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## MOLECULAR AND GENETIC CHARACTERISTICS OF ADENOVIRUS AS CAUSES OF ACUTE DIARRHEA IN THE PEOPLE OF SOUTHERN UKRAINE

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The etiological role of adenoviruses as causative agents of acute diarrhea in the southern region of Ukraine was studied. Adenovirus DNA was detected in 10.61±1.05 % of clinical stool samples. Serotypes of two gene groups: C and F were identified during genotyping and sequencing of genetic material of detected adenovirus isolates. Gene group F (HAdV41), proves their role in the occurrence of this infectious pathology. HAdV41 isolates had a significant advantage 75.0±15.31 % and had a pronounced genetic heterogeneity, showed similarities with serotypes circulating in previous years in different regions of the world: Sweden (1978), Japan (2003, 2006), South Africa (2009–2014), Iraq (2016). The obtained results allowed to establish the geographical origin of circulating adenoviruses, as well as to predict a further increase in the intensity of the epidemic process of adenoviral infections in southern Ukraine.

Key words: acute diarrhea, etiological structure, adenoviral infection, molecular genetic features, epidemic process.

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## МОЛЕКУЛЯРНО-ГЕНЕТИЧНІ ХАРАКТЕРИСТИКИ АДЕНОВІРУСІВ, ЯК ЗБУДНИКІВ ГОСТРИХ ДІАРЕЙ У ЖИТЕЛІВ ПІВДНЯ УКРАЇНИ

Вивчали етіологічну роль аденовірусів, як збудників гострих діарей у жителів південного регіону України. ДНК аденовірусів виявлені в клінічних зразках стільця хворих у 10,61±1,05 % випадків. При генотипуванні та секвенуванні генетичного матеріалу виявлених ізолятів ідентифіковано серотипи двох геногруп: С та F. У 25,0±11,69 % хворих на гостру діарею у вигляді моноінфекції було виявлено аденовіруси геногрупи С: HAdV2 і HAdV5, що поряд з серотипами геногрупи F (HAdV41), доводить їх роль у виникненні даної інфекційної патології. Ізоляти HAdV41 зустрічались із суттєвою перевагою 75,0±15,31 % та мали виражену генетичну гетерогенність, виявляючи подібність з серотипами, що циркулювали у попередні роки у різних регіонах світу: в Швеції (1978 р.), Японії (2003, 2006 рр.), Південній Африці (2009–2014 рр.), в Іраку (2016 р.). Отримані результати дозволили встановити географічне походження циркулюючих аденовірусів, прогнозувати подальше зростання інтенсивності епідемічного процесу аденовірусної інфекції на півдні України.

**Ключові слова:** гострі діареї, етіологічна структура, аденовірусна інфекція, молекулярно-генетичні особливості, епідемічний процес.

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Diarrheal diseases (DD) are the most common acute respiratory diseases in countries with different socio-political systems [6–8], the eighth largest cause of annual death among all age groups, which leads to more than 1.6 million deaths, and the fifth cause of death among children under 5 years worldwide. The disease and death rate from the DD increases in developing countries, where diarrhea caused by various pathogens is one of the major public health problems [9, 10].

International migration, inter-governmental exchange of food and raw materials of animal origin, urbanization, intensification of recreational processes, climatic changes and deterioration of ecological situation contribute to the high-level incidence of the DD [12, 15].

Over the last decades, there has been pronounced dominance of viruses as a reason, which share is brought to 80 % of all DD in children's age [1, 2, 9], which are rapidly spread in organized groups, have a turbulent beginning, non-specific clinical manifestations with rapid development of exsiccosis [11]. 8 different families of viruses: Reoviridae (Rotavirus), Caliciviridae (Norovirus, Sapovirus), Adenoviridae (Mastadenovirus), Astroviridae (Astrovirus), Picornaviridae (Enterovirus, Parechovirus, Kobuvirus), Coronaviridae (Coronavirus, Torovirus), Parvoviridae (Bocavirus), Picobirnaviridae (Picobirnavirus) are associated with acute gastroenteritis [2, 10, 13].

**The purpose** of the study was to clarify the etiological role of adenoviruses as the pathogens of diarrheal diseases in residents of the south of Ukraine and their genetic features for improving the measures of epidemiological surveillance of this group of infections.

**Materials and methods.** To achieve the purpose, we performed study samples of clinical material from patients, hospitalized with the diagnosis of the acute diarrhea in infectious hospitals in three regions.

In the period from July to September 2018, 916 patients were hospitalized in Mykolayiv Regional Center for the Treatment of Infectious diseases (“MRCTID”) and in Odesa City Clinical Infectious Hospital (“OCCIH”) – 5466, In Kherson Regional Infectious Hospital named after. G.I. Gorbachevsky (“KRIH”) – 731, totally 7113 patients.

The method of simple randomization in 419 patients was taken clinical material, including are 89 – from “MRCTID”, 225 – from “OCCIH”, 105 – from “KRIH”. At local laboratory bases, all samples were tested with the help of bacteriological method using the analyzer and corresponding test systems. In those clinical samples where bacterial activators were not found, the PCR-research was conducted. In each sample the genetic material of Adenovirus (AdV) 40 and 41 serotypes (Adenovirus) and other non-intestinal serotypes (Adenovirus resp) were revealed. Such researches were carried out in 134 samples of clinical material of patients “OCCIH”, 25 – from “MRCTID” and 28 – from “KRIH”, in total in 193 samples. Molecular-genetic studies were conducted using the PCR method with a reverse transcription. The study of molecular-genetic characteristics of AdV isolates was carried out by the method of sequencing a fragment of the gene amplification of hexon (a protein of capsid) containing a hypervariabelic region [14].

**Results of the study and their discussion.** At bacteriological research, the pathogens were found in:  $71.91 \pm 4.76$  % of the selected clinical samples in patients from Mykolayiv region,  $40.44 \pm 3.27$  % from Odesa and  $62.86 \pm 4.79$  % from Kherson region. In the rest of the samples:  $28.09 \pm 4.76$  %,  $59.56 \pm 3.27$  % and  $37.14 \pm 4.79$  % (respectively) performed PCR-research for presence of AdV.

In the study of 134 samples of clinical material of patients “OCCIH” in 11 –  $8.21 \pm 2.37$  % DNA of AdV was found. Of the 28 specimens of patients with “KRIH” 7 positive –  $25.0 \pm 6.07$  % were found, of the 25 specimens of patients with “MRCTID” DNA of AdV found in 5 –  $20.0 \pm 8.00$  % of cases. Total in clinical material of 193 patients with dearch in 23 cases  $11.92 \pm 1.05$  % DNA of AdV was detected (fig. 1).

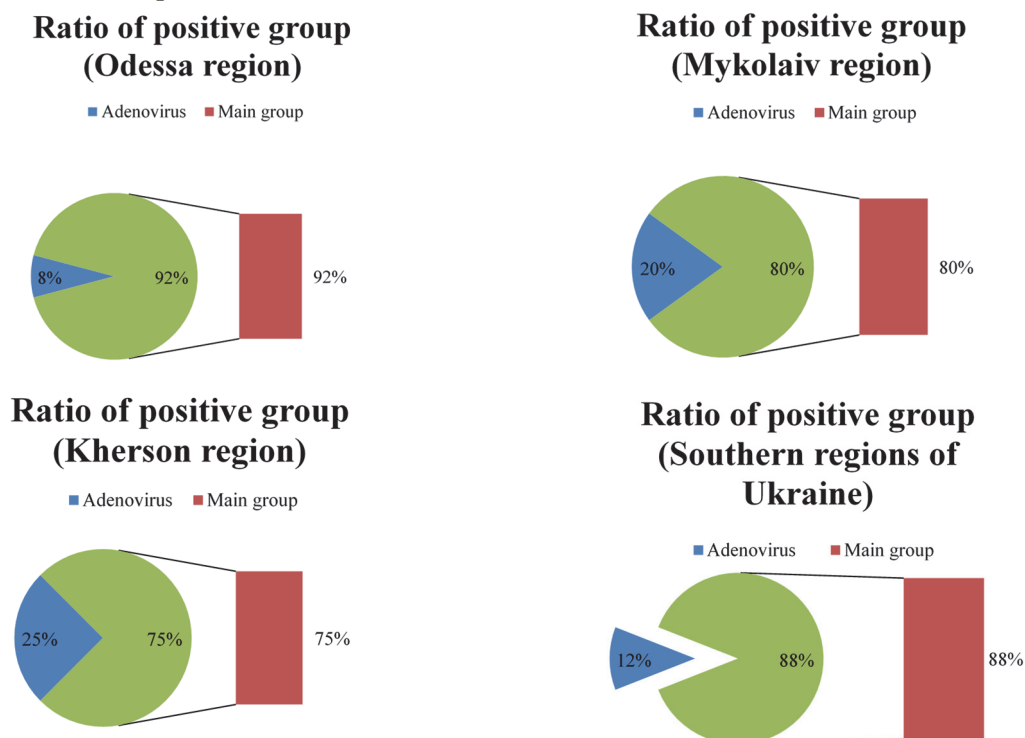


Fig. 1 the frequency of detection of adenoviruses (in %) in clinical samples of patients from Odessa, Mykolaev, Kherson regions and southern Ukraine.

The next stage of this work was the study of molecular-genetic characteristics of the isolates of AdV. As a result of genotyping and DNA sequencing of 8 AdV isolates, which were selected by the blind sampling method, 3 different serotypes were identified: HAdV2, HAdV5 and HAdV41. Among the serotypes of ADH circulating in the region the significant advantage was HAdV41 ( $75.0 \pm 15.31$  %), which is installed in 6 isolates (fig. 2). The other two AdV serotypes, HAdV2 and HAdV5, met at the same frequency in 1 cases ( $12.5 \pm 11.69$  %).

### Ratio of established Adenovirus serotypes

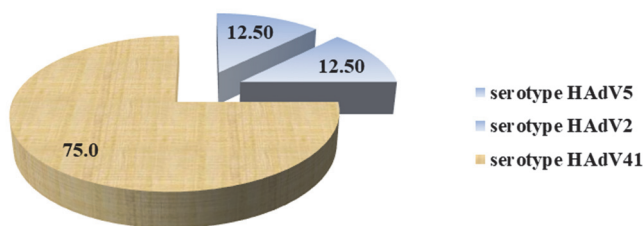


Fig. 2 Frequency of established serotypes of adenoviruses, circulating in southern Ukraine in 2018

At the next stage of this study, the genetic heterogeneity of detected isolates of serotype 41 was evaluated. Within the type there are 2 genotype clusters – GTC1 and GTC2. Phylogenetic analysis of nucleotide sequences of AdV (fig. 3) showed that the investigated isolates belonged to two clusters mentioned above – isolate No. 30, 31 and 35 were included in GTC1, and isolate No. 145, 146 and 162 belonged to GTC2. The share of nucleotide differences between sequences in isolates that belonged to GTC1 and GTC2 was 5.5–5.8 %. All nucleotide sequences in isolates that belonged to GTC2 had 100 % similarity. The nucleotide sequences of the isolates that belonged to the GTC1 differed by 2.3 % among themselves. In this case, the number 35 isolation revealed in the investigated region 100 % similarity with the virus circulating in Sweden in 1978 (Access code GenBank: KX868407) and in Japan in 2006 (Access code GenBank: DQ336391). The other No. 31 was 99 % similar to the headquarters identified in South Africa in 2009-2014, Iraq in 2016, Japan in 2003 (Access codes GenBank: AB103341, MK962806, MK962807, MG925782).

The results show the genetic heterogeneity of the detected AdV in patients with acute diarrhea, which within the framework of the HAdV41 serotype include both different genotypes and different genovaries.

Thus, as a result of genotyping of AdV, circulating was examined in southern Ukraine in 2018. Three different serotypes were detected: HAdV2, HadV5 and HAdV41. Among AdV significant advantages was the serotype HAdV41 (75.0±15.31 %). The other two serotypes met at the same frequency (12.5±11.69 %). The results obtained by us of research of clinical material of patients with the gastrointestinal tract have shown the presence of monofilection of AdV (HAdV2 and HadV5), which along with AdV of genomic group F (HAdV41) show their role in the occurrence of this infectious pathology. This complements the evidence base for the role of the gene group C (HAdV2 and HadV5) in acute diarrhea [3, 15].

It has been found that 11.92 % (23 of 193) of the tested samples of non-bacterial etiology, which were registered in the south of Ukraine in 2018, The reason for acute diarrhea was AdV, including 43.48 % of AdV found in the form of monofilection, 49.42 % with other viruses (Norovirus I/II, Sapivirus, Astrovirus, Enterovirus), and 8.70 % with other viruses and bacterial pathogen (Campylobacter). In most cases, symptoms of intoxication and diarrhea prevailed, vomiting prevailed in the first two days of the disease. The data are matched with the results of research by other authors in different countries, where these authors are among the most common [7, 8]. In a study of 871 clinical specimens of children under 5 years of age who have been diagnosed with gastroenteritis in Bangladesh during 2012-2015, HAdV DNA was found in 93 cases (10.70 %). Among them, 44.0 % of AdV were found in the form of mono-infection, and 56.0 % were found with other virus and bacterial pathogens. Serious illness was marked in 31.6 % of cases. Five types of HAdV (A B, C, D, and F) were identified during the study period, which included 17 different genotypes, with the HAdV-40/41 dominant. Non-intestinal HAdV serotypes were found in a significant part of the samples (15.0 % of genotype C, 15.0 % D, 10.8 % A and 4.3 % B) [5, 10].

The results of our research show a high genetic diversity of patients with AdV acute diarrhea: Intestinal, and non-intestinal genovaries. This confirms the need to further define the role of other HAdV gengroups in the development of diarrheal diseases.

The data obtained by us on molecular characteristics of 8 HAdV isolates, which appeared in patients with acute diarrhea, showed the advantage of serotype 41, which belongs to genotype F. the role of HAdV-F in etiology of infectious pathology of the gastrointestinal tract in the modern period is not questionable.

Detection in patients with acute diarrhea of monoviral AdV infection (HAdV2, HadV5 – Genogroup C) along with AdV Genogroup F (HAdV41), testifies to their role in appearance of this infectious pathology, which is called with results of group of authors [3]. According to the modern classification of human adenoviruses there are seven genomegroups (HAdV – A, B, C, D, E, F, G). Previously, a connection was established between the AdV genomic group and the specific clinical image of the disease [4, 15], This can be the result of different tropical tissues: Viruses of genomic group B1, C and E cause respiratory infections, B2 - infect liver and urinary tract, F and G – are gastroenteritis, Several AdV D are associated with an epidemic of keratoconjunctivitis.

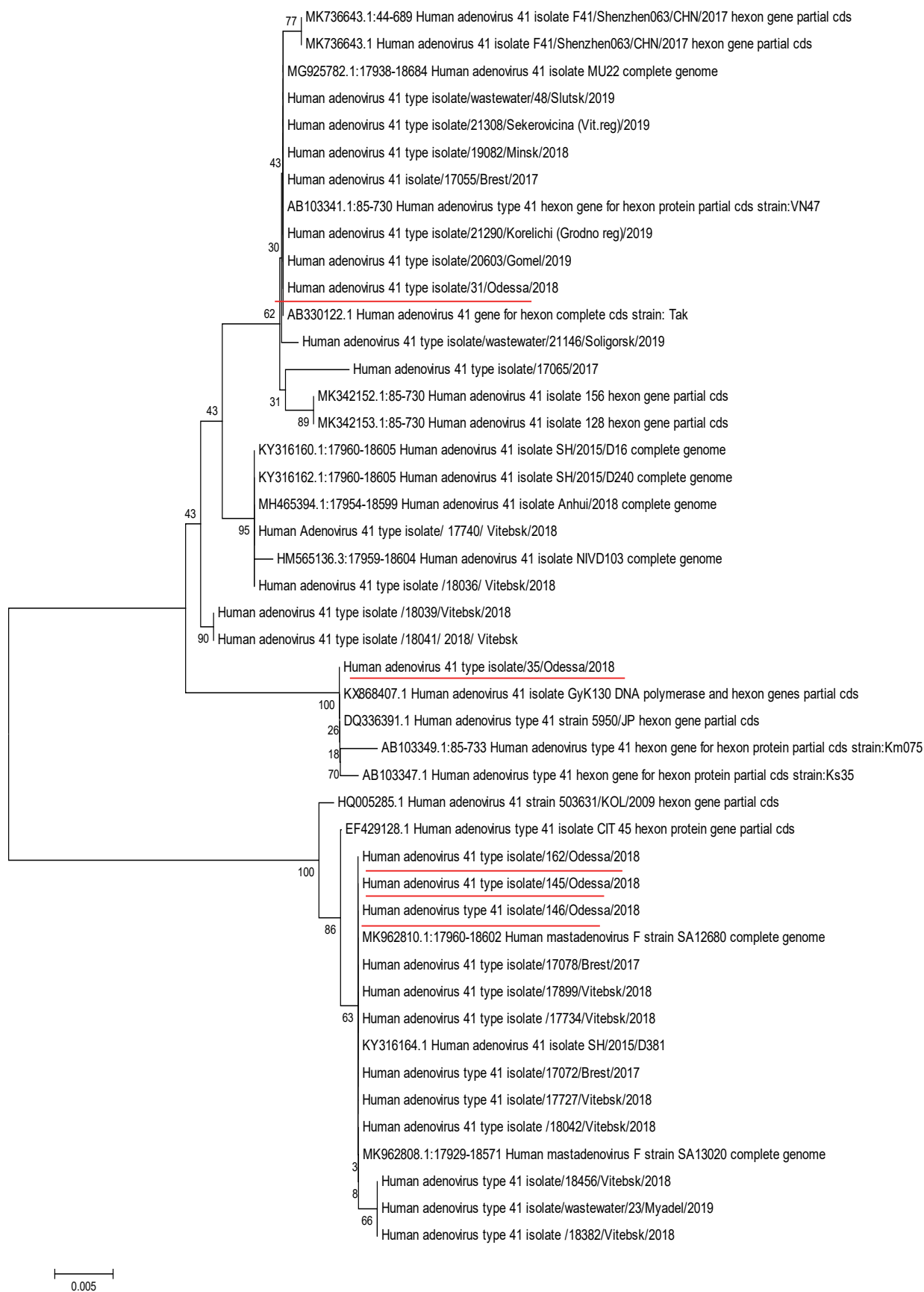


Fig. 3 Phylogenetic analysis of isolates of HAdV41, conducted by a fragment of gene hexon (a protein of capsid) found in clinical material of the patients southern region in 2018

The results of the genotyping of AdV found in 6.3 % of children under 3 living in China showed the presence of a intestinal genotype (HAdV41) in 4.2 % of them, non-intestinal genotypes (HAdV1, HAdV2, HAdV7, HAdV12, HAdV31) – 2.1 %, which is a result of our results [15].

At the same time, in a number of literary sources, the authors disbelieve whether the detection of AdV of other genogroups by accident and reflects their long persistence after previous infection, or non-intestinal AdV can independently cause acute diarrheal diseases [10, 11].

It is necessary to expand the spectrum of diagnostic tests on viruses. In the protocols of providing medical care to patients with acute diarrhea from group fires, a specific study is required to establish arson of viral diarrheal diseases [1]. It is the percentage of cases of viral infections that are ethiologically deciphered that characterizes its real severity and prevalence.

### Conclusions

1. DNA of AdV was found in  $11.92 \pm 1.05$  % of samples of clinical material of patients. On separate territories of the North-West Black Sea region, the frequency of detection of AdV did not differ significantly.

2. 3 different types of AdV serotypes are identified: HAdV2, HadV5 and HAdV41, with the prevalence of the last  $75.0 \pm 15.31$  %, within which genetic heterogeneity is established.

3. Detection in patients with diarrheal diseases of monoviral AdV-infection of genomic C (HAdV2 and HadV5) shows their role in the emergence of this infectious pathology, along with AdV of genomic F (HAdV41).

### References

- Dziublyk IV, Samborska IF, Kotlyk LS, Tykhenko NM, Khatynska ZhV. Molekuliarno-henetychni osoblyvosti norovirusiv v Ukraini. Profilaktychna medytsyna. 2015. 1–2(24). S. 41–46. [in Ukrainian]
- Malyi VP, Kozishkurt EV. Ostrye kishhechnye infektsii s diareynym sindromom. Patogenez, klinika, lechenie. Monografiya. Kiev. «OLFA» 2019. 199 s. [In Russian]
- Afrad MH, Avzun T, Haque J, Haque W, Hossain ME, Rahman AR et.al. Detection of enteric- and non-enteric adenoviruses in gastroenteritis patients, Bangladesh, 2012–2015. J Med Virol. 2018 Apr; 90(4):677–684. doi: 10.1002/jmv.25008.
- Banerjee A, De P, Manna B, Chawla-Sarkar M. Molecular characterization of enteric adenovirus genotypes 40 and 41 identified in children with acute gastroenteritis in Kolkata, India during 2013–2014. J Med Virol. 2017 Apr; 89(4):606–614. doi: 10.1002/jmv.24672.
- Elmahdy EM, Ahmed NI, Shaheen MNF, Mohamed EB, Loutfy SA. Molecular detection of human adenovirus in urban wastewater in Egypt and among children suffering from acute gastroenteritis. J Water Health. 2019 Apr; 17(2):287–294. doi: 10.2166/wh.2019.303.
- Gao Z, Mahe M, Tuohetamu S, Li F, Zhang J, Xia Y et al. Survey of Traveler's Diarrhea: Epidemiology and Testing Reveal the Source. Can J Infect Dis Med Microbiol. 2019 Nov 29; 2019:3569840. doi: 10.1155/2019/3569840.
- GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017 Sep 16; 390(10100):1211–1259. doi: 10.1016/S0140-6736(17)32154-2.
- GBD 2017 Diarrhoeal Disease Collaborators. Quantifying risks and interventions that have affected the burden of diarrhoea among children younger than 5 years: an analysis of the Global Burden of Disease Study 2017. Lancet Infect Dis. 2020 Jan; 20(1):37–59. doi: 10.1016/S1473-3099(19)30401-3.
- Kotloff KL. The Burden and Etiology of Diarrheal Illness in Developing Countries. Pediatr Clin North Am. 2017 Aug; 64(4):799–814. doi: 10.1016/j.pcl.2017.03.006.
- Kozishkurt O, Babienko V, Golubyatnikov M, Amvrosieva T, Maksymenko Yu, Savchuk A Modern etiological structure of acute gastroenterocolitis in the Southern Ukraine. Georgian medical news. 2019; 5 (290): 77–85.. PMID: 31322520.
- Mayindou G, Ngokana B, Sidibé A, Moundélé V, Koukouikila-Koussounda F, Christevy Vouvongui J, et al. Molecular epidemiology and surveillance of circulating rotavirus and adenovirus in Congolese children with gastroenteritis. J Med Virol. 2016 Apr; 88(4):596–605. doi: 10.1002/jmv.24382.
- Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. Clin Infect Dis. 2017 Nov 29; 65(12):45–80. doi: 10.1093/cid/cix669.
- Tang X, Hu Y, Zhong X, Xu H. Molecular Epidemiology of Human Adenovirus, Astrovirus, and Sapovirus Among Outpatient Children With Acute Diarrhea in Chongqing, China, 2017–2019. Front Pediatr. 2022 Mar 3; 10:826600. doi: 10.3389/fped.2022.826600.
- Wang X, Wang D, Umar S, Qin S, Ling Q, Gray GC, et al. Molecular typing of human adenoviruses among hospitalized patients with respiratory tract infections in a tertiary Hospital in Guangzhou, China between 2017 and 2019. BMC Infect Dis. 2021 Aug 3; 21(1):748. doi: 10.1186/s12879-021-06412-0.
- Zhang QL, Wang HB, Wang YL, Zhou JS, Cai J, He RR. Genotypes of adenoviruses in infants and young children with diarrhea. Zhongguo Dang Dai Er Ke Za Zhi. 2016 Aug; 18(8):718–20. doi: 10.7499/j.issn.1008-8830.2016.08.009. [In Chinese]

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