicator, only a wide range of indicators with certain features (**Ashbolt** *et al.*, **2001**).

This review aimed to suggest if viruses could be used as fecal pollution indicators in drinking and treated sewage water samples.

1. Enteric viruses in the aquatic environment

Many groups of enteric viruses, enteric bacteria, and protozoa are transferred by water (Liste *et al.*, 2000). About 150 several serological types of viruses including, adenoviruses (AVs), noroviruses (NoVs), rotaviruses (RVs), enteroviruses (EVs), and polyomaviruses (PVs) cause a variety of diseases. (Hamza *et al.*, 2009; Rodriguez-Lazaro *et al.*, 2012; Tran *et al.*, 2015; Adriaenssens *et al.*, 2019). Enteric viruses replicate in the human and animal intestinal tract; they can tolerate the gut acidic pH, the alkaline activity, and the duodenum proteolytic activity (Greening & Cannon, 2016; Katayama & Vinjé, 2017). Moreover, enteric viruses have high resistance to environmental conditions (temperature, light, salinity), water, and wastewater treatment processes (Gibson, 2014; Kauppinen *et al.*, 2018; Sekwadi *et al.*, 2018). Enteric viruses are host-specific and can be used to distinguish between human and animal sources of fecal contamination (Jiang *et al.*, 2007; Silva *et al.*, 2011). Fecal-oral route is the main

route for the transmission of the enteric virus by contaminated food or water, in addition, to the direct contact with infected individuals (Koopmans & Duizer, 2004; Katayama & Vinjé, 2017). Many diseases are caused by enteric viruses such as conjunctivitis, respiratory infection, non-bacterial gastroenteritis and hepatitis (Lenaerts *et al.*, 2008; Okoh *et al.*, 2010; Fewtrell & Kay, 2015; Graciaa *et al.*, 2018). Some enteric viruses can excrete in infected individuals' feces with a range of up to 10¹¹ viral particles per gram of stool (Bosch, 1998), as well as very low infectious dose (1-10) viral units (Leclerc *et al.*, 2002). Rivers, seawater, and soil can be contaminated by enteric viruses through defects in the wastewater treatment process (La Rosa *et al.*, 2012).

Rotaviruses are the main causative agent of gastroenteritis in kids under the age of five years, with 258 million diarrhea cases (Crawford et al., 2017). There are 111 million diarrheal cases, and about 2 million children hospitalized during the year (Wang et al., 2016; Badur et al., 2019). Rotaviruses are the most RNA enteric viruses detected in rivers and raw sewage water. They are the most resistant RNA enteric viruses to treatment operations in water and wastewater treatment plants (Kukkula et al., 1999; Borchardt et al., 2004; Verheven et al., 2009; El-Senousy et al., 2013a, 2015; El-Senousy & Abou-Elela, 2017). Adenoviruses are the main cause of many diseases, including conjunctivitis, respiratory disease and gastroenteritis (Chitambar et al., 2012). Fever, vomiting, and diarrhea are all symptoms of pediatric gastroenteritis caused by the adenovirus F 40 and 41 serotypes, which is considered the second major causative agent of gastroenteritis in kids after rotaviruses (El-Senousy et al., 2013a; Ogorzaly et al., 2013). In addition, adenoviruses are the main pathogens associated with severe childhood pneumonia (Jonnalagadda et al., 2017). Adenoviruses were detected in drinking water and sewage samples worldwide (Lee & Kim, 2002; He & Jiang, 2005; Verheyen et al., 2009; El-Senousy et al., 2013a; Quintão et al., 2021). Astrovirus is also one of the causes of gastroenteritis (Gofti-Laroche et al., 2003). They follow rotavirus as a major cause of diarrhea in both young and adults (Liste et al., 2000; Macdonald et al., 2015). Astroviruses were also found in sewage and drinking water samples (Abad et al., 1997; Kukkula et al., 1999; El-Senousy et al., 2007; Meleg et al., 2008). Noroviruses are the causative agent of gastroenteritis infections, with 200,000 deaths and 685 million diarrheal cases (Katayama & Vinjé, 2017). Noroviruses were detected in different water types such as treated sewage and drinking water (Kukkula et al., 1999; Borchardt et al., 2004; Meleg et al., 2008; El-Senousy et al., 2013b, 2014). Papillomaviruses and human polyomaviruses have been detected in infected individuals' feces and urine (Rachmadi et al., 2016). Certain polyomaviruses have been detected in seawater, wastewater, river and sediment (Fratini et al., 2014; Di Bonito et al., 2015; Hamza & Hamza, 2018; Samir et al., 2020). Some enteric viruses were detected in water and sewage samples, such as the hepatitis A virus (Borchardt et al., 2004; El-Senousy et al., 2004; Ouardani et al., 2016; Rachida & Taylor, 2020). Enteroviruses (Donaldson et al., 2002; Lee and Kim, 2002; Borchardt et al., 2004; El-Senousy et al., 2004; Ehlers et al., 2005; Tiwari and

Dhole, 2018) were also detected. Other enteric viruses that are considered accusative agents of gastroenteritis have been detected in contaminated rivers and wastewater, with lower titer such as bocaviruses, torque teno virus, and human picobirna viruses (Haramoto *et al.*, 2008; Symonds *et al.*, 2009; Hamza *et al.*, 2011; Adriaenssens *et al.*, 2018).

2. Microbial indicators for water quality

Observing all pathogenic microorganisms in aquatic environments needs a great effort because of the large variety of pathogens that present (bacteria, viruses, and protozoa), the methods required for concentration, analysis, culturing of many pathogens, and the difficulty of identifying, besides the existence in low titer in aquatic environments. On the other hand, detecting only one pathogen can give a false impression if another pathogen not tested is present (Scott et al., 2005; Stoeckel & Harwood, 2007). The water quality microbiological indicator selection is the process to select one species or group of microorganisms transferred to water through the feces of infected individuals but can be easily detected to measure than other harmful pathogens that pose to risk human health (Berg, 1978; Bosch, 2007). The perfect indicators are detected whenever a pathogen is present (Payment et al., 2003). The destruction and removal of indicators against the target pathogen have an important role in the selection of any indicator system (Berg, 1978). There is no unique indicator, but a variety of indicators with a specific characteristic. The difference between these indicators is controlled by a wide range of factors that affect their ability to be stable and transport through the environment, such as the size, tolerance to environmental factors, abundance in feces, and the nature of the hydrological process (Anderson et al., 2005; Yates, 2007). The ideal indicator should include the following characteristics: (i) should be completely related to the origin of the pathogen (specific to a host species) and must be absent in non-contaminated areas, (ii) detected with concentration higher than pathogens concentration, (iii) very simple for detection and quantification (easy, cheap, rapid methods), (iv) does not replicate outside the host, (v) related with human diseases, (vi) high abundant in feces of host individuals, (vii) more resistant than pathogen to environmental factors (persistence, survival, fate, transport, temp, pH, salinity, light) and disinfection processes in water and wastewater treatment, (viii) must not be pathogenic (safe for those who are monitoring) (Bosch, 1998; Walker et al., 2020).

2.1 Fecal indicator bacteria (FIB)

FIBs are used to determine the fecal pollution in different aquatic environments which are related to other pathogenic intestinal bacteria. Total coliform, fecal streptococcus, fecal coliform, and *E. coli* are used to assess contamination since they are easy and low-cost to detect (Bitton, 2005; Fong & Lipp, 2005; Fong *et al.*, 2010; Tawfik *et al.*, 2012; El-Senousy *et al.*, 2013a; Ogorzaly *et al.*, 2013). The first time that fecal coliform was

recommended as FIB by the **EPA** (1976), it was used to detect pathogens in recreational waters (**Cabelli** *et al.*, 1983; **Dufour**, 1984). The coliform group includes *E. coli* and *E. aerogenes* which are found in contaminated water by infected individuals' feces. *E. coli* provides a good indicator for fecal pollution (**Messner** *et al.*, 2017). The correlation between the pathogens and FIB can be changed in an aquatic environment due to a wide range of parameters such as the environmental survival of pathogens, dilution, and water flow characteristics (**Devane** *et al.*, 2014; **Boehm** *et al.*, 2015; **Ahmed** *et al.*, 2018; **Nelson** *et al.*, 2018). According to WHO, the acceptable levels of *E. coli* and coliform bacteria must be null for 100 ml of water and about 126 CFU/100 ml for recreational and domestic water (**Gunda & Mitra, 2016**).

2.2 Correlation between FIB and enteric viruses

Globally, E. coli and Enterococci are the most common fecal indicators used; when compared with viruses, they are less tolerant to environmental conditions, like UV irradiation, sun irradiation, pH and temperature (Gerba et al., 1979; Wyer et al., 1995; Borchardt et al., 2004; Harwood et al., 2005). Enteric viruses have been detected during the drinking water and wastewater treatment processes, with higher incidence rates than bacterial indicators; furthermore, they are also greater persistent in the aquatic environment (Kim et al., 2009; Staley et al., 2012; Lin & Ganesh, 2013; Prez et al., 2015; Sidhu et al., 2017). Many environmental studies reported that, there is no relationship between FIB and human enteric viruses (Baggi et al., 2001; Kageyama et al., 2003; Haramoto et al., 2007; Espinosa et al., 2009; Kitajima et al., 2009; Jurzik et al., 2010; Kuo et al., 2010; Simmons et al., 2011; Wu et al., 2011; Flannery et al., **2012**). The defect of FIB in a lot of cases to detect viral contamination in drinking water and treated sewage samples indicates that the bacterial indicators may fail to express fecal pollution in treated sewage and drinking water. We could explain this statement as viruses, especially that enteric viruses are usually excreted in infected or carrier persons' feces, thus it always expresses the fecal contamination in sewage and subsequently water. Consequently, the failure of FIBs to indicate the enteric virus's presence in a lot of cases may indicate failure in expressing the fecal pollution which is the cause of viral contamination of sewage and water. The addition of at least one viral indicator besides bacterial indicators can help providing more adequate water quality results and more trust in the safety of water quality (Toribio-Avedillo et al., 2021). The drinking water quality standard, which was issued by WHO includes twenty-eight microbiological indicators; it contains eight kinds of viruses, twelve kinds of bacteria, six kinds of protozoa, and two kinds of parasites (WHO, 2011). It is agreeable that, FIB concentration above the level of detection is supposed to detect fecal contamination. However, the detection of FIB to evaluate the effect of pathogenic contamination in natural waters is difficult because this FIB may multiply in the natural aquatic environment under suitable conditions (Ishii et al., 2006; Vogel et al., 2007). Moreover, it's not easy to distinguish between the source of fecal pollution origin as human or animal-infected individuals by using bacterial indicators. Additionally, the incidence of some pathogens, such as adenoviruses, human enteroviruses, *Giardia* spp., Salmonella spp., *Cryptosporidium*, and coliphages are more stable than FIB in aquatic environments; thus, the detection of FIB in different types of waters does not indicate the incidence of pathogens (Bonadonna *et al.*, 2002; Payment & Locas, 2011; Sidhu & Toze, 2012). One of the most important tasks is to identify the fecal contamination source markers (Tran *et al.*, 2015).

3. Viral indicators for water quality

The optimal viral indicator must have similar stability, and higher resistance to environmental conditions or treatment processes than the pathogen, and it must be detected throughout the year in aquatic contaminated environments. Furthermore, during a viral outbreak or pandemic, the viral indicator can determine the ratio of infected people (Xagoraraki & O'Brien, 2020). Adenoviruses are used as viral indicators for contamination to monitor water quality due to their higher persistance in the environment compared to the FIB (Simmons *et al.*, 2011; Rachmadi *et al.*, 2016; Messner *et al.*, 2017; El-Senousy, 2021; Rashed *et al.*, 2022). Norovirus can be used also as a viral indicator because of its higher resistance to treatment processes (Duizer *et al.*, 2004; Jimenez & Chiang, 2006), high persistance in the environment, long-term stability (Wu *et al.*, 2005; D'Souza *et al.*, 2006) and a low dose of infection (Teunis *et al.*, 2008). NoV has also been reported in recreational water (Maunula *et al.*, 2004; Sartorius *et al.*, 2007) and in many outbreaks of contaminated drinking water (Maunula *et al.*, 2005; Hewitt *et al.*, 2007). The viral indicators for water quality can be classified into two types:

3.1 Viral indicators used as fecal pollution indicators

Enteric viruses are considered a promising fecal pollution indicator due to their host specificity and prevalence in host feces (Sidhu & Toze, 2009; Payment & Locas, 2011; Tran *et al.*, 2015). In addition, they can differentiate between human or animal fecal pollution sources by identifying the sequence of common genes in the genus (Fong *et al.*, 2005; Ahmed *et al.*, 2010). Human adenovirus and polyomavirus (JC and BK) have been suggested as fecal indicators and targets for microbial source monitoring markers based on their distribution in the population $(10^3 - 10^7 \text{ gc/l})$, high resistance to environmental factors, and human host specificity (Pina *et al.*, 1998; Albinana-Gimenez *et al.*, 2009; Ahmed *et al.*, 2010; Wolf *et al.*, 2010; Wyn-Jones *et al.*, 2011; McQuaig *et al.*, 2012; Hewitt *et al.*, 2013; Liang *et al.*, 2015). De Giglio *et al.* (2017) detected enterovirus, rotavirus, and norovirus (fecal pollution indicators) in groundwater. On the other hand, FIBs were poor to indicate the presence of viruses in groundwater. Many studies suggested coliphages as an adequate fecal indicator in several types of water according to their characteristics, occurrence, fate, and epidemiological relationship in the

environment (**Blanch** *et al.*, **2006**; **Lee** *et al.*, **2011**). F-specific RNA coliphages (F - RNA) were suggested to express fecal pollution in groundwater and surface water. Moreover, by using their genotyping or serotyping groups, it is easy to identify the source of fecal contamination (**Havelaar** *et al.*, **1993**). In addition to the results of some studies subgroups I and IV of F - RNA are typically related to animal feces and subgroups II and III are correlated with human fecal pollution (**Ibarluzea** *et al.*, **2007**; **Lee** *et al.*, **2011**).

3.2 Viral indicators used as surrogates for viral pollution

Aichi virus (AiV1) has been found at a higher concentration in wastewater and the environment, compared to NoVs due to the morphological and prevalence similarity (Hata *et al.*, 2013; Kitajima *et al.*, 2014). AiV can be used as a viral indicator to detect viral contamination in different types of waters (Kitajima & Gerba, 2015). Garcia *et al.* (2022) suggested that adenovirus, pepper mild mottle virus (PMMoV), and crAssphage may be used as viral indicators. Some studies showed that many groups of coliphages have similarities in structure, morphology, size, persistence, and survivability in the environment to enteric viruses when compared to FIB (Cole *et al.*, 2003; Love *et al.*, 2008). The removal of somatic coliphages is still regarded as a reflection of the elimination of human viruses, while the use of F-specific coliphages as human viruses index is restricted (Ottoson *et al.*, 2006). Havelaar *et al.* (1993) suggested that F- RNA coliphages are a suitable microbial marker for human viral pollution in the aquatic environment. PMMoV was suggested as a viral indicator that was detected with high concentrations to evaluate enteric viruses' detection in different types of water. (Hamza *et al.*, 2011; Kitajima *et al.*, 2018; Garcia *et al.*, 2022).

4. Survival of viral indicators in the environment

Viruses are obligatory host-specific and cannot multiply outside their hosts, thus the viral particles may survive or be damaged when they are suspended in the environment; this means that the viral concentration will be the same or decreased (**Pinon & Vialette, 2018**). The effect of these factors changed according to the type of environment (**Rzeżutka and Cook, 2004; Pinon and Vialette, 2018**). Enteric viruses in the soil can persist for more than 100 days at 20 to 30°C, up to 120 days in fresh water and sewage, and up to 130 days in seawater (**Wetz** *et al., 2004*). **Kocwa-Haluch (2001)** showed that with a wide range of pH (3 to 10) and low degrees of temperatures enteric viruses can persist for a long time. Rotavirus is one of the more persistent viruses in aquatic environments; it can survive for 16 days with a 2-log₁₀ reduction in the initial count, using cell-based techniques for the detection of the virus in unpolluted lake water (**Pancorbo** *et al., 1987*). **Espinosa** *et al.* (2008) reported that, the survival of rotavirus by using quantitative polymerase chain reaction in surface water and groundwater samples may reach 4-log₁₀ and 3-log₁₀ reduction, respectively, in periods ranging between 150 to 180 days. Many environmental conditions such as temperature, sunlight, and salinity

have an impact on viruses' survival in the environment (**Rzeżutka & Cook, 2004; Pinon & Vialette, 2018**).

a. Temperature

The biological processes such as occurrence, penetration, attachment, viability, and multiplication depend on the degree of environmental temperatures (Sobsey & Meschke, 2003; Jończyk et al., 2011). High temperatures may destroy nucleic acids, and the viral capsid protein, or inactivate the enzymes of replication (Bitton, 1980). The effect of temperature can increase the activity of viral cells at ambient temperature, but decay occurred rapidly at higher temperatures while at a low temperature above freezing (Shahid et al., 2009; Paluszak et al., 2012). Several studies reported that enteric viruses and coliphages have been surviving for long periods in natural environments at lowtemperature degrees and rapid inactivation at higher temperature degrees (Long and Sobsey, 2004; Fong & Lipp, 2005). Abad et al. (1997) noted that, the logarithmic reduction of astroviruses in drinking water can reach $2 \log_{10}$ for 30 days at 20°C and 60 days at 4°C. Adenovirus can be persisted in groundwater for 132 days at 4°C, when the temperature increases to 20° C, the decay occurred more rapidly in 36 days with the same reduction (1 log₁₀) (Ogorzaly et al., 2010). Porcine rotavirus and MS2 coliphage have low inactivation rates constant at temperatures ranging from 14 to 42°C, the inactivation rates increased 10-fold when the temperature increased to 50°C (Romero et al., 2011). Seo et al. (2012) examined the log_{10} of MS2 coliphage and murine NoV at a range of temperatures between 24 to 85°C. The result showed that the reduction of MS2 coliphage was lower than murine NoV at the range of 24°C and 60°C, while both viruses inactivated rapidly at temperatures higher than 60°C.

b. Sunlight

In addition, sunlight is the most common factor in virus inactivation. In dark conditions, coliphages and enteric viruses have lower reduction than in sunlight conditions (Sobsey & Meschke, 2003; Fong & Lipp, 2005; Jończyk *et al.*, 2011). The main composition of sunlight, besides the visible light is UV light that is responsible for the damage of the genetic materials of the viral cell by forming pyrimidine dimers or other photo products (Lytle & Sagripanti, 2005; Love *et al.*, 2010; Silverman *et al.*, 2013). Johnson *et al.* (1997) observed that, the inactivation of polioviruses increased to $3\log_{10}$ when exposed to sunlight than dark after 24 h incubation in the marine environment. Sinton *et al.* (2002) reported that, the reduction rates of bacteriophages are ten times higher in sunlight conditions than in dark conditions. Under sunlight conditions, the reduction rates of somatic coliphages, poliovirus type 3, and F-DNA phages were equal to or higher than the reduction rates of F-RNA phages and adenovirus type 2 in seawater (Love *et al.*, 2010). Silverman *et al.* (2013) observed that, the GI of F-RNA phage reduction rates were equal to or less than adenovirus type 2 and significantly below poliovirus type 3 in

all examined waters under sunlight and dark conditions. This study emphasized that the reduction rates of dark conditions were less than the reduction rates of sunlight conditions.

c. Salinity

Salinity has an impact factor on the reduction rates of enteric viruses by increasing or decreasing, according to the salt concentration, the salt type, temperature, and the specific viruses found (Nguyen *et al.*, 2011; Seo *et al.*, 2012). The salinity effect depends on monovalent salts that provide strong steric and electrostatic stabilization that have a strong inactivation effect by aggregating all the particles of viruses (Mylon *et al.*, 2010; Nguyen *et al.*, 2011). Hurst and Gerba (1980) showed that, the results of reduced rates of simian rotavirus, coxsackievirus, poliovirus, and echovirus, in fresh and estuarine water for two different years were more rapid in estuarine water than in fresh water in one year, and become similar in the second year. Seo *et al.* (2012) observed that, MS2 RNA coliphage was more resistant to NaCl than murine NoV under different concentrations of NaCl at several temperatures ranging between 24°C to 50°C.

5. Correlation between viral indicator and pathogen

The correlations between the indicator and pathogen detection are controlled by some factors, such as detection methods, sample size, number of positive samples of pathogens, and pathogen sources. Furthermore, it might be difficult to assess the health hazards of decisions based on the results of indicators (Wu et al., 2011). The fundamental objective of the viral indicator is to serve as a monitoring system for detecting pathogens. The indicator should be detected at an equal or greater number than the pathogen. Many studies' results have been used to help find the suitable viral indicator correlated with the pathogen. Payment and Franco (1993) reported a significant correlation between enteric viruses and Cryptosporidium oocysts, Giardia cysts, and C. perfringens counts. Also, the study showed that somatic coliphages and C. perfringens can be used to assess the virological and parasitological quality of treated drinking water. Detection of enteric viruses' genomes has been easy than the isolation by cell culture such as the detection of the enterovirus genomes Furthermore, the results of detecting viral contamination in surface water showed that somatic coliphages were not suitable for the detection of viral contamination and pathogens (Hot et al., 2003). Another study done by Ottoson et al. (2006) represents the significant relation between coliphages, enterococci, and E. coli in untreated wastewater and no correlation between pathogens reductions and indicators (P>0.05). Total coliphages can be used as a viral index indicator rather than using Fspecific phages. This study agrees with other studies that suggested using viral indicators for pollution detection, for example, polyomaviruses (Bofill-Mas et al., 2000), adenoviruses (Pina et al., 1998), and enteroviruses (Hot et al., 2003). Tonani et al. (2013) found that no statistical significance in *Cryptosporidium* count decreases with

pathogens; besides, there is a significant decrease in the count of adenovirus, rotavirus, and *giardia* (*P*<0.05). Additionally, there was no significant seasonal detection observed in protozoa (oo) cysts distribution in the collected sewage samples. **Tian** *et al.* (2017) reported that, the human NoV rate was detected in all positive and negative bacterial pathogens samples without any positive relationship between the incidence of human NoV and pathogenic bacteria (Listeria, Salmonella, O157 *E. coli* STEC and non-O157 STEC).

As a result, the study of **Tandukar** *et al.* (2018) on 8 viruses (human adenoviruses, rotavirus A, Aichi virus 1, human cosaviruses, enteroviruses, caliciviruses, and noroviruses GI and GII) found a positive relationship between human enteric viruses and theses viruses (P < 0.05); no positive correlation was detected between FIB and *Cryptosporidium* or *Giardia* (P > 0.05). Furthermore, the detection ratio of fecal markers was lower than the human *bacteroidales*, besides that these fecal markers have a significant relationship with human enteric viruses. Finally, this study suggested that the use of the viral index, bacterial indicators, and human *bacteroidales* could be used as good indicators for human fecal contamination detection in rivers.

Another study in 2020, compared four water-borne enteric viruses (enterovirus, astroviruses, hepatitis A virus, and rotaviruses) with fecal bacterial and bacteriophages indicators of fecal pollution in wastewater treatment plants. The incidence rates in influent samples of EV, AV, HAV, and RV were 100%, 75%, 12.5%, and 12.5%, respectively; however, enteroviruses RNA was detected in half of all the outlet samples. The positive samples of the enteric virus had a high concentration of bacteriophages in inlet and outlet samples. The most abundant phages in the samples were *E. coli* phages, which had titer ranging between (7-8) log pfu/ml. The fecal bacteriological indicators were detected in high concentrations in all outlet samples: 1.92×10^3 cfu/ml, 1.32×10^3 cfu/ml, and 3.20×10^3 cfu/ml for shigella spp., salmonella spp., and *E. coli*, respectively. According to these results, a positive relation was recorded between the detection and cultivation of pathogenic bacteria (salmonella, fecal coliform, and *E. coli*), and the detection of EV and their specific bacteriophages. Thus, the study introduced the non-pathogenic coliphages as a good indicator for viral pollution to assess water quality (**Janahi** *et al.*, **2020**).

Bailey *et al.* (2021) found that *cryptosporidium*, adenoviruses, and *giardia* were found in 100%, 81%, and 41%, respectively, of all samples; furthermore, the incidence of total coliphage, somatic coliphages, and F+ coliphages were detected in 77%,77%, and 32%, respectively, of all samples. *E. coli* was detected in half of the samples, while total coliforms and enterococcus were found in 95%, and 64%, respectively, of all samples. This study investigated that, the presence or absence of an indicator is not always accurate in predicting the presence of pathogens in the samples, and these results noted that many cases of false-positive or negative results used only one indicator for the

detection of pathogens. Consequently, in the prediction of pathogen presence or survival in surface water, no one signal indicator was perfect in detection. This study suggested that enteric pathogens, including salmonella spp., adenoviruses, *cryptosporidium*, and *giardia* may be used as indicators for drinking water sources. From previous studies, we try to answer one question to help in determining the viral indicator that can suit for viral detection.

6. Which viral indicators are suitable?

Most viruses don't have the complete requirements to be a universal indicator. Thus, it is necessary to select the suitable indicator according to the distribution in the aquatic environment, seasonal variation, stability to environmental conditions, and resistance to the treatment process. The suitable viral indicators should have the ability for long-term detection of viral pollution in aquatic environments throughout the year (Walker et al., 2020). Papillomaviruses, coronaviruses, and influenza viruses have been detected in wastewater with high titer but not or less detected in a contaminated environment; this is due to the rapid damage of these viruses (Bosch et al., 2016). Other viruses have clear peaks during the seasons of the year, such as rotavirus peaks in autumn and winter (Villena et al., 2003; El-Senousy et al., 2004, 2013a, 2014), AiV peaks during spring and winter in wastewater (Kitajima et al., 2014), the peaks of sapoviruses and noroviruses in winter, and the enteroviruses peaks in summer (Prevost et al., 2015; **Cooper** et al., 2018). The suitable indicator must be able to distinguish between human and animal sources of contamination (Scott et al., 2002), such as zoonotic enteric viruses (hepatitis E virus, torque teno virus, rotavirus, and astrovirus), which are present due to the activities of agriculture contaminated with human wastes in the aquatic environment (Bosch et al., 2016). Human AdVs are found in polluted environments without any seasonal variation which is detected all over the year, many studies have suggested human adenovirus as an effective indicator (Kitajima and Gerba, 2015; Rachmadi et al., 2016). PMMoV has been proposed as useful viral pollution for wastewater pollution (Kitajima et al., 2018; Symonds et al., 2018). It is detected with a high concentration in wastewater samples before and after the treatment process over the year (Myrmel et al., 2015; Schmitz et al., 2016). Coliphages are usually found in high titer in several types of water and used proposed as a viral indicator to detect enteric viruses in contaminated water (McMinn et al., 2017). A result of the previous studies that suggested different viral candidates as a viral indicator for viral pollution of water and wastewater discussed the following topics:

I. Bacteriophages

Viruses that infect bacteria are called bacteriophages (phages). Phages were discovered in the early 1900s and originated from the intestinal tract of humans (d'Herelle & Smith, 1926; Ashbolt *et al.*, 2001). These phages may be detected in several environments

where the bacteria can grow, like in different types of water, soil, and can detect inside other higher individuals (Clokie et al., 2011; Dutilh et al., 2014; Dorevitch, 2016). Bacteriophages are divided into three taxonomic groups: somatic coliphages, F-specific (DNA, RNA) coliphages, and bacteriophages that can infect Bacteroides spp. (Jofre et al., 2016; Jebri et al., 2017). Bacteriophages are suggested as fecal and viral indicators for fecal contamination in several aquatic environments and assessing the viral pollution. Coliphages are phages that infect *Escherichia coli*; they have been suggested as alternatives to FIB and as a surrogate to enteric viruses to detect viral contamination. Bacteriophages infect intestinal bacteria in a similar way to enteric viruses (Hilton & Stotzky, 1973; Gerba, 1987; Sobsey et al., 1995; Chung et al., 1998; Contreras-Coll et al., 2002; Skraber et al., 2004; Mocé-Llivina et al., 2005; McMinn et al., 2017; Toribio-Avedillo et al., 2019). Bacteriophages have the most ideal features of viral indicators such as being excreted in feces and not replicating in the environment till the presence of their hosts, being stable against the environmental conditions, more distributed in the environment, giving high accuracy results (Tufenkji and Emelko, 2011).

Somatic coliphage can infect *E. coli* and coliform bacteria by adhesion to specific receptors on the cell wall of bacteria (**Muniesa** *et al.*, 2003). Several trials, laboratory experiments, and validation testing suggested that somatic coliphages such as PRD-1, phix174, T-4, and T-7 can be used as viral surrogates to enteric viruses to detect viral pollutions (**Lucena & Jofre, 2010**). Hot *et al.* (2003) showed no positive relationship between somatic coliphages and HAdVs, EVs, Norwalk I and II viruses.

F coliphage is another new approach to detect and quantify pathogens and fecal pollution in the aquatic environment (**Griffith** *et al.*, **2016**). They are recommended for use as a fecal and viral indicator of water contamination because of the similarity in shape and size to enteric viruses, detection in sewage contamination, and the difficulty to multiply outside the host in the environment (**Duran** *et al.*, **2003**). Many studies detected these phages in recreation water, groundwater, surface water, rivers, harbors and wastewater (**Yamahara** *et al.*, **2012**; **Vijayavel** *et al.*, **2014**; **Rashed** *et al.*, **2022**). **Stewart- Pullaro** *et al.* (**2006**) reported that, somatic coliphages have been found at high concentrations than male-specific phages in raw water sources and wastewater. Especially, F-RNA phages, and somatic coliphages have been demonstrated as excellent fecal viral indicators (**Jofre** *et al.*, **2016**; **Jebri** *et al.*, **2017**). HSP 40 phage can infect the *bacteroides fragilis*. *Bacteroides fragilis*, the anaerobic bacteria found with high titer in the human intestinal tract, execrated through the feces of infected individuals, and die rapidly when released into the environment. HSP 40 phage is represented as a unique indicator for fecal pollution in polluted water (**Duran** *et al.*, **2003**).

There are some disadvantages of bacteriophages that prevent them to be viral indicators such as some coliphages present in low numbers than bacterial indicators (**Payment &**

Locas, 2011). To differentiate between the origin of fecal pollution as an animal or human fecal contamination was recorded a failure (Hot *et al.*, 2003; Jiang & Chu, 2004). Some types of bacteriophages are present in contaminated water with high concentrations than other types like somatic coliphages detected in raw and wastewater with high titer than male-specific phages (Stewart- Pullaro *et al.*, 2006). In some studies, there is no relationship between viral contamination and coliphages in sewage water (Carducci *et al.*, 1999), surface water (Hot *et al.*, 2003) and groundwater (Long & Dewer, 2008).

II. Pepper mild mottled virus (PMMoV)

PMMoV is RNA plant virus that infects the leaves of a pepper plant. It is a member of Tobamo virus genus, and it is responsible for economic losses of infected pepper worldwide (Fauquet et al., 2005). Zhang et al. (2006) was the first study that identified the PMMoV in feces by using viral metagenomics techniques. PMMoV is suggested by many studies as a fecal indicator (Hamza et al., 2011; Symonds et al., 2018). It has been detected at a significantly high concentration, with a higher prevalence than enteric viruses and pathogenic viruses in human feces (Rosario et al., 2009; Hamza et al., 2011; Haramoto et al., 2013; Symonds et al., 2018), It was detected in several types of water such as surface water and sewage water (Rosario et al., 2009; Kitajima et al., 2018; Shrestha et al., 2018; Symonds et al., 2018; Tandukar et al., 2020). PMMoV is also present in wastewater in some places such as Florida, Germany, New Zealand, and Vietnam, with a range from 10^6 to 10^{10} gc/l. (Rosario et al., 2009; Hamza et al., 2011; Kitajima et al., 2014; Schmitz et al., 2016; Gyawali et al., 2019). On the other hand, it was less prevalent with a titer range between $(10^3 - 10^6 \text{ gc/l})$ in Spain, Arizona, and the UK (Kitajima et al., 2014; Rusinol et al., 2015; Schmitz et al., 2016; Cooper et al., 2018). **Rashed** et al. (2022) detected PMMoV in four samples of drinking water, with a complete absence of the infectious units of phix 174 bacteriophage virus and adenoviruses in these samples. This is due to the higher resistance of PMMoV to treatment processes (chlorine disinfection) than phix174 bacteriophage virus and adenoviruses, which can give false positive results so it cannot be used as a viral indicator for all types of waters (Shirasaki et al., 2018, 2020).

III.Adenoviruses

Adenoviruses have been considered the second viral pathogen after rotavirus which leads gastroenteritis (Fong *et al.*, 2010). They infect children less than five years (Lennon *et al.*, 2007). Ad40 and Ad41 enteric serotypes are found under species F (Rigotto *et al.*, 2011), which are responsible for most cases of gastroenteritis (Logan *et al.*, 2006). Adenoviruses sub-species B is responsible for 5–7% of conjunctivitis and respiratory diseases in kids (Wold & Horwitz, 2007). Compared to other interic viruses, adenoviruses are more resistant to environmental degradation (Hijnen *et al.*, 2006), and

ultraviolet disinfection (Linden et al., 2007). In addition, they are more resistant to pH conditions (Thurston-Enriquez et al., 2003), and chlorine in the water treatment process (Thurston-Enriquez et al., 2005; Rashed et al., 2022). They are detected in several types of water, such as drinking water, wastewater, groundwater, swimming pools, recreational waters, rivers, and polluted water (Pina et al., 1998; Bofill-Mas et al., 2006; Haramoto et al., 2007; Katayama et al., 2008; Miagostovich et al., 2008; Wong et al., 2009; Dong et al., 2010; El-Senousy et al., 2014). It's a human host specificity that cannot replicate outside of the host (Fong et al., 2005; Wong et al., 2012). Pina et al. (1998) worked on wastewater treatment plants and found that adenoviruses were detected throughout the year. On the other hand, the concentrations of fecal coliform were below regulatory standards, thus the proposed adenoviruses as a viral indicator. Another study was done by Jiang et al. (2001) on California beaches exposed to an urban runoff in which adenoviruses genomes concentrations ranged from 0.9 to 7.5×10^3 genomes/l. AdV was more resistant to water and sewage treatment processes in treatment plants than RV (El-Senousy et al., 2013a). it is important to detect the HAdV infectious units to know the recent contamination because HAdV infectious units persist in water less than the genomes (Donia et al., 2010; El-Senousy et al., 2014; Prevost et al., 2016; Rashed et al., 2022). HAdV is suggested as a viral water quality indicator by several studies (Puig et al., 1994; Pina et al., 1998; Albinana-Gimenez et al., 2006; Hundesa et al., 2006; Bosch et al., 2008; Jurzik et al., 2010; Okoh et al., 2010; El-Senousy et al., 2013a; Rames et al., 2016; Iaconelli et al., 2017; Lun et al., 2019; Ibrahim et al., 2021; Rashed et al., 2022).

IV. Human polyomavirus (HPyVs)

HPyVs are non-enveloped DNA viruses, are found under the family *Polyomaviridae* (Bofill-Mas *et al.*, 2001; Johne *et al.*, 2011), consist of five serotypes JCV, BKV, KIV, MCV, and WUV (Kean *et al.*, 2009). HPyV is mainly excreted in the feces and urine of infected individuals (Rachmadi *et al.*, 2016). HPyVs were detected in different aquatic environments such as in sources of drinking water (Albinana-Gimenez *et al.*, 2006), in river water (Haramoto *et al.*, 2010; Hamza *et al.*, 2014; Rusinol *et al.*, 2015), in drinking water sources (Albinana-Gimenez *et al.*, 2006), in wastewater (Hamza *et al.*, 2014; Kitajima *et al.*, 2014), stormwater (Sidhu *et al.*, 2012), swimming water (La Rosa *et al.*, 2012) and seawater (Moresco *et al.*, 2012). Fratini *et al.* (2014) detected that the major pathway of the infection by the HPyV by inhalation or ingestion of contaminated water with HPyV. HPyVs have been concentrated in different types of water by many concentrated methods such as virus adsorption and elution (Karim *et al.*, 2009; Haramoto *et al.*, 2010), ultrafiltration (Liang *et al.*, 2015), and skim milk flocculation (Calgua *et al.*, 2013). Also, several molecular methods detections used to detect HPyV in concentrated samples including PCR (Sidhu *et al.*, 2012), qPCR (Wong *et al.*, 2012;

Liang et al., 2015), immunofluorescence (Calgua et al., 2011), microarray and cell culture technique (Schowalter et al., 2012).

JCV and BKV were detected in drinking water sources in Spain with titer ranging between $(2.6 \times 10^1 \text{ gc/l} - 4.62 \times 10^3 \text{ gc/l})$ and $2.1 \times 10^1 \text{ gc/l}$ respectively (Albinana-Gimenez *et al.*, 2006, 2009). While detected in Japan with titer $(2.90 \times 10^2 \text{ gc/l} - 1.3 \times 10^3 \text{ gc/l})$ and $2.50 \times 10^2 \text{ gc/l}$ respectively, (Haramoto *et al.*, 2012).

McQuaig *et al.* (2009) suggested that HPyVs especially BKV and JCV serotypes are a good indicator of pathogenic viruses due to the high resistance to different degrees of temperature which is similar to AdV resistance. Also, several studies suggested HPyV as a viral indicator due to its high stability in environmental waters with little seasonality (Bofill-Mas *et al.*, 2001, 2006; Rachmadi *et al.*, 2016), resistance to UV (Nims and Plavsic, 2013; Calgua *et al.*, 2014) resistant to acidic conditions (Bofill-Mas and Girones, 2003) and more resistant to the treatment process in water and sewage plants than other types of viruses such as AdV type 2 and MNV-1 (Hata *et al.*, 2018).

CONCLUSION

From this review, we conclude some points to select the best viral indicator

• No correlation between fecal indicators bacterial and viral indicators.

• No single viral indicator can be used for the detection of pathogenic viruses in all water bodies.

• Use of one or more viruses as viral indicators according to the prevalence of these viruses in the aquatic environment for each country which can change from country to country.

• Detection of infectious units of viral indicators is necessary to determine the recent contamination than detection of genome copies which are more persistent than infectious units.

• PMMoV is more resistant to the water treatment process than other enteric viruses so it cannot be used as a viral indicator for all types of water.

• Bacteriophages can be used as viral indicators, while some studies, showed that bacteriophages do not always correlate with human enteric viruses and it is difficult to differentiate between the source of contamination (human or animal fecal contamination).

• Till now the studies suggested adenoviruses as viral indicators which are more tolerant to the water treatment process than other viruses, rapidly detecting the infectious units and easy differentiating between human and animal contamination.

• Most of the water quality criteria don't have any of the viruses to express the pollution of water and wastewater with enteric viruses so, more studies are needed to suggest one or grouped viral indicators that can be used as viral indicators.

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