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MOLECULAR CHARACTERIZATION AND GENOTYPING OF ROTAVIRUS FROM DIARRHEIC CHILDREN IN TIKRIT PROVINCE

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Abstract:

Out of 34 rotavirus-positive cases, (31.25%) were males while (11.25%) were females resulting in a male to female ratio of (1:77:1) and the highest rate of infection was found in male which was 25 (31.25%) and the lowest rate of infection was found in females which was 9(11.25%). The typical symptoms associated with acute viral gastroenteritis are diarrhea, vomiting and fever. The children suffering from rotavirus diarrhea had either fever (72.5%) followed by fever and respiratory symptoms (11.8%) and the clinical symptoms in diarrheic children showing respiratory symptoms without fever (3.9%) The molecular studies revealed that (34) of the isolates, independent of the host, exhibited the [G1] genotype and represents first record of [G1] genotype in Iraqi children and the species of Rotavirus was confirmed by analyzing the nucleotide sequences of the Beg9 and End9 gene. Nucleotide sequences of the Rotavirus from diarrheic children were deposited in the GenBank database under accession numbers (LC699251- LC699252). It revealed that, (20) isolates under the accession number (LC699251) showed 99.34% identity to Rotavirus (MH560416). Furthermore, thirteen isolates under the accession number (LC699252) showed 99.32% identity with Rotavirus (MW058363). The similarity LC699251 and LC699252 was 99.29% due to nucleotide changes (C → T) at position 288, 361 and 447 and (G → A) at position 333 and 757 and (A→ T) at position 739 and (A → C) at position 765 and 772. The phylogenic are a useful for health and educational authorities responsible for designing and implementing effective measures for disease control

Keywords: Rotavirus, PCR, genotyping, Iraq

1- INTRODUCTION

Diarrheal disease is one of the major causes of morbidity and mortality in children. Rotavirus A (RVA) is the most common etiological agent causing gastroenteritis in infants and young children worldwide (Wahyuni et al., 2021) and it causes severe acute gastroenteritis among children and is responsible for approximately 60% of inpatients and 41% of outpatients in pediatric hospitals, respectively (Zhou et al., 2016, Sabit et al., 2021). RVA is responsible for 2% mortality of all causes in children under 5 years old (Ahmed, 2018). Rotavirus is a non-enveloped virus and is classified as a member of the family Reoviridae (Troeger et al., 2018). The genome consists of 11 segments of double-stranded RNA (dsRNA) which encodes six viral structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and six non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6). Encompassing the dsRNA are 3 protein layers, the core–shell is formed by VP2, the middle player is composed by VP6 and the outer capsid consists of VP7 and VP4 proteins (Ahmed et al., 2006, Bonura et al., 2022, Lu et al., 2022). Rotavirus is classified into nine recognized species (RVA-RVI) and a tentative species (RVJ) according to the antigenicity of VP6 protein (Wahyuni et al., 2021). Among those, RVA is the most widespread species in humans (Lorestani et al., 2019). Traditionally, binomial classification of RVA is adopted based on the nucleotide sequence of genomic segments of the VP7 (G genotypes) and VP4 (P genotypes) (Lu et al., 2022). Despite, the implementation of RV-1 rotavirus vaccine, data on circulating RVA genotypes in vaccinated cases are not available and specific RVA genotype determination after the use of rotavirus vaccines has not been possible(Cho et al., 2020). This is mainly due to limited capacities in the clinical laboratories to establish and maintain molecular biology and sequencing facilities (Bonkoungou et al., 2010). The virus genotypes emerged due to mutations, the transmission of viruses from animal to human and the

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conduction of new animal strains resulting in the introduction of new antigenic variants (Damasceno et al., 2020, Lestari et al., 2020). Identification of new variants of Rotavirus is measured through the distribution of important elements of epidemiological surveys, vaccine administration, prevalence studies of genotype, disease distribution, and efficacy program for monitoring (Ali et al., 2021). The aim of the study to the detect rotavirus from diarrheic children in stool specimen with phylogenic tree in Tikrit province.

2- MATERIALS AND METHODS

2.1 Study design

From October 2021 to February 2022, a total of 80 stool specimens were collected from children < 3 years who were diagnosed with acute diarrhea. All of the enrolled specimens were routinely collected and stored at – 70 °C prior to investigation. The diagnosis of acute diarrhea was more loose, watery, thin stools with a paste-like texture, or the presence of mucous stools within 24 h, possibly accompanied by vomiting (Amadu et al., 2019, Boni-Cisse et al., 2018), abdominal pain, fever, and nausea (Castells et al., 2020, Lu et al., 2022)

2.2 RNA extraction

The stool sample was initially diluted in phosphate buffer saline (PBS). Further, a stool sample (1g or 100µl) was taken in a labeled eppendorf tube and mixed with 900µl PBS. Vortexed for few seconds till suspension and then centrifuged at 14,000 rpm for 15 minutes. After centrifugation, the clear liquid at top of the lysate was collected by micropipette and stored at -70°C until use. Furthermore, RNA was isolated using commercial kit from Qiagen- Germany according to manufacturer protocols, in a type II Biosafety cabinet.

2.3 Reverse transcription

The synthesis of first strand cDNA from RNA templates and combines all the reagents necessary for successful cDNA synthesis in a convenient individually aliquot and lyophilized in single-tube, two-step format. RTase, which is an RNA-dependent DNA polymerase that is used in cDNA synthesis with long RNA template. During cDNA generation random hexamer primers were used to exposure to all regions of the RNA when cDNA was produced having different lengths of cDNA. The first strand of cDNA can be directly used as a template in PCR.

2.4 Nucleotide Sequencing and Phylogenetic Analyses

Thirty four positive specimens detected by PCR contained sufficient RNA for further whole genome characterizations. The amplified products were commercially sequenced in both directions (Macrogen Corporation – Korea) and the generated sequences were examined using Accelrys Gene 2.5 program (Accelrys, Cambridge, UK) and compared against the NCBI database through the use of BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). A total of 34 samples were sequenced to determine the intraspecific genotype. Sequencing of the Beg9 and End9 gene (Gouvea et al., 1990) was done using the National Center for Biotechnology Information BLAST programs and databases (Accelrys\Accelrys Gene 2.5) (http://accelrys-gene.software.informer.com/). The genome sequences were aligned with reference sequences using CLUSTAL X (version 1.83) software (Thompson et al., 1994), and the phylogenetic trees were constructed by the neighbour joining method. To confirm the reliability of the phylogenetic tree analysis using Molecular Evolutionary Genetic Analysis (MEGA 6) (Tamura et al., 2013) and BioEdit software (Hall et al., 2011). The gene sequences described in the present study have been deposited in the GenBank database under accession numbers (LC699251- LC699252).

2.5 Data analysis and organization

Statistical analysis was performed using the statistical program Graph pad version 16.0. Variation in proportions of Rotavirus (+) in gender-wise was tested using the chi-square test. A P-value less than 0.05 were considered statistically significant.

3- RESULTS AND DISCUSSION

The data indicated that, the majority of the children suffering from rotavirus diarrhea had either fever (72.5%) or fever and respiratory symptoms (11.8%). The prevalence of rotavirus diarrhea in children showing respiratory symptoms without fever was (3.9%) (Table 4). There is no significant association between rotavirus diarrhea and these symptoms ($P >$ 0.05). The result agreed with (Athiyyah et al., 2019), which explained that, the typical symptoms associated with acute viral gastroenteritis are diarrhea, vomiting, fever, and dehydration. The prevalence's of vomiting, watery stool, and some dehydration were significantly greater in the RVA-positive children than in the RVA-negative children As well as, (Bonkoungou et al., 2010) revealed that, out of 217 outpatient children, 48 (22.1%) were infected and of the 230 inpatient children, 103 (44.8%) were infected with rotavirus. The rotavirus infection prevalence was significantly higher among hospitalized children (p = 0.0001) illustrating a significant relationship between rate of hospitalization and severity of illness. In addition, fever was the symptom most commonly reported in association with

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rotavirus diarrhea (82.1%), followed by vomiting (72.8%) and dehydration (48.3%).

(Table 1): Sign of rotavirus diarrhea in children

Other Symptoms of Rota virus	No. of cases (34)	Percentage (%)
Fever	25	73.53
Respiratory symptoms	1	2.94
Fever and Respiratory symptoms	8	23.53

Among the 80 children studied, 47 (58.75%) were males while 33 (41.33%) were females, respectively, giving a male to female ratio of (1:42:1) for all diarrhea cases. Similarly, of the 34 rotavirus-positive cases,(31.25%) were males while (11.25%) were females resulting in a male to female ratio of (1:77:1). According to results of PCR test, Rotavirus detected in rate of (42.5%) from children with acute diarrhea, in Tikrit province, Out of (34) stool samples from child, the highest rate of infection was found in male which was 25 (31.25%) and the lowest rate of infection was found in females which was 9(11.25 %) as represented in (Table 2). There is a significant difference between them (P<0.05). The result agreed with (Dey et al., 2020) in Bangladesh which revealed that, the highest rate of infection was found in male (55.6%) as compared with females (44.4%). As well as (Nasser et al., 2021), in Diyala province revealed that, the highest rate of infection was found in males (54%) as compared to females which was (46%). Furthermore, (Ojobor et al., 2020) showed that, there was no significant difference in the burden of rotavirus disease between male and female children but the preponderance of the disease was more in males. While (Ndze et al., 2012), reported that, there is significant difference was found in males than females. The reasons for the difference in detection rate between males and females are however, not known (Fenjan et al., 2020).

(Table 2): Gender wise distribution of Rotavirus PCR result

Gender	No. of cases	(%)	No of Positive samples	(%)	No of Negative samples	(%)	P value
Male	47	58.75	25	31.25	22	27.5	0.0239
Female	33	41.25	9	11.25	24	30	
Total	80	100	34	42.5	46	57.5	

The RNA was extracted from each of 80 samples isolated from different diarrheic children. Thirty four samples showed amplification of a 1062-bp fragment with the *Beg9* and *End9* gene which are specific for Rotavirus. After amplification the products were electrophoresed and visualized using UV transluminator, which showed clear and visible bands. The size of the bands was the same in viruses isolated from children which were about 100bp on 1.5% agarose gel after staining with ethidium bromide as shown in figures (1). These agreed with (Lorestani et al., 2019) expressed that, the initial screening for presence of human rotavirus genome in 345 stool samples was performed by direct dsRNA extraction of samples with RNX-Plus reagent and RNA–PAGE analysis. Also (Pardo-Mora et al., 2018) reported that, PCR amplification of the 341bp-fragment of the VP7 gene in the nine samples that developed a cytopathic effect allowed to confirm the presence of rotaviruses.

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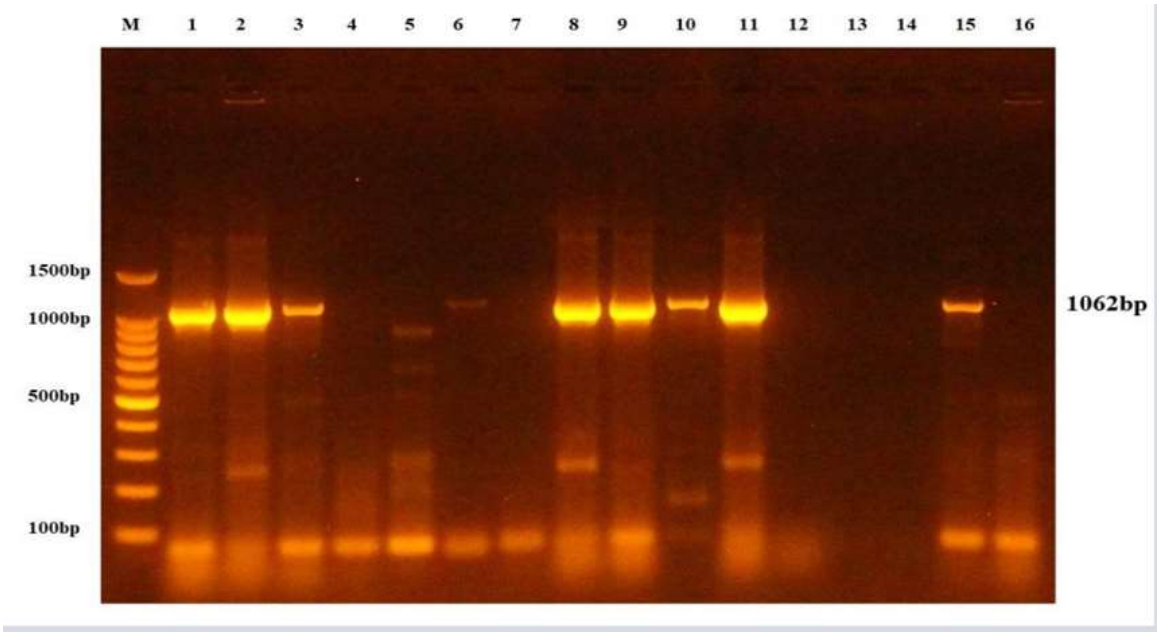

Figure 1: PCR Results of Rotavirus amplicon using *Beg9* and *End 9* gene. Lanes denote Template DNA isolated from stool samples (L100), DNA Marker Size (100-bp DNA ladder). In the *Beg9* and End9 gene, PCR only 1062 bp Fragment was Amplified.

All PCR products were sequenced and the accuracy of the sequencing data was confirmed by sequencing in both directions. Sequence analysis for 34 isolates was successfully performed on PCR products of Rotavirus. The obtained result (LC699251 and LC699252) compared with the previously published sequences under accession number MH560416 and MW058363 as shown in Figure (2a and b). It revealed that, 20 isolates under the accession number (LC699251) showed 99.34% identity to Rotavirus (MH560416) due to nucleotide changes (A → G) at position 158, 334 and 790 and (T → C) at position 457. Furthermore, thirteen isolates under the accession number (LC699252) showed 99.32% identity with Rotavirus (MW058363) due to nucleotide changes (A → G) at position 22, (T → C) at position 343, (A → G) at position 740, (A → T) at position 721 and (A → C) at position 748 and 755. (Figure 3) shows, the similarity LC699251 and LC699252 was 99.29% due to nucleotide changes (C → T) at position 288, 361 and 447 and (G → A) at position 333 and 757 and (A→ T) at position 739 and (A → C) at position 765 and 772. The rotavirus capsid is composed of three concentric protein layers. Proteins VP4 and VP7 comprise the outer layer. VP4 forms spikes, is the viral attachment protein, and is cleaved by trypsin into VP8 and VP5. VP7 is a glycoprotein and the major constituent of the outer protein layer (Nair et al., 2017). Both VP4 and VP7 induce neutralizing and protective antibodies. To gain insight into the virus neutralization mechanisms, the effects of neutralizing monoclonal antibodies (MAbs) directed against VP8, VP5, and VP7 on the decapsidation process of purified OSU and RRV virions were studied (Harastani et al., 2020). Changes in virion size were followed in real time by 90° light scattering. The transition from triple-layered particles to double-layered particles induced by controlled low calcium concentrations was completely inhibited by anti-VP7 MAbs but not by anti-VP8 or anti-VP5 MAbs (Ludert et al., 2002). On the other hand, (Aldawmy et al., 2021) expressed that, by using Blast software for all local samples of VP7 derived from diarrheic children samples derived nucleotide sequences were aligned for matching with the reference database (GenBank) revealing the local human samples revealed (100% identity) completely match for VP7 human sample with reference VP7 gene GenBank sequence accession ID: K681838.

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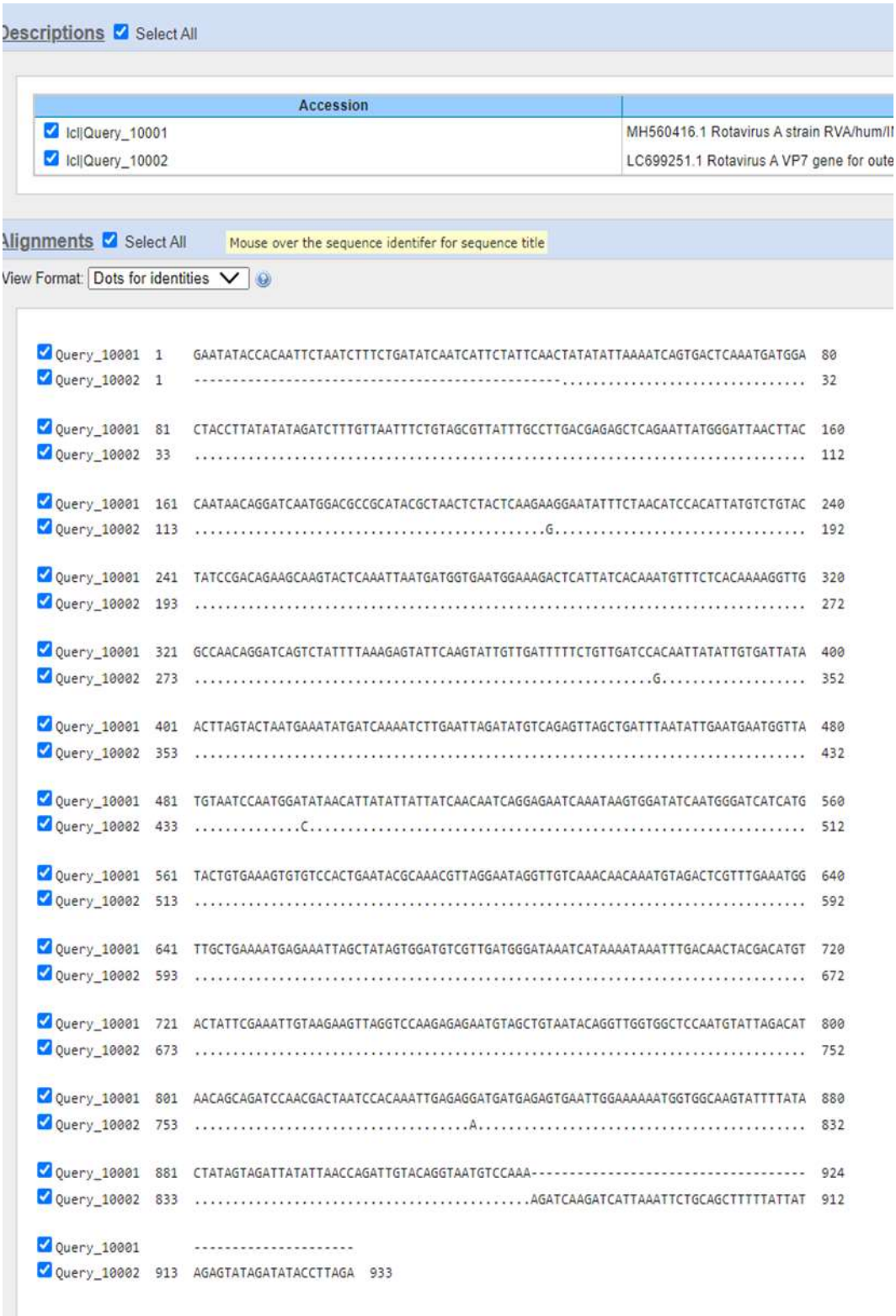

Descriptions ☑ Select All

Accession	
☑ lcl|Query_10001	MH560416.1 Rotavirus A strain RVA/hum/I
☑ lcl|Query_10002	LC699251.1 Rotavirus A VP7 gene for oute

Alignments ☑ Select All Mouse over the sequence identifer for sequence title

View Format: Dots for identities

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Query_10002  1    ----------------------------------------------.....................................  32

Query_10001  81   CTACCTTATATATAGATCTTTGTTAATTTCTGTAGCGTTATTTGCCTTGACGAGAGCTCAGAATTATGGGATTAACTTAC  160
Query_10002  33   .....................................................................................  112

Query_10001  161  CAATAACAGGATCAATGGACGCCGCATACGCTAACTCTACTCAAGAAGGAATATTTCTAACATCCACATTATGTCTGTAC  240
Query_10002  113  ..............................................G......................................  192

Query_10001  241  TATCCGACAGAAGCAAGTACTCAAATTAATGATGGTGAATGGAAAGACTCATTATCACAAATGTTTCTCACAAAAGGTTG  320
Query_10002  193  .....................................................................................  272

Query_10001  321  GCCAACAGGATCAGTCTATTTTAAAGAGTATTCAAGTATTGTTGATTTTTCTGTTGATCCACAATTATATTGTGATTATA  400
Query_10002  273  ...........................................................G.........................  352

Query_10001  401  ACTTAGTACTAATGAAATATGATCAAAATCTTGAATTAGATATGTCAGAGTTAGCTGATTTAATATTGAATGAATGGTTA  480
Query_10002  353  .....................................................................................  432

Query_10001  481  TGTAATCCAATGGATATAACATTATATTATTATCAACAATCAGGAGAATCAAATAAGTGGATATCAATGGGATCATCATG  560
Query_10002  433  ..............C......................................................................  512

Query_10001  561  TACTGTGAAAGTGTGTCCACTGAATACGCAAACGTTAGGAATAGGTTGTCAAACAACAAATGTAGACTCGTTTGAAATGG  640
Query_10002  513  .....................................................................................  592

Query_10001  641  TTGCTGAAAATGAGAAATTAGCTATAGTGGATGTCGTTGATGGGATAAATCATAAAATAAATTTGACAACTACGACATGT  720
Query_10002  593  .....................................................................................  672

Query_10001  721  ACTATTCGAAATTGTAAGAAGTTAGGTCCAAGAGAGAATGTAGCTGTAATACAGGTTGGTGGCTCCAATGTATTAGACAT  800
Query_10002  673  .....................................................................................  752

Query_10001  801  AACAGCAGATCCAACGACTAATCCACAAATTGAGAGGATGATGAGAGTGAATTGGAAAAAATGGTGGCAAGTATTTTATA  880
Query_10002  753  ....................................A................................................  832

Query_10001  881  CTATAGTAGATTATATTAACCAGATTGTACAGGTAATGTCCAAA----------------------------------  924
Query_10002  833  ..........................................AGATCAAGATCATTAAATTCTGCAGCTTTTTATTAT  912

Query_10001       --------------------
Query_10002  913  AGAGTATAGATATACCTTAGA  933
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Figure (2a): Representative Variable Regions of Partial Nucleotide Sequences (LC699251) of 912bps Fragment of the *Beg9* and *End9* gene for 20 stool samples Isolated from Different diarrheic children in Tikrit Province

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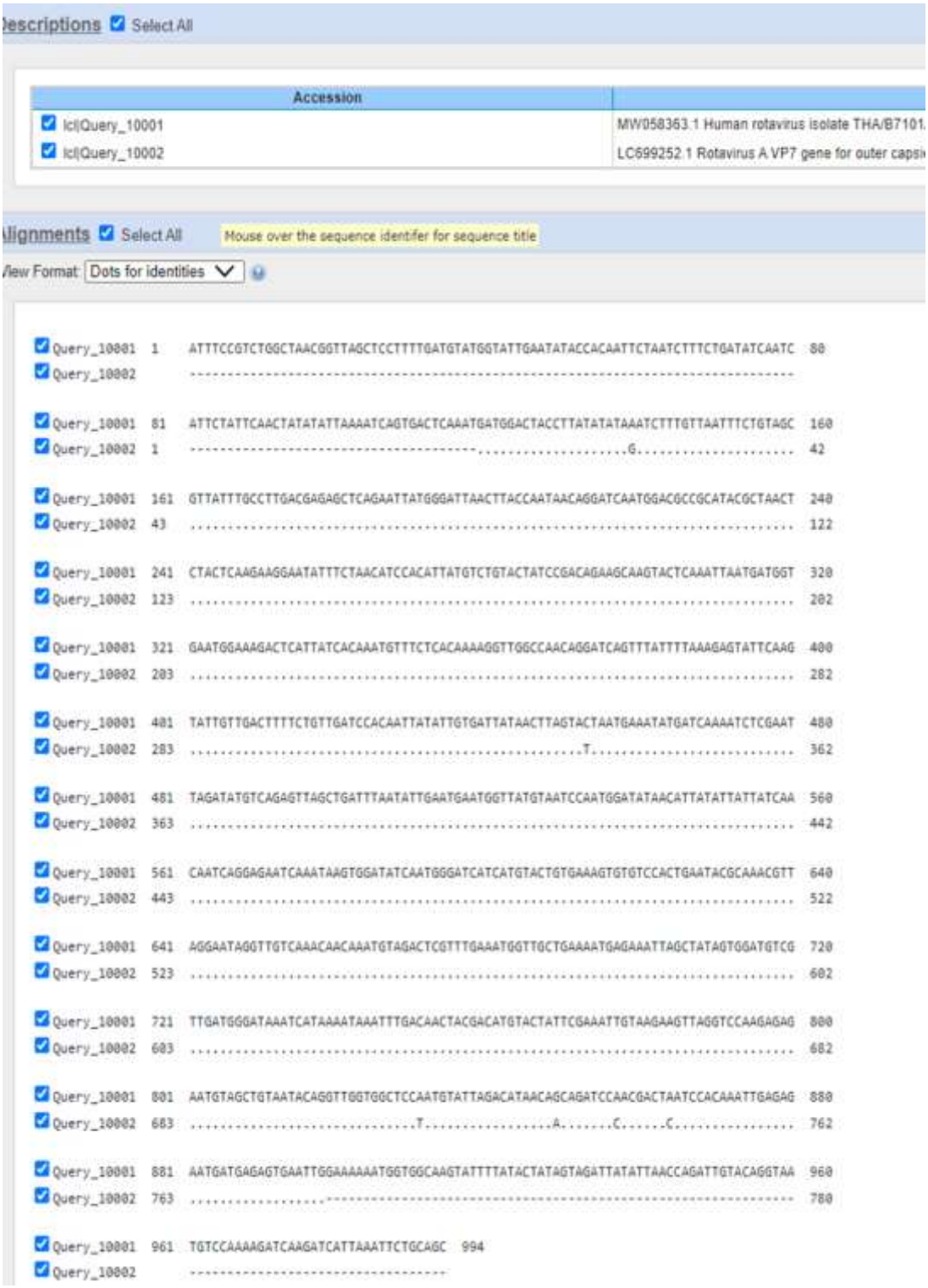

Figure (2b): Representative Variable Regions of Partial Nucleotide Sequences (LC699252) of 912bps Fragment of the Beg9 and End9 gene for 14 stool samples Isolated from Different diarrheic children in Tikrit Province

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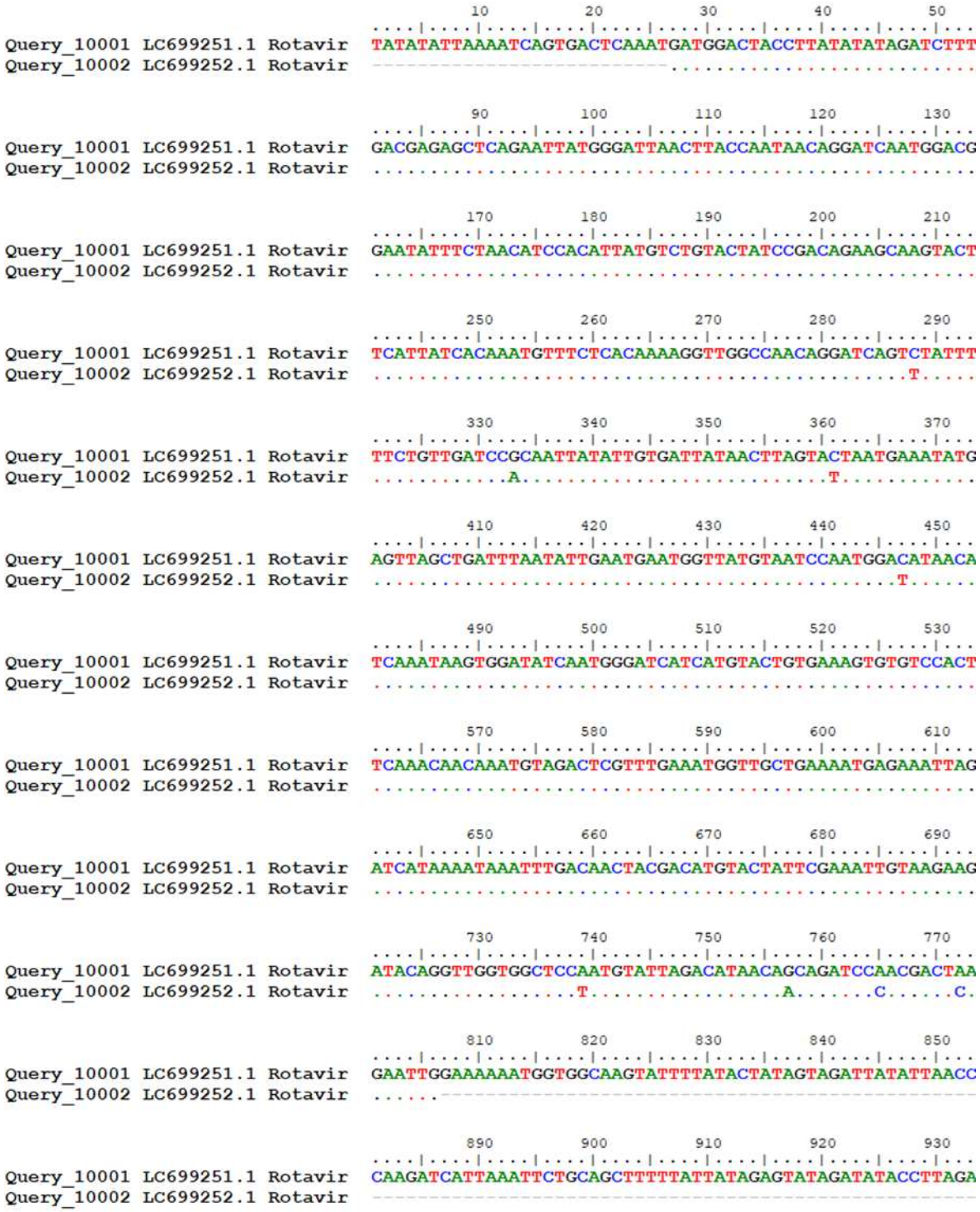

Figure (3): Representative Variable Regions of Partial Nucleotide Sequences (LC699251 and LC699252) of 912bps Fragment for 33 stool samples Isolated from Different diarrheic children in Tikrit Province

The phylogenetic tree was constructed depending on the multiple sequence alignment for the current results (LC699251) with the Rotavirus references (MW058363.1, C541520.1, LC582538.1,

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MH560416.1, KM288563.1, KX638538.1, EU984109.1 and GU377170.2) for the classification (Figure 4). Furthermore, figure (5) shows multiple sequence alignment for the current results (LC699252) with the references of (EU984109.1, KX638538.1, MN836887.1, MN836885.1, MZ955388.1, GQ452920.3, MW058322, MW058234, GQ117002, LC541520, LC541518 and LC541517). The phylogenetic analysis demonstrated that the isolates share close homology with some reference strains isolates. Rotavirus is one of the most viral species among children. The virus transmission among a range of child could increase the chance of genetic variability within different populations of the virus in the world (Sashina et al., 2021). The results of the PCR revealed that from a total of 34 patients, showed positive VP7 by RT-PCR. [G1] was mostly the predominant serotype, of all VP7-positive isolates. The result agreed with (Shams et al., 2020) which explained that, the dominant G-P combination was G1P (32%), followed by G2P (11%), G9P (11%), and G3P (11%). Further, G4P, G4P, G9P, G9P, and G12P were detected in 25% of all evaluated specimens. Also (Lorestani et al., 2019), which revealed that, the results of P and G typing in this study revealed that genotype G1P (Estes and Cohen, 1989) was dominant with the prevalence of 57.82%, followed by the genotypes G2P. In a recent study, the result disagreed with (Azaran et al., 2018), which reported that, the prevalence rate of 28.13% for genotype G9P in patient with gasteroentritis during 2015 to 2016. Furthermore, the result partially agreed with (Amadu et al., 2019) revealed that, Genotyping by RT-PCR was done on 25 samples. The most prevalent type-able VP7 G types were G9 (28%), G1 (24%), followed by G12 (20%), G2 (12%), and G10 (4 %); on the other hand, the most prevalent type-able VP4 P types are P8 (48%), P4 (24%), and P6 (16%).

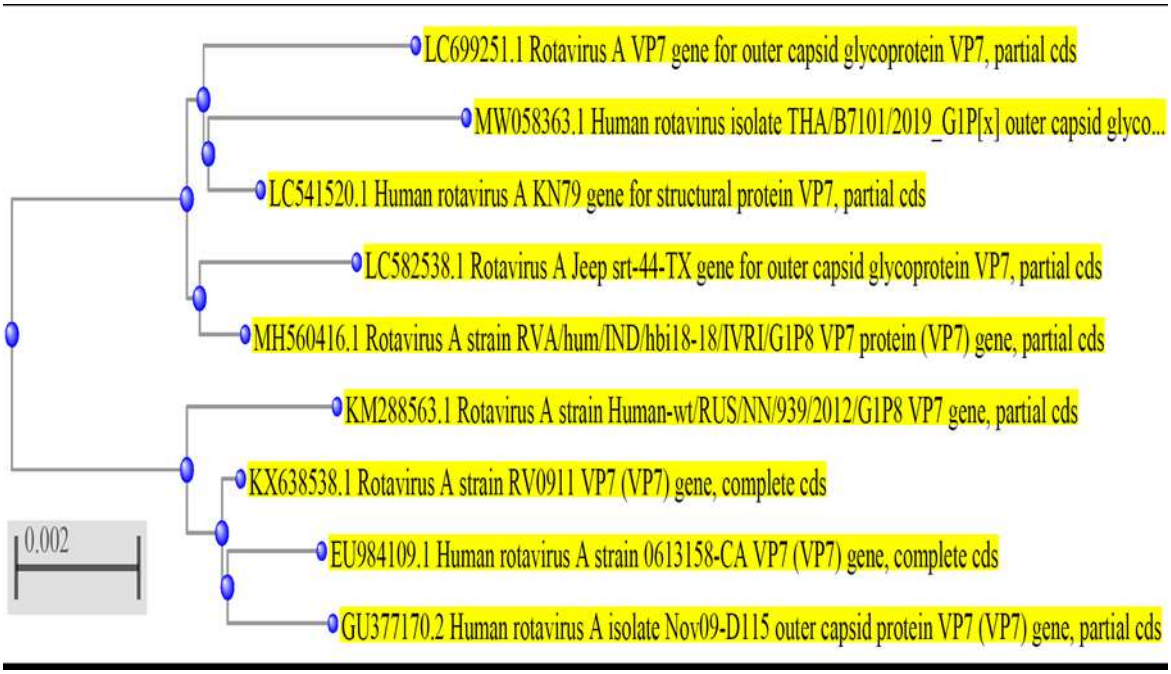

Figure (4): Phylogenetic Tree of Representative Sequences of Rotavirus (LC699251) and References Sequences of other Genotype Deposited in GenBank

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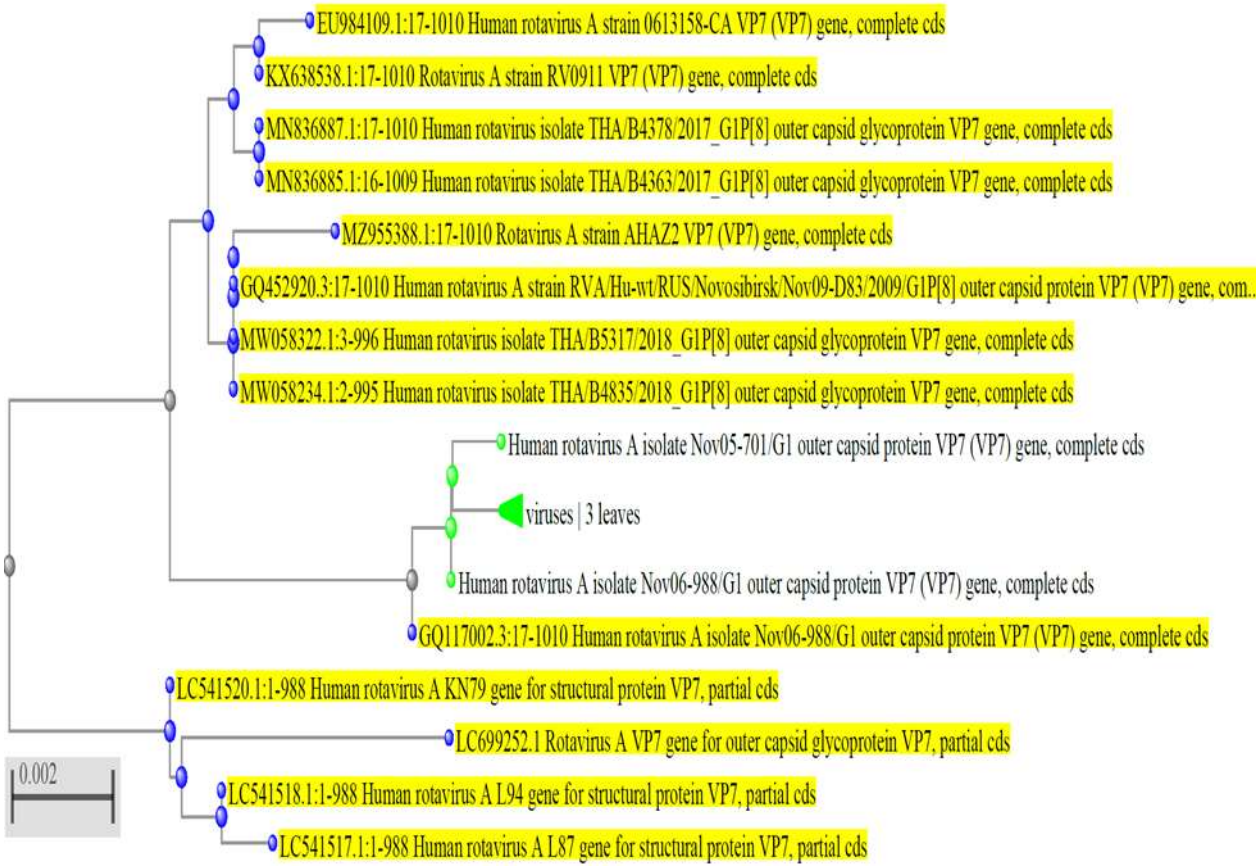

Figure (5): Phylogenetic Tree of Representative Sequences of Rotavirus (LC699252) and References Sequences of other Genotype Deposited in GenBank

4- CONCLUSIONS

This study provides information on the epidemiology and the extent of rotavirus infections in Tikrit Province. Our results indicate that gastroenteritis caused by rotavirus in the country is an important health problem during the cold season. These data will be useful for making an informed decision about the introduction of rotavirus vaccine in Tikrit and will provide a baseline against which the impact of the vaccine introduction can be measured in the future. Furthermore, the nucleotide sequences of G1 genotypes revealed high homology to previously reported similar genotypes especially from India and Thailand.

REFERENCES

1. Ahmed, A. F. M. A. 2018. Assessment Of The Effectiveness Of Immunization Programme Among Children Under Five Years And Their Mothers In Shendi Locality From 2016–2017. Abdallah Ahmed Adam Belal.
2. Ahmed, H. M., Coulter, J. B. S., Nakagomi, O., Hart, C. A., Zaki, J. M., Al-Rabaty, A. A., Dove, W. & Cunliffe, N. A. 2006. Molecular Characterization Of Rotavirus Gastroenteritis Strains, Iraqi Kurdistan. Emerging Infectious Diseases, 12**,** 824.
3. Aldawmy, F. K., Thwiny, H. T. & Abo Almaali, H. M. 2021. Epidemiological And Molecular Study Of Rotavirus Infection Among Human And Animal In Karbala And Basrah Provinces. Iraqi Journal Of Veterinary Sciences, 35**,** 403-410.
4. Ali, S., Khan, S., Khan, S., Rauf, M., Khan, M., Majid, A., Dawar, F., Akbar, N. U., Ullah, R. & Bari, A. 2021. Molecular Detection And Prevalence Of Rotavirus With Acute Gastroenteritis Among The Children Of Rural And Urban Areas. Brazilian Journal Of Biology, 83.
5. Amadu, D. O., Abdullahi, I. N., Emeribe, A. U., Musa, P. O., Olayemi, L., Yunusa, T., Okechukwu, C. E. & Salami, M. O. 2019. Molecular Characterization Of Rotavirus Genotype-A In Children With Acute Diarrhea Attending A Tertiary Hospital In Ilorin, Nigeria. Int J Health Allied Sci, 8**,** 187-92.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-15, October 2022
ISSN: 2749-3644

6. Athiyyah, A. F., Utsumi, T., Wahyuni, R. M., Dinana, Z., Yamani, L. N., Sudarmo, S. M., Ranuh, R. G., Darma, A., Raharjo, D. & Matsui, C. 2019. Molecular Epidemiology And Clinical Features Of Rotavirus Infection Among Pediatric Patients In East Java, Indonesia During 2015–2018: Dynamic Changes In Rotavirus Genotypes From Equine-Like G3 To Typical Human G1/G3. Frontiers In Microbiology, 10**,** 940.
7. Azaran, A., Makvandi, M., Teimoori, A., Ebrahimi, S., Heydari, F. & Nikfar, R. 2018. Distribution Of Rotavirus Genotypes Circulating In Ahvaz, Iran In 2016. Iranian Biomedical Journal, 22**,** 107.
8. Boni-Cisse, C., Meite, S., Mlan, A. B., Zaba, F., N'guessan, R., Lepri, N. A. & Lartey, B. 2018. Genotypic Characterization Of Rotavirus In Children Under 5 Years Circulating In Côte D'Ivoire From 2010 To 2013. Virology Journal, 15**,** 1-6.
9. Bonkoungou, I. J., Sanou, I., Bon, F., Benon, B., Coulibaly, S. O., Haukka, K., Traoré, A. S. & Barro, N. 2010. Epidemiology Of Rotavirus Infection Among Young Children With Acute Diarrhoea In Burkina Faso. BMC Pediatrics, 10**,** 1-6.
10. Bonura, F., Mangiaracina, L., Filizzolo, C., Bonura, C., Martella, V., Ciarlet, M., Giammanco, G. M. & De Grazia, S. 2022. Impact Of Vaccination On Rotavirus Genotype Diversity: A Nearly Two-Decade-Long Epidemiological Study Before And After Rotavirus Vaccine Introduction In Sicily, Italy. Pathogens, 11**,** 424.
11. Castells, M., Caffarena, R. D., Casaux, M. L., Schild, C., Miño, S., Castells, F., Castells, D., Victoria, M., Riet-Correa, F. & Giannitti, F. 2020. Phylogenetic Analyses Of Rotavirus A From Cattle In Uruguay Reveal The Circulation Of Common And Uncommon Genotypes And Suggest Interspecies Transmission. Pathogens, 9**,** 570.
12. Cho, H.-K., Hwang, S. H., Nam, H. N., Han, K., Kim, B., Kong, I., Park, K. & Lee, J. 2020. Incidence Of Intussusception Before And After The Introduction Of Rotavirus Vaccine In Korea. Plos One, 15**,** E0238185.
13. Damasceno, J. L., Andrade, G., Santiago, M. B., Martins, C. H. G. & Pires, R. H. 2020. Rotavirus: A Review And Emergence Of New Genotypes. Revista Brasileira Multidisciplinar, 23**,** 173-189.
14. Dey, S. K., Sharif, N., Sarkar, O. S., Sarkar, M. K., Talukder, A. A., Phan, T. & Ushijima, H. 2020. Molecular Epidemiology And Surveillance Of Circulating Rotavirus Among Children With Gastroenteritis In Bangladesh During 2014–2019. Plos One, 15**,** E0242813.
15. Estes, M. K. & Cohen, J. 1989. Rotavirus Gene Structure And Function. Microbiological Reviews, 53**,** 410-449.
16. Fenjan, M. N., Jarullah, B. A. & Abdulrahman, S. J. 2020. Molecular Identification And Phylogenetic Analysis Of Rotavirus In Children Suffered From Diarrhea Under Five Years Old In Thi-Qar Provience, Of Iraq. International Journal Of Pharmaceutical Research, 12.
17. Gouvea, V., Glass, R., Woods, P., Taniguchi, K., Clark, H., Forrester, B. & Fang, Z.-Y. 1990. Polymerase Chain Reaction Amplification And Typing Of Rotavirus Nucleic Acid From Stool Specimens. Journal Of Clinical Microbiology, 28**,** 276-282.
18. Hall, T., Biosciences, I. & Carlsbad, C. 2011. Bioedit: An Important Software For Molecular Biology. GERF Bull Biosci, 2**,** 60-61.
19. Harastani, H. H., Reslan, L., Sabra, A., Ali, Z., Hammadi, M., Ghanem, S., Hajar, F., Matar, G. M., Dbaibo, G. S. & Zaraket, H. 2020. Genetic Diversity Of Human Rotavirus A Among Hospitalized Children Under-5 Years In Lebanon. Frontiers In Immunology, 11**,** 317.
20. Lestari, F. B., Vongpunsawad, S., Wanlapakorn, N. & Poovorawan, Y. 2020. Rotavirus Infection In Children In Southeast Asia 2008–2018: Disease Burden, Genotype Distribution, Seasonality, And Vaccination. Journal Of Biomedical Science, 27**,** 1-19.
21. Lorestani, N., Moradi, A., Teimoori, A., Masodi, M., Khanizadeh, S., Hassanpour, M., Javid, N., Ardebili, A., Tabarraei, A. & Nikoo, H. R. 2019. Molecular And Serologic Characterization Of Rotavirus From Children With Acute Gastroenteritis In Northern Iran, Gorgan. BMC Gastroenterology, 19**,** 1-9.
22. Lu, L., Zhong, H., Jia, R., Su, L., Xu, M., Cao, L., Liu, P., Ao, Y., Dong, N. & Xu, J. 2022. Prevalence And Genotypes Distribution Of Group A Rotavirus Among Outpatient Children Under 5 Years With Acute Diarrhea In Shanghai, China, 2012–2018. BMC Gastroenterology, 22**,** 1-10.
23. Ludert, J. E., Ruiz, M. C., Hidalgo, C. & Liprandi, F. 2002. Antibodies To Rotavirus Outer Capsid

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Glycoprotein VP7 Neutralize Infectivity By Inhibiting Virion Decapsidation. Journal Of Virology, 76**,** 6643-6651.
24. Nair, N., Feng, N., Blum, L. K., Sanyal, M., Ding, S., Jiang, B., Sen, A., Morton, J. M., He, X.-S. & Robinson, W. H. 2017. VP4-And VP7-Specific Antibodies Mediate Heterotypic Immunity To Rotavirus In Humans. Science Translational Medicine, 9**,** Eaam5434.
25. Nasser, A. T., Hasan, A. S., Saleh, A. K. & Saleh, M. K. 2021. Immunological And Molecular Detection Of Rotavirus Genotype In Children With Gastroenteritis In Diyala-Iraq. GSC Advanced Research And Reviews, 6**,** 194-208.
26. Ndze, V. N., Akum, A. E., Kamga, G. H., Enjema, L. E., Esona, M. D., Banyai, K. & Therese, O. A. M. 2012. Epidemiology Of Rotavirus Diarrhea In Children Under 5 Years In Northern Cameroon. Pan African Medical Journal, 11.
27. Ojobor, C., Olovo, C., Onah, L. & Ike, A. 2020. Prevalence And Associated Factors To Rotavirus Infection In Children Less Than 5 Years In Enugu State, Nigeria. Virusdisease, 31**,** 316-322.
28. Pardo-Mora, D., Vargas-Bermúdez, D. S., Oliver-Espinosa, O. & Jaime-Correa, J. 2018. Molecular Characterization Of Rotaviruses Isolated From Calves With Bovine Neonatal Diarrhea (BND) In Colombia. Infectio, 22**,** 99-104.
29. Sabit, H., Tombuloglu, H., Rehman, S., Almandil, N. B., Cevik, E., Abdel-Ghany, S., Rashwan, S., Abasiyanik, M. F. & Waye, M. M. Y. 2021. Gut Microbiota Metabolites In Autistic Children: An Epigenetic Perspective. Heliyon, 7**,** E06105.
30. Sashina, T., Morozova, O., Epifanova, N., Kashnikov, A., Leonov, A. & Novikova, N. 2021. Molecular Monitoring Of The Rotavirus (Reoviridae: Sedoreovirinae: Rotavirus: Rotavirus A) Strains Circulating In Nizhny Novgorod (2012–2020): Detection Of The Strains With The New Genetic Features. Problems Of Virology, 66**,** 140-151.
31. Shams, S., Nasab, S. D. M., Heydari, H., Tafaroji, J., Ahmadi, N. & Afzali, E. S. 2020. Detection And Characterization Of Rotavirus G And P Types From Children With Acute Gastroenteritis In Qom, Central Iran. Gastroenterology And Hepatology From Bed To Bench, 13**,** S128.
32. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology And Evolution, 30**,** 2725-2729.
33. Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994. CLUSTAL W: Improving The Sensitivity Of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties And Weight Matrix Choice. Nucleic Acids Research, 22**,** 4673-4680.
34. Troeger, C., Khalil, I. A., Rao, P. C., Cao, S., Blacker, B. F., Ahmed, T., Armah, G., Bines, J. E., Brewer, T. G. & Colombara, D. V. 2018. Rotavirus Vaccination And The Global Burden Of Rotavirus Diarrhea Among Children Younger Than 5 Years. JAMA Pediatrics, 172**,** 958-965.
35. Wahyuni, R. M., Utsumi, T., Dinana, Z., Yamani, L. N., Wuwuti, I. S., Fitriana, E., Gunawan, E., Liang, Y., Ramadhan, F. & Lusida, M. I. 2021. Prevalence And Distribution Of Rotavirus Genotypes Among Children With Acute Gastroenteritis In Areas Other Than Java Island, Indonesia, 2016–2018. Frontiers In Microbiology, 12**,** 672837.
36. Zhou, Y., WU, J., GENG, P., KUI, X., XIE, Y., ZHANG, L., LIU, Y., YIN, N., ZHANG, G. & YI, S. 2016. Microrna Profile Analysis Of Host Cells Before And After Wild Human Rotavirus Infection. Journal Of Medical Virology, 88**,** 1497-1510.