SHORT COMMUNICATION

ROTAVIRUS GENOTYPING: A PROMISING DIAGNOSTIC TOOL OF RESEARCH FOR PEDIATRIC GASTROENTERITIS

Tayyab Un Nisa
Department of Microbiology, University of Karachi, Pakistan.

ABSTRACT

Every child in his early five years' age usually suffers at least once with rotavirus diarrhea worldwide. Diagnosis of rotavirus diarrhea is carried out by various methods available worldwide: including electron microscopy, antigen detection, nucleic acid detection and amplification (PCR). However, reverse transcription-polymerase chain reaction (RT-PCR) method for detection of rotavirus and its genotyping is considered as the gold standard diagnostic tool, and commonly used for determining the rotavirus gastroenteritis burden as well prevalence of virus type in children for surveillance and outbreak investigation. RT-PCR genotyping methods are also used as alternatives for rotavirus serotyping. Nested RT-PCRs are usually performed for rotavirus genotyping, in which extracted viral ribonucleic acid (RNA) from infected or suspected pediatric fecal specimens are processed for conserved region of viral genome. The PCR is performed by using consensus primers for the gene 9 and 4, responsible for expression of VP4 and VP7 consecutively. Rotavirus genotyping also helps in taking appropriate decision about the introduction and administration of rotavirus vaccines.

Keywords: Rotavirus Genotyping; Pediatric Gastroenteritis; Enteric Viruses.

Corresponding Author:
Tayyab Un Nisa
Department of Microbiology,
University of Karachi, Pakistan.
Email: tayyabunnisa@yahoo.com

INTRODUCTION

Human enteric viruses are a major public health risk, transmitted from person-to-person, or through food and water. Viral gastroenteritis outbreaks are most often linked to adenovirus, norovirus, astrovirus, sapovirus and rotavirus. Rotavirus infections in newborn, toddlers and young kids causes severe clinical consequences such as moderate to severe diarrhea (MSD), vomiting, high grade fever, dehydration, electrolyte imbalance and metabolic acidosis. Rotavirus is declared as one of the five top most global pediatrics gastroenteritic pathogens in children <5 years of age[1].

Rotavirus is extremely contagious, having a very low infectious dose. Even <100 viruses can cause brutal infection. Rotaviruses are transmitted mostly by the fecal-oral route as well as by respiratory droplets, fomite and are shed in high concentration from stool of infected child[2,3]. The objective of this paper is to determine the efficacy and efficiency of Rotavirus genotyping in comparison with other methods, and then explaining importance of applying genotyping technique as an effective means of rotavirus surveillance.

DISCUSSION

Clinical features of rotavirus infection and lab based diagnosis of rotavirus infection is essential component of consistent rotavirus surveillance. Stool specimen is the choice for lab-based diagnosis of rotavirus infection. The enzyme linked immunosorbent assay (ELISA) is widely available test for rotavirus antigen detection. This test is designed to check the presence of viral VP6 (structural viral protein) antigen, which is present in all group A Rotaviruses. There are several ELISA kits available in market, which are cheaper, user friendly, quick, and are with high sensitivity (90-100%). These assays are qualitative and semi-quantitative and are perfect for early clinical diagnosis as well as rotavirus surveillance[3].

Latex agglutination or latex fixation is less sensitive and specific than ELISA, but still used due to low cost[4]. It is preferable technique for a research setting for confirmation of the non-typable strain, but not suitable for high number of samples or any

surveillance study. A comparative diagnosis of rotavirus infection with latex agglutination, electron microscopy and Real-time reverse transcription-PCR show that Real-time PCR is many fold sensitive than other test.

Electron microscopy (EM) is an expensive and laborious technique. The scanning electron microscopy (SEM) technique was mostly restricted to topographical identification. Results of electron microscopy were affected by staining procedure and physical properties of stains [5]. This technique is used mainly in research settings.

Immune detection (ELISA) was considered as a reference method for Rotavirus detection in a comparative report of electron microscopy and other immune detection techniques[6]. As of conventional electron microscopy and scanning electron microscopy (SEM) it was difficult to study the infected cell organelle and intracellular processes of viral infection. These days, with the expanded use of transmission electron microscopy (TEM), electron microscopy has become valuable means of structural and non structural viral protein detection; and interaction of these proteins with viral replication and assembly, Transmission electron microscopy and immunoaffinaty capture EM used as detection techniques of the viral protein interaction through replication intermediates forms of rotavirus assembly. Moreover, EM in combination with disc centrifugation and capillary electrophoresis was found to be of great importance for estimating the double layer protein and triple layer protein ratio in describing immune effectiveness of a rotavirus vaccine preparation[7,8].

Polyacrylamide gel electrophoresis (PAGE) and silver staining are equally sensitive to ELISA but are laborious[9]. PAGE is used to analyze the genome variation based on size of viral nucleic acid. Nucleic acid amplification by Real Time PCR or multiplex probe based rapid RT-PCR are available for Rotavirus identification and determination of viral load and viral type respectively[11]. These assays were tested on large specimen numbers for epidemiological studies as screening tests[12]. NSP3 region was selected by other scientists as a target of detection of rotavirus (Group A) through real time RT-PCR[13,14]. Multiplex PCR for genotyping, and nested RT-PCR with nucleotide sequencing is proven as an standard alternate of serotyping, Latex agglutination and electron microscopy. These techniques are used for the identification of prevalent as well non-typable rotavirus G and P genotypes[15-18].

However, a good multiplex typing protocol is needed for consistently reproducible results. Limitations with any multiplex real-time PCR should be taken under consideration. Rotavirus genotyping can also be performed by doing VP4 and VP7 typing through conventional PCR to evaluate the circulating viral type in community. Identification of new or less common genotypes through RT-PCR can be counter checked and confirmed through Nucleotide sequencing[19].

Table 1: Comparison of rotavirus detection techniques.

TECHNIQUE	ADVANTAGES	DISADVANTAGES	SUITABILITY
Antigen detection, enzyme linked immunosorbent assay (ELISA)	Quick, less time and labor consuming	Can only answer of positive / negative	Surveillance studies and routine diagnosis
Latex agglutination (LA)	Quick, less time and labor consuming	Can only be good if type specific sera available	Research and confirmation of diagnostics
Electron microscopy(EM)	Provide surface structure information, precise	Cumbersome, Expensive, information. Do not type the strain	Only for research
Nucleic acid detection (PAGE)	Confirmation of specific strain type	Laborious	Only for research
Nucleic acid amplification (Real Time PCR)	Quick and accurate	Expensive than conventional PCR Take time for type specific identification	Surveillance studies and routine diagnosis
Reverse Transcription RT-PCR genotyping methods	Quick and accurate	Less expensive, Take time for type specific identification	Surveillance studies and routine diagnosis in a resource limited setting
Nucleic Acid Sequencing	Quick and accurate	Most expensive	Confirmation of new or less common strain. Confirmation of RT-PCR based genotyped strains

The review of different techniques and the working expertise of rotavirus genotyping show that Real time rotavirus detection has an edge over ELISA, PAGE, Agglutination and Electron microscopy. All these are excellent with good specificity and sensitivity for Rotavirus detection in a research setting for rapid detection [Yes/ No] results; and the genotyping to detect the circulating strain of rotavirus in a community [Surveillance Study]. Rotavirus real time PCR detection and rotavirus genotyping are the most promising tools (Table 1).

Real time PCR is commonly used than Reverse Transcription PCR assay, which defines the presence or absence of Rotavirus with its genotypes under investigation.[10] One of the reported assays, representative of dominant diagnostic tools for usual detection of rotavirus in fecal samples, claimed improved detection over ELISA and semi nested RT-PCR[11], although limitations with the multiplex genotyping RT-PCR for Rotavirus type detection cannot be ignored.

While comparing serotyping with the genotyping of the rotavirus, it is worth mentioning that although there is a defined association between P serotyping, which is monoclonal antibody based, and P genotyping; but reverse transcription PCR of Rotavirus detection is not evident of the serotypes of the tested strains for every case. This is confirmation of the fact that Reverse transcription PCR typing methods can be used to classify the common VP4 genes (VP4 genotype) in circulation but cannot be evident of serotypes[20,21]. Reverse transcription PCR of Rotavirus also comes up with many non-typable strains which can be resolved with the correct primer designing as well as by performing the comparative analysis of 2 sets of primers reported from different settings[22]. Moreover, the reverse transcription PCR of rotavirus is used as the standard technique in any of the research setting for the prevalence of circulating viral strain. Selection of the primer is a very important issue to address emerging virus type as well as genetic mutations in similar virus type.

CONCLUSION

Despite of all above mentioned limitation related to primer design, primer selection and the new rotavirus strains emergence in a community, the rotavirus genotyping is still considered as the promising tool for rotavirus gastroenteritis in any research setting. Considering this fact, it is suggested that there is a dire need to design and develop new primers for development of RT-PCR for defining circulating rotavirus genotypes. Moreover, sequencing of rotavirus gene 9 and gene 4 may be helpful in defining new genotypes.

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CONFLICT OF INTEREST

Author does not have any conflict of interest to declare.

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