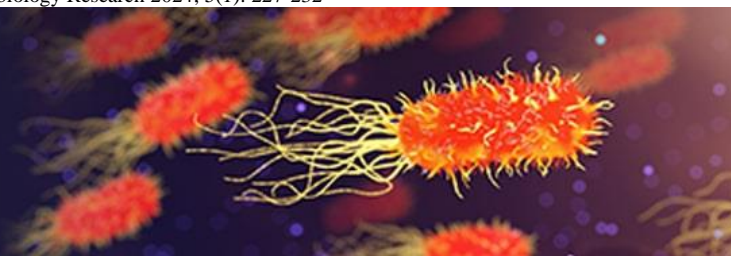


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Etiology of viral gastroenteritis and their diagnosis with recent advanced molecular techniques

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Abstract

Acute Gastroenteritis (AGE) in under 5 years' children is mainly caused by enteric viruses and the Rotavirus is most common pathogen causing viral diarrhea with the emergence of Norovirus followed by administration of Rotavirus vaccine. The causative agents of AGE have conventionally been identified with their biological markers as specific antigens and by targeting their specific genes. With recent advances in the Next Generation Sequencing, techniques like Metagenomics can play important role to discover such pathogenic enteric viruses. This overview the causes of AGE and describe a large number of recently available multi-parameter commercial test which will help in the prediction of prognosis, and virulence for the active infections and support the accurate and rapid clinical diagnosis of AGE and improve the patient condition and reduce both morbidity and mortality associated with infections, especially in children under 5 yr. of age worldwide.

Keywords: Acute gastroenteritis (AGE), enteric viruses, viral diarrhea, under-5 children, next-generation sequencing (NGS)

Introduction

Acute gastroenteritis (AGE) is the second leading cause of mortality in children under 5 yr of age worldwide, particularly in developing countries [1]. According to a WHO report, nearly 8% of under-5 deaths are due to diarrheal disease [2, 3]. In India, diarrheal disease accounts for 9.9% of mortality in under-5 children with 500,664 annual deaths in children [4]. According to National Family Health Survey (NFHS-4, 2015-2016) In India, the prevalence of diarrhea in under 5 children was 9.2% and in Madhya Pradesh (M.P) 9.5% [3, 5, 6]. Since AGE is an important cause of mortality and morbidity worldwide, many times these cases go unnoticed due to limited data representing a significant threat to public health, particularly in children under-5 of age [4, 7-10].

A wide variety of enteric pathogens causing AGE includes viruses, bacteria, parasitic, and fungi [11]. Viral gastroenteritis is the most frequent cause of mortality and morbidity in under-5 yr. of children under (36.4%) followed by bacterial (25%), parasitic (3.5%) in resource-limited regions such as low and middle-income countries [12, 13, 14, 15].

Enteric viruses that are most commonly associated with AGE belong to 4 families; Reoviridae (Rotavirus Group A), Caliciviridae (Norovirus and Sapovirus), Adenoviridae (Adenovirus 40/41), Astroviridae (Astrovirus) [16, 17, 18, 19]. Amongst these viruses, Rotavirus is the leading cause of AGE in under-5 yr children contributing to 35.5% of all cases in India [20], and around 2 million hospitalizations, and 453,000 deaths of under-5 yr children worldwide [11]. The incidence and severity of Rotavirus infections have decreased in countries that added the Rotavirus vaccine to their routine immunization schedule earlier [21, 22]. And therefore Norovirus is emerging as the second leading cause of AGE [36] responsible for 218,000 deaths in the pediatric population every year and 1.1 million hospitalizations of children over the world [37].

AGE is commonly acquired through the fecal-oral route, person to person, fomites, contaminated surfaces, poor sanitation, and unhygienic water sources [23, 24]. Though AGE is a self-limiting and preventable disease it can lead to the most frequent and dangerous complications like dehydration associated with electrolyte imbalance and acidosis with significant emergency visits, admission to hospitals, and ultimately death [13, 18]. All of these can lead to a high burden of AGE disease in developing countries where outbreaks can occur [13].

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Therefore, the identification of the most predominant circulating viral agents will be useful in the development of strategies to diagnose the disease and control the progression of the disease [13]. Routinely used conventional methods for diagnosis of AGE include electron microscopy, virus isolation, antigen detection, and enzyme immunoassay [13, 25]. These methods are labor-intensive, time taking with limited sensitivity and specificity compared to nucleic acid amplification technique (NAAT) [13]. Whereas recently developed advanced techniques are simple, rapid, and more reliable for making a diagnosis than conventional methods [26]. Thus, this review aims to study the etiology of viral gastroenteritis and its diagnosis by advanced molecular diagnostic methods that are currently available.

Methods

We performed a literature search using electronic databases search engines including PubMed, Google Scholar by using search terms; etiology, acute viral gastroenteritis and diagnosis of acute gastroenteritis. We retrieved a total of 1373 articles from PubMed and 2830 from Google Scholar. We included 37 relevant studies of both original research papers and review papers published within the past 10 years and set up a brief review of our own experience on the current status of etiology of viral gastroenteritis and recent advancement in the diagnosis techniques of age.

Etiology of acute viral gastroenteritis

The viral gastroenteritis agents are mainly divided into 4 families that are commonly associated with AGE; Reoviridae (Group A Rotaviruses), Caliciviridae (Norovirus, Sapovirus), Adenoviridae (Adenovirus 40/41), and Astroviridae (Astrovirus) [16, 17, 18]. Amongst these viruses, Rotavirus and Norovirus are the predominant cause of AGE.

Rotavirus is the leading cause of AGE in children below 5 yr of age with estimated 200,000 deaths occurring around the world, especially in developing countries, and accounts for 35.5% of diarrheal cases in India [2, 27]. The Rotavirus infection is characterized by sudden onset of vomiting followed by watery diarrhea, abdominal cramps, and fever and often lead to complication like dehydration [28]. However, in 2006 after the introduction of rotavirus vaccines Rotateq R and Rotatrix R, there was a significant reduction of 67% of rotavirus infection in developed and high-income countries [27, 29].

Further more, this changed the etiology of viral gastroenteritis with Norovirus which is more common in the USA, Europe, and other developed countries [30]. Whereas in India, under the Universal Immunization program in 2016 Rotavirus vaccine ROTAVAC was launched by the Government of India in 4 states (U.P, Haryana, H.P, Odisha with later on, in 5 additional states (Rajasthan, M.P, Assam, Tripura, Tamil Nadu) was implemented on 2017. Since it has been 7 years of the ROTAVAC vaccine was introduced in India, the effect on the current etiology of viral gastroenteritis should be assessed and monitored [30].

Most of the existing literature suggests, that Norovirus is the second leading cause of AGE. But few studies suggest Adenovirus as the second predominant cause of AGE [31]. According to the GBD study conducted in India in 2019 resulted in 8864.36 deaths per 1 lakh population caused by Adenovirus which is relatively higher than Rotavirus-associated infections causing death around 2059.74 per 1

lakh population [1]. Though these death rates are reduced due to the effective administration of the Rotavirus vaccine in the country [1].

According to (Sidoti *et al* 2015) study suggesting Human Astrovirus are the second most common cause of AGE in children with varying incidence from 4.3%-8.6% after Rotavirus [31] Astrovirus infections occur mainly in the rainy and winter season in temperate climate countries [32]. They are the cause of sporadic, community-acquired, and nosocomial infections and present with mild watery diarrhea, vomiting, abdominal cramps, and fever lasting for a short period of 1-3 days [31].

Sapovirus are the members of caliciviridae family and responsible for minority of sporadic cases of gastroenteritis in children [31] cause around 12.7% of AGE globally [33]. Sapovirus-causing gastroenteritis is less severe and mortality is rare [31]. Thus detection of the specific causative agents by the advanced methods will help in the timely management of patient treatment and reduce the chances of occurrence of comorbidities [16].

Conventional methods: Stress on limitations

Conventional diagnostic methods were the main foundation for diagnosis of viral infections in the past two decades [31]. Traditionally, these methods were depended on virus identification, isolation and demonstration of virus specific antibody response. However, these techniques were not significant in the diagnosis of viral gastroenteritis [25].

Conventional diagnostic methods include Electron microscopy (EM) based on direct visualization of the virus. Since the method requires expensive equipment and skilled professionals, they are not practically feasible for epidemiological and clinical studies [31]. Other method is Immune EM (IEM) which is useful only at the early phase of gastroenteritis and lack virus detection in low concentration [31]. Later on IEM have modified to solid-phase IEM (SIEM) to express viral particle (SPIEM). Other techniques include Immune adherence hemagglutination assay (IAHA) which was further replaced by Radioimmunoassay (RIA) which requires precautions from radioactive substances that were used. Other procedure is Virus isolation, which was not useful for diagnosis of enteric viruses, as they are slow and technically inconvenient [31].

Hence, the conventional diagnostic methods are time consuming, labour intensive, requires trained personnel, well set up laboratories and costly. These methods were limited to research laboratory and not suitable for routine diagnosis. These diagnostic methods often lack sensitivity and specificity compared to molecular techniques. Therefore, to overcome the limitations of conventional diagnostic methods, several molecular techniques were developed which are more rapid, accurate, cost-effective, commercially available test increasingly used in clinical diagnosis of viral gastroenteritis [8, 25, 31, 34].

Recent advanced techniques for the diagnosis of viral gastroenteritis

According to the literature (Hasan *et al* 2021) biomarkers are used in various methods for the detection of etiological agents of AGE. The methods used for the detection of antigens are Enzyme immunoassay (EIA), Enzyme-linked immunosorbent assay (ELISA), and Immunochromatography (ICT) [8, 16, 17].

The molecular methods includes detection of genes of causative agents of AGE by methods such as real-time PCR (RT-PCR) and multiplex real-time PCR, LAMP and NASBA based on nucleic acid amplification techniques (NAAT). Such methods are more rapid, cost-effective and reliable with high degree of sensitivity and specificity. Next generation sequencing (NGS) is the other molecular based diagnostic technology for the identification and sequencing of genome which is accepted worldwide. Thus, recently developed advanced diagnostic methods has overcome the shortcomings of traditional diagnostic tools and will tend to replace conventional diagnostic methods in the near future [16, 31, 34].

Next-generation tools for identification of enteric viruses Metagenomics

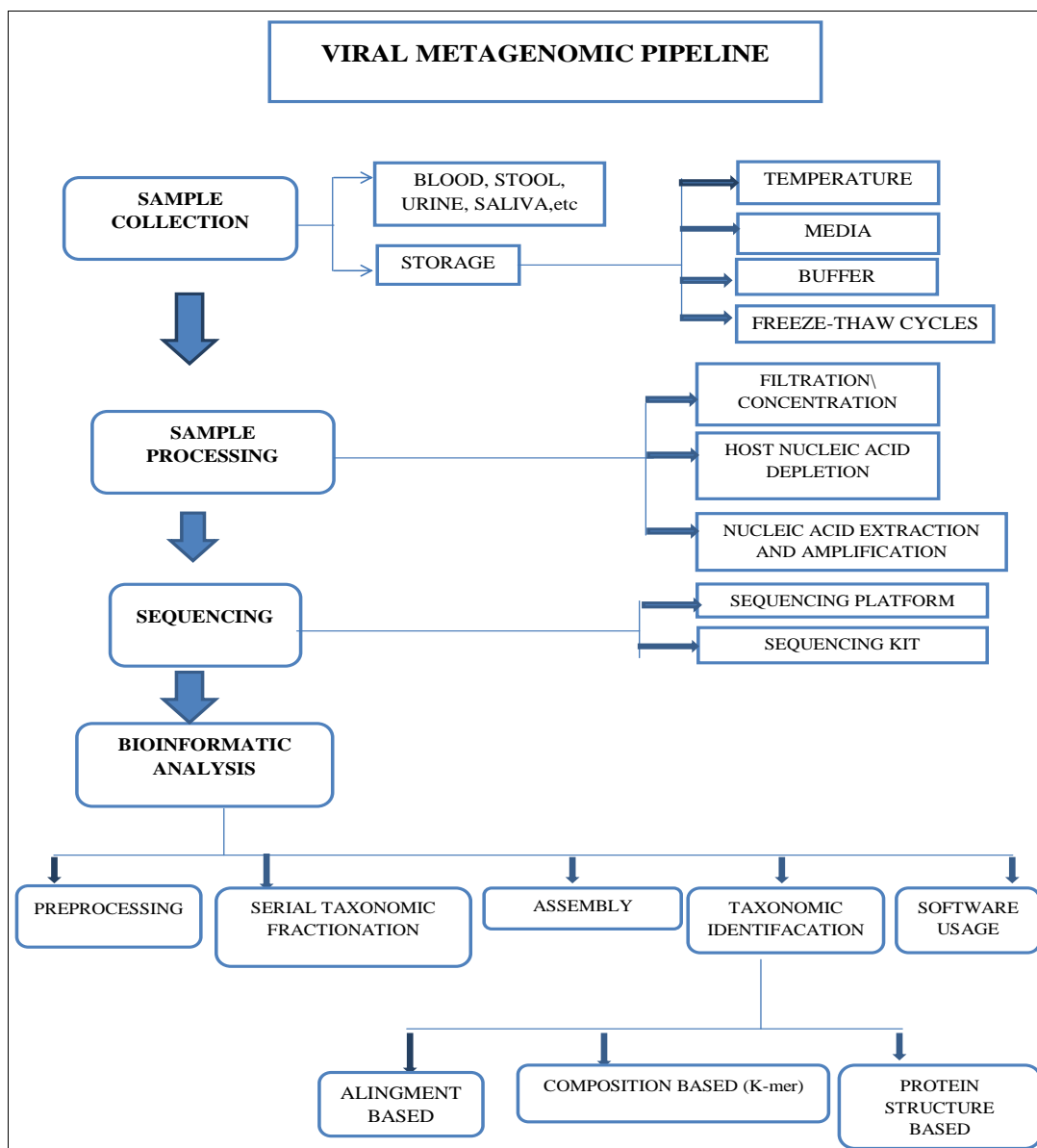
Next-generation sequencing (NGS) based Metagenomics method is a target-independent analysis for simultaneous detection of all microorganisms and their genomic characterization present in a sample allowing many gene fragments to be sequenced simultaneously and independently [16, 32]. Several recent works of literature have reported the use of Metagenomics in identifying and characterization of viruses [35]. One of the studies overviews

the application of the metagenomic next-generation sequencing technique first applied to detect predominant genotype in Rotavirus positive samples. Also, the NGS approach was performed on fecal samples for the diagnosis and whole-genome sequencing of norovirus infections [32]. Similarly, Astrovirus is identified into “classical” and “novel”, recombinant genotypes and Adenovirus is differentiated into serotypes 40 and 41 [32].

Table 1: Next-generation sequencing platform

Potential Biomarkers			
Rotavirus	Norovirus	Astrovirus	Adenovirus
G1P [8]	GI genotypes GII genotypes GII.4	MAstV-1	HAdV A
G9P [8]		MAstV-6	HAdV B
G3P [8]		MAstV-8	HAdV C
G3P [6]		MAstV-9	HAdV D
G8P [8]			HAdV E
			HAdV F
			HAdV G

According to the article (Geng-Hao Bai *et al* 2022), Metagenomics has a wide clinical application. This Viral Metagenomics pipelines include sample collection, sample processing, sequencing, and bioinformatics analysis [35].



Each step of processing is accompanied by contamination risk and metabolism of nucleic acid which may affect the analyses. Therefore, sample processing is an important step in Viral Metagenomics [35].

Therefore, modifications and improvements made in sequencing and identification of genomes have helped to develop more improved versions technology to Illumina, Ion-torrent, and the latest development being Nanopore technology which is based on single-molecule sequencing technologies detecting DNA sequences using nanopore that can generate full-length genome haplotype [35]. This can be performed using a portable MiniIon device, the benchtop GridIon and the high sample number Prometh Ion [35].

Thus Next Generation DNA Sequencing (NGS) is beneficial and is increasingly applied in the molecular characterization of various pathogens. It is a useful method but due to its high cost, its usage is limited to molecular epidemiology studies. Considering its performance and reliability it can be modulated into a cost-effective technique in the upcoming future [35].

Mass spectrometry

Modern mass spectroscopy techniques are based on matrix-associated laser desorption ionization-time of flight mass spectrometry-based system (MALDI-TOF-MS). The combination of PCR with mass assay i.e. Multiplex PCR with MALDI-TOF-MS used for simultaneous detection of 8 enteric viruses (Piao *et al*, 2012). The limitation of this method is time-consuming, laborious, and depends on operator skills. Thus, this requires up-gradation to automation. Another technique is Surface Enhanced Raman Spectroscopy (SERS), (Driskell *et al* 2010) detected Rotavirus strains by identifying their molecular signature [16].

Biosensors

The objective is the detection limit may be a whole virus or any protein. Calicivirus biosensors developed with a detection limit of 1.6×10^5 PFU/ml (Liu *et al*, 2007). The advantage is that its efficiency is not affected by low concentration of ions and molecules and the drawback is that technology is costly to be used practically in resource-poor countries [16].

Microarray

Microarray techniques are used for surveillance analysis of bulk samples and reservoir tracing in endemic regions. A study by (Buss *et al*, 2015) used Film Array GI Panel. This test consists of extraction of nucleic acid, reverse transcription, amplification, and analysis available within 1 hr per specimen. This method can be used for the diagnosis of Norovirus, Sapovirus, and Adenovirus. They can be used simultaneously to detect hundreds of viruses (Wang *et al*, 2020) [16].

Aptamers

Aptamers (oligonucleotides or peptides) are alternatives to antibodies. As they are thermostable and economical therefore they are acceptable in resource-limited countries (Song *et al*, 2012). This method is linked with ELISA, and RTPCR and used as Aptamers linked immune sorbent assay and SPR-based aptasensors. The advantage is their high sensitivity, portable, easy, and fast detection (Kieboom *et al* 2015) This method is used in the diagnosis of Norovirus

strains (Abarca *et al*, 2014) [16].

Future perspectives

There are many sensitive and specific techniques available for the diagnosis of viral pathogens but their applications have not been surveyed and explored in detection in the detection of enteric viruses. The technique can be modified for the identification of suitable enteric viruses and used for efficient diagnosis. Some examples are given: Tissue-based immunoassay and slide ELISA (SELISA) based on solid-phase assays, Tissue immunoblotting assay (TIBA), Peptide nucleic acid (PNAS), PNA-PCR Assay, Single nucleotide polymorphism (SNP) arrays, NASBA-CRISPER [16].

Conclusions

Acute gastroenteritis (AGE) is caused by enteric viruses, Rotavirus is the main leading cause of infection in children worldwide. And Norovirus is an emerging and second-leading causative agent for AGE in both children and adults. As with the emergence of other causative agents, there is a lack of the most reliable parameters. Therefore, more vigorous diagnostic and predictive biomarkers are needed to be considered. NGS-based Metagenomics is novel biomarker detection in clinical microbiology with wider integrated holistic approaches but shortcomings to be eliminated in all methods. Multiplex is the rapid detection with multiple targets which requires multiple testing technique procedures whereas in the syndromic disease Metagenomics is the new viral diagnostic approach with low cost, rapid detection technology. However, Biosensors, Aptamers, and microassays can replace the NGS in the upcoming future. Thus in this review, we described the etiology of AGE and their diagnosis by commercially available multiparametric integrated NAAT that identifies viruses with the scope of routine use of molecular-based tests in clinical setup in near future.

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Not available

Author's Contribution

Not available

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