CHAPTER 1

INTRODUCTION

1.1. Gastroenteritis and rotavirus

Diarrhoeal diseases are one of the leading causes of deaths in children worldwide. Currently, there are five enteric viruses known to cause gastroenteritis in humans: rotavirus, astrovirus, adenovirus, norovirus and sapovirus. Among these, group A rotavirus (RVA) is the most common viral causative agent of diarrhoea worldwide. Rotavirus (RV) particles are excreted in the stools of infected individuals and the main modes of transmission are through fecal-oral route, close-contact, and fomites.

RVs are non-enveloped RNA viruses and belong to the family *Reoviridae*. The 11-dsRNA genome segments of rotavirus encode six structural proteins and six non-structural proteins [1]. The mature infectious rotavirus particles are made up of three layers of capsid proteins: outer (VP7 and VP4), middle (VP6), and core (VP2). Rotavirus is further divided into nine serogroups (A-I) based on group specific VP6 antigen [2,3]. The dual genotyping system (G-P typing) is used based on variations in the nucleotide sequences of the VP7 (**G**lycoprotein) and VP4 (**P**rotease sensitive protein) genes [4,5,6].

1.2. History of rotavirus discovery

Since the mid-20th century viruses were assumed of being significant causes of gastroenteritis (GE) as the etiology remained unknown frequently [7]. Efforts made to identify the etiological agents were unsuccessful in the 1950, the "golden age" of virology, when several new viruses were discovered using newly developed animal tissue culture technology [8]. Kapikian et al. (1972) first identified Norwalk virus (or Norovirus) a 27 x 32 nm particle in feces after a sharp outbreak of acute group I (GI) in Norwalk, Ohio [9] as a cause of GI using immune electron microscopy [10]. Another breakthrough occurred when a second virus measuring 70 nm in size was identified by electron microscopy of thin sections of duodenal mucosa from infants suffering from diarrhoea [11]. This particle was subsequently designated as rotavirus and within 5 years, RVs were recognized as the primary cause of infectious diarrhoea in newborns and young children globally accounting for approximately one third of severe diarrhoea cases [12]. The word ‘rota’ derived from

Latin word means 'wheel-like'. The name was suggested when the structure was observed to be a wheel-like particle and it was later officially recognized by the International Committee on Taxonomy of Viruses (ICTV) [13].

1.3. Burden of rotavirus diarrhoea: hospitalization, mortality, and economic burden

Rotavirus is highly contagious afflicting infants and young children. An estimated 453,000 children under the age of 5 die annually from rotavirus diarrhoea and developing nations in Asia and Africa account for more than half of all child deaths [14,15]. In 2019, it resulted 45.5 million disability-adjusted life year (DALYs) in children under five-year-old [37]. In 2013, an estimated 215,000 of global death were due to RV with more than 90% occurring in developing countries like India. Of these, 47100 RV deaths occurred in India, making up 22% of all RV deaths worldwide [16]. In 2017, three countries-Nigeria, India, and the DRC (Democratic Republic of the Congo) were reported to account for half of all RV deaths, with India contributing 16% of all global deaths among children under the age of five. This is according to a recent report by the Rotavirus Organization of Technical Allies (ROTA) council [17]. According to the published report of national rotavirus surveillance network (NRSN), RV causes approximately 34% of all deaths in children [18,19,20].

Numerous studies have been conducted in different countries to assess the economic cost of rotavirus disease treatment [17]. For a cohort of infants born in 2011, there were an estimated 11.37 million cases of rotavirus GE in India, totalling 3.27 million outpatient and 872,000 inpatient admissions, costing Rs. 10.37 billion ($171 million) from birth to age five, or Rs. 382 ($6.30) for each child born that year [21]. A middle-income nation like Malaysia reported a cost of $33.5 million in 2013, or $19 for every child under the age of five [17,22]. Similar to this observation, in high-income nations like Germany (2012) and Sweden (2015), each hospitalised child cost the health care system more than $2,000 on average [17,23,24].

1.4. Global rotavirus strains distribution

Globally, the most common RVA genotypes are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] [25,26]. Prior to vaccination, G1P[8] was the most common strain in India, followed by G2P[4], G9P[4], G9P[8], and G12P[6], which together accounted for 49.5% of cases. In the post-vaccine era, G3P[8] was the most common G-type, followed by G1P[8], G2P[4], G9P[4], and G1P[6] [27]. As a result of co-infection with animal and other human

strains, there have been instances of other strains, in particular, unusual, rare, or novel strains due to sequential point mutations (antigenic drift), genomic rearrangement (intragenic recombination), and genetic reassortment (antigenic shift) [28,29,30].

1.5. Rotavirus vaccine in universal immunization program (UIP)

Vaccination is considered the most reliable preventive measure to avoid serious consequences of rotaviral gastroenteritis that can even lead to death. Two global oral live attenuated RV vaccines, Rotarix (monovalent, GSK Biologicals) and RotaTeq (pentavalent bovine-human reassortant, Merck) are available since 2006 for immunization of Indian children [31]. Rotavac™, developed by the Bharat Biotech International, Hyderabad is based on rotavirus strain 116E with G9P[8] genotype which has been licensed in 2015 and included in Universal Immunization Program (UIP) of 16 states [32,33]. Another indigenous vaccine, RotaSIIL® (Serum Institute of India) was licensed in 2017 and introduced in UIP in Jharkhand. This suggests the need for continuous surveillance of circulating rotavirus strains during pre- and post-vaccination period to measure the efficacy of the oral vaccines in the population.

1.6. Lack of rotavirus surveillance studies in North-eastern states (NER) of India

There is scarcity of rotavirus surveillance studies in north-east region which is geographically as well as ethnically distinct from the rest of India. As opposed to the tropical climate of the northern, southern, or eastern states where rotavirus surveillances have been previously conducted, the region has both temperate and tropical climatic conditions. It is critical to look at the prevalence and circulating strains of rotavirus in each state of India to comprehensively understand the rotavirus disease burden in the country. Systematic search of literature on health conditions is the first step towards obtaining an idea about its burden in society. In Manipur, during the year 1979 to 1988, few rotavirus outbreaks were reported with evidence supporting zoonotic transmission [34,35]. A hospital-based surveillance study conducted in Manipur by Mukherjee et al. (2010) in children with acute diarrhoea have found the prevalence of G12 genotype strains [36]. There is a scarcity of research on diarrhoea and comprehensive epidemiological considerations pertaining to such investigations as relevant to northeast India require urgent attention. This emphasizes the need to understand the rotavirus strain diversity, the mechanism by which these strains emerged over time and their long-term impact on vaccination.

The present study investigates the prevalence and frequency of rotavirus strains circulating in Manipur, a state in northeast India before the inclusion of indigenous RV vaccine(s) in national immunization program. The findings will help in implementation of programs for improvement of child health, and it also provides a reference to measure the effectiveness of an indigenous oral vaccine in post-immunization schedule in Manipur and other northeastern states. Further, comprehensive investigations of the prevalence and diversity of four common enteric viruses after introduction of vaccines for rotavirus-led diarrhoea will be helpful to provide further understanding of efficacy of vaccines in children under five-year-old of Northeast India. Moreover, it is known that both double and triple layer particles are shed in stools of patients. But the present ELISA based diagnosis method is based on VP6 which is middle layer capsid protein. It limits detection of only double layer particles during diagnosis. A mobile, indigenous, more sensitive and economical diagnostic tool based on VP6 and outer layer protein VP4 and VP7 will be of immense application for monitoring of rotavirus surveillance in developing countries. The present study has been carried out with the following objectives.

1.7. Objectives

1. Molecular characterization of group A rotavirus (RVA) in children under five-year-old in Imphal, Manipur, India
2. Characterization of unusual or untypeable rotavirus isolates by electropherotyping and whole genome sequencing
3. Identification of B- and T-cell epitopes in the rotavirus proteome and development of chimeric antigen for diagnosis of rotavirus

1.8. Bibliography

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