WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 1-11. Research Article ISSN 2277–7105

VIRAL AGENT THAT CAUSING DIARRHOEA AMONG CHILDREN IN AL-NAJAF PROVINCE, IRAQ

Aqeel A. AL-Sadawi*, Mahdi Al-Ammar and Nabil S. Tuwaij

University of Kufa-Faculty of Science-Department of Biology.

Article Received on
25 May 2017,

Revised on 14 June 2017,
Accepted on 04 July 2017

DOI: 10.20959/wjpr20178-8947

***Corresponding Author**
Aqeel A. AL-Sadawi
University of Kufa-Faculty of Science-Department of Biology.

ABSTRUCT

Enteric viruses are major etiologic agents of acute gastroenteritis among infants and young children worldwide. The main objective of the study was to determine investigate the role of enteric viruses in acute diarrhea in the country. A total of 200 stool specimens were collected from children aged 1 month to 8 years with clinical signs of diarrhea who attended to AL-Zahra Teaching Hospital for children in Al-Najaf province, during the period from 1, October, 2016 to20, January, 2017. All specimens were tested for detect enteric viruses using_rapid test device (chromate-graphic immunoassay). The result showed that at least one viral agent was detected in 24/200(12%) specimens were positive for enteric viral agent compare 176(88%) were negative. The data revealed highest frequency among males 15(62.5%) compared with females 9(37.5%). The results showed the diarrhea was more frequency in children less than 2 years 12(50%) compared with other ages. The results gave 29 virus (some more one in same specimen) as following, the prevalence of adenovirus was10(34.4%), astrovirus 3(10.4%) and 8(27.6%) was for rotavirus and norovirus. Positive specimens for adenovirus was confirmed by polymerase chain reaction (PCR) using specific primer. However, the molecular results showed 8(80%) of adenovirus carry the Hexon gene.

KEYWORDS: Astrovirus, rotavirus and norovirus.

INTRODUCTION

Diarrhoea can be defined as the passing of loose or liquid stools three or more times within a 24-hour period. Diarrheal diseases account for one in nine child deaths worldwide more than AIDS, malaria, and measles combined (Anteneh *et al*., 2017 and Hashi *et al.* 2016). It is the

major cause after pneumonia in children, mainly in developing countries(WHO, 2013). About 20% million children annually in Asia, Africa, and America; and 60% of these death occur in the first 2 years of life(cited by Ali *et al*., 2009).

Enteric viruses including rotavirus, norovirus, astroviruses and adenovirus types (40 and 41) specially infect the enterocytes of the small intestine and it mainly affect in children less than two years of age, leading to persistent watery diarrhoea, and it can cause severe dehydration. The norovirus is considered a major cause of severe gastroenteritis in infants and young children worldwide and it is recognized as the important cause of foodborne outbreaks (Sdiri-Loulizi et al., 2009; Nair et al., 2010). New sensitive and specific diagnostic methods, such as direct PCR analysis of fecal specimens, have been used to identified viruses pathogens (Kim et al., 2007). Therefore the aim of this paper to the prevalence the viral agents that responsible for diarrhea in Al-Najaf province using phenotypic and molecular methods.

MATERIALS AND METHODS

The study was done at Laboratories of Bacteriology and Molecular in Biology Department, Faculty of Sciences, University of Kufa, Iraq.

specimens collection and bacterial identification

The study population consist of 200 clinical stool specimens collected from children suffering from diarrhoea with age ranged between (1month-8years) who attended to AL-Zahra Teaching Hospital for children in Al-Najaf province, during the period from 1, October, 2016 to20, January, 2017. Stool amount from 2 gm to 5 gm in disposable containers were used for viral detection. In this study 0.5 - 3 (ml) of venous blood was collected from patients and healthy control group of children, which left to at room temperature to clot, the clotted specimens were centrifuged at 1500 r.p.m for 15 minutes to obtain pure serum and kept at deep freeze -80 ^{o}C for serological detection.

Viral detection

Estimation of the Serum Level of Immunoglobulin IgG, IgM, and Complement C3 andC4

Principle

The inoculated antigen (protein) in the plate well, diffusing in the agar gel radially, reacting with specific antibody incorporated in agar gel and form precipitin ring around the well. The

area within the ring is directly proportional to the initial concentration of analyzed antigen, and by comparing the diameter with a standard curve the precise concentration of antigen will be determined.

Procedure

Plate: Agarose gel containing mono specific antiserum to human plasma protein IgG, IgM, C3, C4. The envelope from plate its has been removed and left to stand for few minutes so that any condensed water in the wells can evaporate. The wells were filled with 5µl of sample and /or control. Then the plate has been closed with lid, after the sample has diffused into gel for a few minute, left to stand and then overturned into the envelope at room temperature for 48h. The diameters of the precipitating ring is measured and compared with standard table for each immunoglobulin. according to the instruction of Manufacturer Company of radial immunodiffusion.

Rapid Test Device

The rapid test device(CerTest) card is a one step colored chromato-graphic immunoassay for the qualitative detection of enteric virus types(*Rota , Adeno , Astero* and *Norovirus*). It can be used directly with stool specimens. Rapid test device was carried according to restriction manual of manufactur-ing company (CerTest-Spain), (Weinberg and Walker, 2005).

Viral DNA Extraction

Viral RNA/DNA were extracted using Viral Nucleic Acid Extraction Kit (Primer Design Ltd PrecisionTM Viral RNA/DNA extraction kit). according to the instruction of Manufacturer Company**.**

PCR amplification and gel electrophoresis

DNA of all isolates were subjected to PCR to detect Hexon genes for *Adenovirus*. The specific primers and reaction conditions that used in the work are shown in tables 1 and 2. Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products(Chaudhary and Payasi, 2014).

Table 1: Specific primers used in the present study.

Target gene	Sequence	Bp	Reference
Hexon for *Adenovirus*	F 5′- GCCACCGATACGTACTTCAGCCTG-3′ R 5′ -GGCAGTGCCGGAGTAGGGTTTAAA -3′	261	Rohayem et al., (2004)

Table (2): The thermal cycler conditions.

Gene Name	Temperature (°C) / Time					Cycles Number
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extension		
Hexon	94°C / 4 min	94°C /30 std	60°C /62 std	72°C/1min	72°C/7min	35 cycles

RESULTS AND DISCUSSION

The present study included 200 clinical stool specimen, 24(12%) were positive(causes virus) and the other 176(88%) isolates were considered negative results (Figure 1).

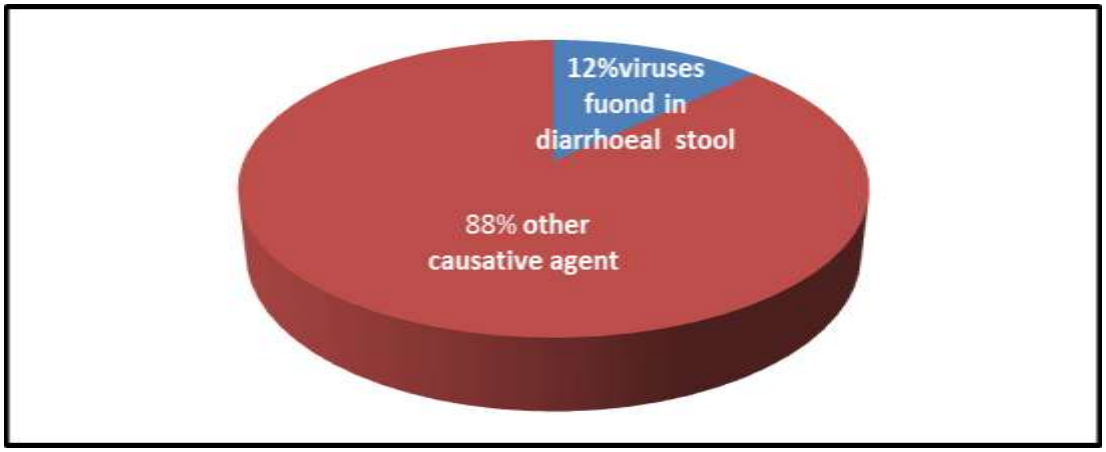

Figure (1): The occurrence of viruses isolated from 200 children with diarrhoea.

Regarding the characteristic of diarrhoeal clinical specimens it revealed highest frequency among male compared with female, as show in table(3). This result had the same opinion with Ibraheem (2016) who found that male was higher than females. Other studies by Al-Mayahi and Saleem (2005) and Barros and Lunet (2003) found no significant difference between male and female .The male is more than female because of environmental condition such as;(PH) and nutrition.

According this study diarrhoea is common in children understudy were less than 2 years of age 12(50%). However, Ali *et al.* (2009) who observed that children at this age were more susceptible to enteropathogenic diarrhea than older.

The higher infection percentage in these two groups of infants may be due to diminish of immunity, as the amount of trance-placental antibodies of the child starts dwindling after 6 months of age. Many researchers explained that the diarrhoea is one of the most common health problems during infant / early child age because their immunity system is less capable of controlling gastrointestinal infection Mirza *et al*. (1997).

Table 3: Distribution of the diarrhoea patients with hospitalization, gender and age.

Patient profile	**Status**	NO.(%) of sample
Hospitalization	outpatients	17(70.83%)
	inpatients	7(29.17%)
Gender	Male	15(62.5%)
	Female	9(37.5%)
Age group (years)	1month – 2years	12 (50%)
	2 - 4 years	6 (25%)
	4 - 6 years	4(16.66%)
	6 - 8 years	2(8.34%)

Serological test

Statistical differences were recorded in mean concentration in serum of IgG ,IgM ,C3 and C4 in patients (1926.3, 357.33, 286.28 and 83.80) respectively, when compared with it's mean concentration in sera of control group (1023 , 160.42 , 124.72 and 31.32) respectively ,as show in Table (4).

Table 4: Concentration of IgG, IgM, C3 and C4 in serum of patients infected with enteric virus and control group.

	IgG		**IgM**		**C3**		**C4**	
Group	**Patients**	**Control**	**Patients**	**Control**	**Patients**	**Control**	**Patients**	**Control**
Number of samples	15	5	15	5	15	5	15	5
Range	1816 – 2828	489-1254	291-438.6	55.6-177	193.66 – 374	82.5-148	56.5 – 149	19-43
Mean	1926.3	1023	357.33	160.42	286.28	124.72	83.80	31.32
SD.±	700.8	528.8	101.9	60	65.5	25	33.7	15.4
S.E.	180.9	227	26.3	26.8	16.9	11.1	8.7	6.8
N.V	190-1510		12-210		90-180		20-50	

The recent work demonstrated that IgG level in sera of infected patient was more than of control group, and these results were also established by Arinapour and Mohapatra (2003) who indicated the increasing in IgG concentration among patients indicator for enteric virus. Generally the elevated levels of IgG and IgM which documented in patients of the study may be due to the systemic sensitization of B – cells to viral antigen Valenzuela *et al.,* (2001).

The results like with Hess (2000) found high level of immunoglobulin in serum acute viral gastroenteritis. Few studies are available on the serum immunoglobulin levels in viral infections. Grohmann *et al*., (1993) significantly raised serum IgG and IgM levels. In another study was a significant fall in the serum IgG , IgM , C3 and C4 levels.

Rapid Test Device

Of a total (200) stool samples collected, only 24(12%) specimens were positive for viruses*; (Rota, Adeno, Astero and Norovirus)* were identified by this method according to the presence of red colored line in the test reaction zone and blue control reaction zone indicating to positive result figure (2 A,B,C). While it is absent according to the red color in the test reaction zone for other samples of 176(88%) but a green colored line was visible in the control reaction zone indicating to control only or negative result figure (2D).

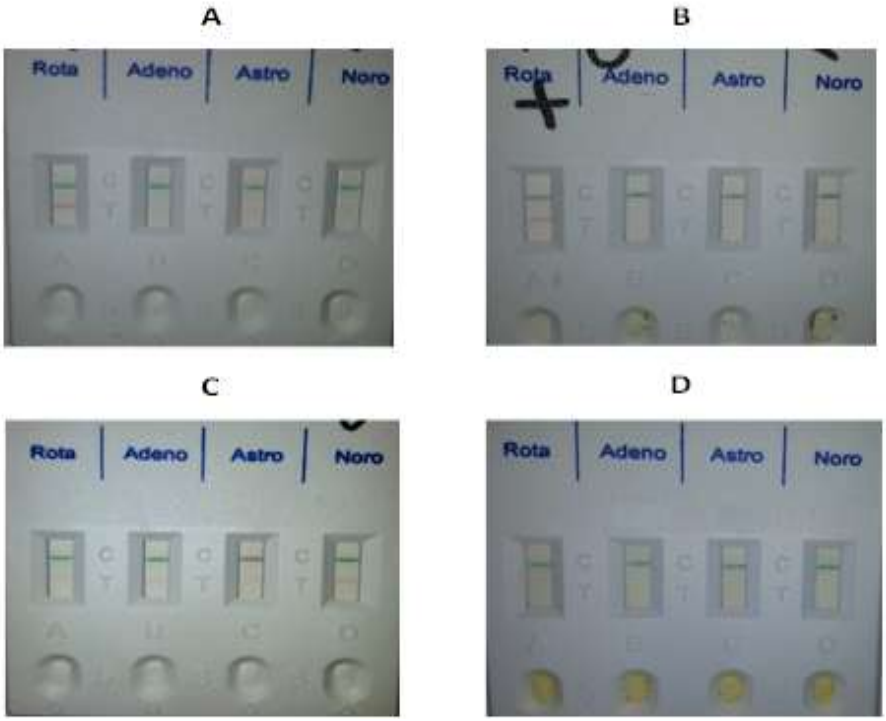

Figure (2): Rapid test device for detection of enteric virus(*Rota, Adeno, Astero* and *Norovirus*). A- positive *Rotavirus* B- positive *Rotavirus* and *Norovirus*. C- positive *Rota, Adeno ,Astero* and *Norovirus* . D- negative ruselt.

Resulting in only 24 positive specimens of viruses from 200 stool examined specimens, all patient children of the 24 specimens gave 4 types of viruses such as;(*Rota, Adeno, Astero* and *Norovirus)* table(5)**.**

Table 5: The positive results of viruses isolates from Diarrhoeal children.

Viruses species	No.	Percentage(%)
Adenovirus ssp	10	34.4%
Rotavirus ssp	8	27.6%
Asterovirus ssp	3	10.4%
Norovirus ssp	8	27.6%
Total	29	100%

Rapid *adenovirus* diagnostic tests are immunoassays that can identify the presence of *adenovirus* viral antigens in fecal specimens of diarrhea as a rapid diagnostic test Tran *et al.*, (2010). In previous studies done in Iraq, *Rotavirus* infections were high than *adenovirus*, *Norovirus* and *Asterovirus* in infants and young children Mahmood *et al*., (2015).

The *Norovirus* detection in children by rapid test resulted in only 8(27.6%) positive samples of *norovirus* from 200 stool. Increased in relative frequency of *norovirus* gastroenteritis cases may be due to implementing a rotavirus vaccination Patel *et al*.,(2009). A previous study conducted in Europe showed that *norovirus* is an important cause of acute gastroenteritis, *Norovirus* became the main viral cause of acute gastroenteritis among children younger than five years of age Bucardo *et al.*,(2014) ; Levidiotou *et al.*, (2009).

Regarded to *Adenovirus* detection in children by rapid test resulted in only 10(34.4%) positive samples of *Adenovirus* from stool. A rapid test is an easy and accurate test performed to diagnose a case. The findings of the present study are lesser than other study in Iran–Tehran that also indicate the spread of the *adenovirus* in the rate 8% of patients with diarrhea Rezaei *et al*., (2012). However, the findings of the present study of prevalence rates are lower than those reported in Canada (20.3%) Higgins *et al.*,(2011). The *Astrovirus* detection in children by rapid test raveled in only 3(10.4%) positive samples of *Astrovirus* from 200 stools. This study agreement with a study by (Mahmood *et al.*, 2015)in Baghdad who detected 3% of *Astrovirus* isolates from diarrhoea sources. Compatible results were reported in the America Koo *et al.*,(2012).

The *Rotavirus* detection in children by rapid test revealed in 8(27.7%) positive samples of *Rotavirus* from 30 stool. This study like with a study by Hussan (2012) in Baghdad who detected 36.4% of *Rotavirus* isolates from diarrhoea sources. After introduction of the Rotarix vaccine, which was correlated with an increase in the incidence of other viruses in many countries Rooney *et al.*,(2014).

Rotavirus, *Norovirus* and *adenovirus* are considered highly contagious agents which distribute in hospitals, schools, cruises and restaurants because transmission of this agents by direct person to person contact, aerosolization and contaminated water or food. Also these viruses can be transmitted by droplet through vomitus infected person in rooms, as a results of surviving fomites droplet on surfaces for long time these viruses have stable particles in the environment and extremely resistant to disinfections so this leads to outbreak infections especially in hospitals Kirby *et al.*,(2011).

Molecular detection of *Adenovirus*

The molecular result showed that the *Adenovirus* isolate carry the gene *Hexon* and they were 8(80%) isolate and other were negative as in figure (3). The results of this study are similar to a study by Moattari (2014) who detected 21.95 % of *Adenovirus* isolates from diarrhoea sources by *hexon* gene.

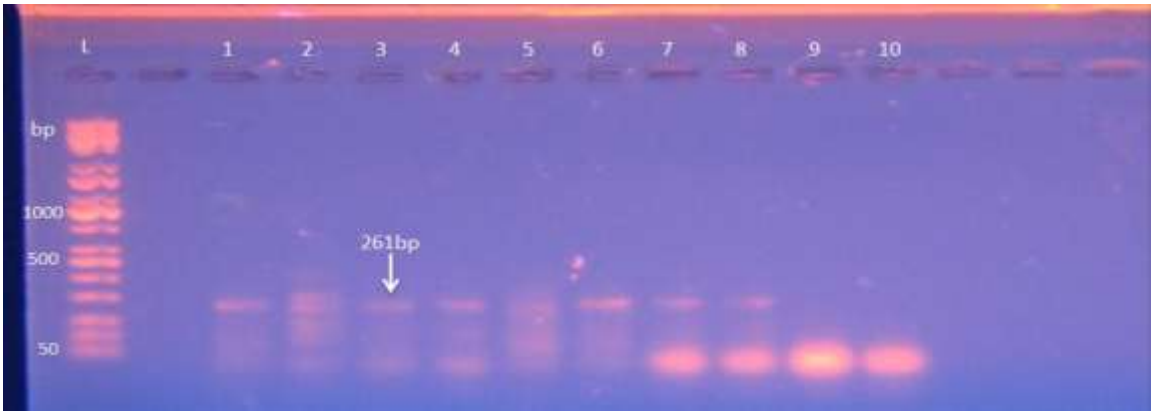

Figure (3): Ethidium bromide stained agarose gel PCR amplification products of Adenovirus isolates that amplified with *hexon* gene primers with product 261 bp. (1.5%agrose gel, 75 V, 1.20 hours). Lane (L), DNA molecular size marker (50-bp ladder), Lanes (1-8) show positive results with *hexon* gene, Lanes (9 and 10) show negative results with *hexon* gene.

REFERENCES

1. Ali, C. I.; Mahmood, A. R.; Jafar, N. A. and Khorsheed, S. (2009). Prevalence of enteropathogenic diarrhea in Children up to 2 years in Kirkuk province. Tikrit Med. J.; 15(2): 124-131.
2. Al-Mayahi, H. T. and Saleem, M. B. (2005). Diarrheal diseases among hospitalized children of less than two years of age, B. Med. J, 23(1): 1.

3. Anteneh, Z. A.; Andargie, K. and Tarekegn M. (2017). Prevalence and determinants of acute diarrhea among children younger than five years old in Jabithennan District, Northwest Ethiopia, 2014. BMC Public Health, 17: 99.
4. Arinapour, N. and Mohapatra, T.M. (2003). Study of anti – amoebic antibody in amoebiasis using ELISA and RID techniques. Iranian J. publ. Hlth, 32 (4): 13–18.
5. Barros, H. and Lunet, N. (2003). Association between child-care and acute diarrhea: a study in Portuguese children. Rev. Saud. Publica, 37(5): 607.
6. Bucardo, F.; Reyes, Y.; Svensson, L. and Nordgren, J. (2014). Predominance of norovirus and sapovirus in Nicaragua after implementation of universal rotavirus vaccination. PLoS ONE, 9: e98201.
7. Chaudhary, M. and Payasi A.(2014). Molecular characterization and in vitro susceptibilities of β-lactamase producing *Escherichia coli, Klebsiella species, Acinetobacterbaumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus* to CSE1034and other β-lactams. Asian Pac J Trop Med, 7(1): 217-223.
8. Grohmann GS, Glass RI, Pereira HG, Monroe SS, Hightower A.W., and Weber R.(1993). Enteric viruses and diarrhea in adenovirus -infected patients. Enteric Opportunistic Infections Working Group. N Engl J Med, 329; 14–20.
9. Hess, M. (2000). Detection and differentiation of adenoviruses: a review. In Avian Pathol, 29: 195-206.
10. Higgins, R.R.; Beniprashad, M.; Cardona, M.; Masney, S.; Low, D.E. and Gubbay, J.B. (2011). Evaluation and verification of the Seeplex Diarrhea-V ACE assay for simultaneous detection of adenovirus, rotavirus, and norovirus geno-groups I and II in clinical stool specimens. J. Clin. Microbiol, 49: 3154-62.
11. Hashi, A., Kumie, A. and Gasana, J. (2016) Prevalence of Diarrhoea and Associated Factors among Under-Five Children in Jigjiga District, Somali Region,Eastern Ethiopia. Open Journal of Preventive Medicine, 6: 233-246.
12. Hussan, B.M. (2012). Determination of the prevalence of viral aetiology of diarrhea in children less than 5 years of age in Baghdad province. *Quality Management Journal*, 8(14): 163-176.
13. Ibraheem, M.F. (2016). Etiology and Clinical Manifestations of Infectious Bloody Diarrhea in Children Welfare Teaching Hospital, The Iraqi Post-gradual Medical Journal, 15: 1.
14. Kim D.R.; Ali, M. and Clemens J.D. (2008). The high burden of cholera in children. comparison of incidence from endemic areas in Asia and Africa. Negl. Trop. Dis, 2: 173.

15. Kirby, A.; Dove, W.; Ashton, L.; Hopkins, M. and Cunliffe, N. A. (2011). Detection of norovirus in mouthwash samples from patients with acute gastroenteritis. J. Clin. Virol, 48(4): 285–287.
16. Koo, H.L.; Neill, F.H.; Estes, M.K.; Munoz, F.M.; Cameron, A.; Dupont, H.L. and Atmar, R.L.(2012). Noroviruses the most common pediatric viral enteric pathogen at a large university hospital after introduction of rotavirus vaccination. J. Pediatric. Infect. Dis. Soc, 2: 57-60.
17. Levidiotou, S.; Gartzonika, C.; Papaventsis, D.; Christaki, C.; Priavali, E.; Zotos, N.; Kapsali, E. and Vrioni, G.(2009). Viral agents of acute gastroenteritis in hospitalized children in Greece. Clin. Microbio.l Infect, 15: 596-598.
18. Mahmood, D.B., Al-suhail, R., Al-Hamdani, F.G. and Salih, R.A. (2015). Detection of Adenovirus antigen and its correlation with clinical signs among children with gastroenteritis. Iraqi Journal of Science, 56, 2C: 1633-1638
19. Mirza, N.M. Caulfield, L.E.; Black, R.E. and Macharia, W.M. (1997). Risk factors for diarrheal duration. Am. J. Epidemiol, 146: 85-776.
20. Moattari, A., Emami, A., Pirbonyeh, N. and Yaghoobi, R. (2014). Detection of Adenovirus Infection Among Children With Acute Respiratory Disease During 2010-2012 in Shiraz, Iran. Arch Pediatr Infect Dis, October; 2(4): e15930.Muller, E.C.; Morais, M.A.; Gabbay, Y.B.and Linhares, A.C.(2010). Detection of adenovirus in children with severe acute gastroenteritis in the city of Belém, Pará state, Brazil. Rev. Pan. Amaz. Saude, 1: 49.
21. Nair, G.B.; Ramamurthy, T.B.; hattacharya, M.K.; Krishnan, T.; Ganguly, S.; Saha, D.R.(2010). Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrheal patients in Kolkata, India. Gut. Pathog, 2: 4.
22. Patel, M. M.; Hall, A. J.; Vinje, J. And Parashar, U. D.(2009). Noroviruses: A Comprehensive Review. J. Clin. Virol, 44: 1-8.
23. Rezaei, M.; Amir, S.; Rosita, E.; Seyed, D.; Hosna, G.; Marziyeh, R. and Shahab, M. (2012). Molecular Epidemiology of Acute Gastroenteritis Caused by Subgenus F (40, 41) Enteric Adenoviruses in Inpatient Children. LAB. MEDICINE, 43: 1.
24. Rooney, B.L.; Pettipas, J.; Grudeski, E.; Mykytczuk, O.; Pang, X.L.; Booth, T.F.; Hatchette, T.F.; LeBlanc, J.J.(2014). Detection of circulating norovirus genotypes: hitting a moving target. Virol. J, 11: 129.

25. Sdiri-Loulizi, K.; Gharbi-Khelifi, H. and Rougemont, A. (2009). Molecular epidemiology of human astrovirus and adenovirus serotypes 40/41 strains related to acute diarrhea in Tunisian children. J. Med. Virol, 81: 1895-1902.
26. Tran, A.; Talmud, D.; Lejeune, B.; Jovenin, N.; Renois, F.; Payan, C.; Leveque, N. and Andreoletti, L.(2010). Prevalence of rotavirus, adenovirus, norovirus and astrovirus infections and coinfections among hospitalized children in northern France. J. Clin. Microbiol, 48: 1943-1946.
27. Valenzuela, O.; Ramos, F.; Moran, P.; Gonzalez, E.; Naladez, A.; Gomez, A.; Melendro, E.I.; Ramiro, M.; Munoz, O. and Ximenez, C. (2001). Persistence of secretary anti – amoebic antibodies in patients with past invasive intestinal or hepatic amoebiasis. Parasitol. Res, 87(10): 849-852.
28. Weinberg A., Walker M. L. (2005). Clinical and Diagnostic Labor-atory Immunology", 12(3): 367-370.
29. World Health Organization(WHO) (2013). Diarrhoeal Disease Fact Sheet N330. Retrieved 18 June 2014.