



Article

# Molecular Genotyping of Human Adenovirus Infections from Gastrointestinal Tract and Conjunctivitis Patients

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**Abstract:** Human adenovirus is a common human viral pathogen especially of eye infection and gastroenteritis. This study includes detection of most circulating human adenovirus types implicated in both of conjunctivitis and gastroenteritis in Iraqi patients. Conjunctival swabs and stool samples taken separately from both of Keratoconjunctivitis and diarrheal patients with adenovirus infection. Viral DNA were taken and primers constructed specifically and then used to multiply the targeted gene. 10 positive specimens sent for sequencing for more analysis. The results revealed the out of around 80 patients of 10 samples give positive results for hexon gene and then sent for partial sequencing of hexon gene. The results showed that the local human adenovirus conjunctivitis isolates (IQC-1, IQC-3, and IQC-5) revealed close linked to human adenovirus type 8. The locally adenovirus conjunctivitis isolates (IQC2 and IQC4) and the local gastroenteritis isolates (IQ11 and IQ12) revealed closed linked to human adenovirus type 56. The locally gastroenteritis isolates (IQ13, IQ14) revealed close linked to human adenovirus type 29. The locally gastroenteritis isolates (IQ15) revealed close linked to human adenovirus type 15 at total genetic changes (0.20-0.050%). The current study demonstrated the potential implication of human adenovirus type D8 and D56 in eye infections especially conjunctivitis and HAdV-D29, D56, D15 in gastroenteritis.

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## 1. Introduction

Human adenovirus (HAdVs) is a common virus that is associated with a wide range of human infections, involving upper respiratory problems, high fever, pneumonia, gastroenteritis, and conjunctivitis [1].

HAdV belongs to the genus Mastadenovirus that belongs to the Adenoviridae family, contains non-enveloped dsDNA, and possesses an icosahedral structure virus. Approximately, there are over 100 serotypes were recognized and categorized into 7 species (HAdV A to G) since the first detection in 1953 by the team of Rowe et al, This classification is based on the biological properties and genetic features with progression of phylogenetic analysis and bioinformatics techniques. Variable serotypes in turn exhibit variable tissue tropisms and may lead to different clinical manifestations ranging from mild to severe diseases represented by multiple-organ failure in immunocompromised individuals [2], [3], [4], [5].

HAdV can cause infections at any human age, but most frequently occurs in pediatric groups, predominantly in kids and infants [6], due to their inadequately developed immunity. The children are more sensitive to diarrheal manifestations [7]. These quite

stable viruses are distributed globally. Infections incidence are usually occurred by the breathing in droplet that aerosolized from an infected person, typically conjunctively inoculation route faecal-oral spread. HAdVs usually persist in the completely gastrointestinal tract (GIT) of an infected person, and the identification of the load of viruses in the feces offers early evidence of HAdV infections [8].

Diarrheal illnesses associated with enteric serotypes of HAdVs are prevalent among children, particularly infants. Enteric serotype of HAdv is described by its capability for stability in the intestines and transmission by consumption of the contaminated food and drink. The majority of the clinical symptoms, such as "low-grade fever, vomiting, abdominal pains, and dehydration," developed after an incubation period of (7-10) days [9].

The period of incubation related adenoviral eye infections ranges 5 to 12 days, manifestations related to Adenovirus induced conjunctivitis infection include (redness of conjunctiva, edema of conjunctiva, and flowing) include inflammation path analogous to host immune responses that occurred with different viral infections such as herpes ocular infections, conjunctival-pharynx fever, conjunctival-keratitis outbreak, and follicular non-specific conjunctiva inflammation [10]. Adenovirus illnesses of the surface of the eye are frequent, highly contagious, and lead to optical morbidity. There is no particular antiviral factor available for ocular adenoviral infections. Instead, supportive treatment and proper hygiene protocol are recommended to minimize its transmission; the topical antibiotics have no role [11].

In this study, we aim to evaluate the molecular characteristics of circulating HAdV in some Iraqi patients to get an adequate knowledge of the circulating genotypes of HAdV related to gastroenteritis and conjunctivitis burden in this region.

## 2. Methodology

Clinical swabs were collected from keratoconjunctivitis and gastroenteritis patients with adenovirus suspected infections. Extracting of viral DNA were applied and specifically pairs of primer were designed for hexon gene detection was used to multiply the required gene, only 10 positive specimens forwarded to be sequenced. All ten HAdV were further analyzed in NCBI-BLAST [National Centre for Biotechnology Information. The multiple alignment analysis was constructed using (ClustalW alignment tool). The construction of phylogenetic tree was performed using (UPGMA tree) in (MEGA 6.0 edition).

## 3. Results and Discussions

The method of DNA sequencing was performed to recognize genetic variations in the hexon gene of regional HAdV eye conjunctivitis and GIT infection isolates and NCBI-Blast-associated Human adenovirus genotypes isolates.

The genetic relationship revealed by the phylogenetic tree demonstrated that the local HAdv conjunctivitis (IQC.1, IQC.3, and IQC.5) were revealed closely linked to NCBI-BLAST HAdv type 8. The locally HAdv eye conjunctivitis (IQC.2 and IQC4) and the locally HAdv GIT (IQ11 and IQ12) were revealed to be closely linked to NCBI-BLAST HAdv type 56. The locally HAdv GIT (IQ13, IQ14) were revealed to be closely linked to NCBI-BLAST HAdv type 29. The locally HAdv GIT (IQ15) was shown to be closely linked to NCBI-BLAST HAdv type 15 at total genetic variation (0.20-0.050%), as appeared in Figure 1.

The homological of sequence identity that existed between locally HAdV eye conjunctivitis and GIT infection isolates and NCBI-BLAST related HAdV-genotypes

revealed genetic similarity sequence identity extended from (99.12-99.84%) as appeared in table (1).

Lastly, the locally HAdV eye conjunctivitis and GTI infection isolates were admitted into NCBI Genbank and recognized by accession numbers (OP558007.1-OP558016.1).

OP558008.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558010.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558013.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558012.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558014.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558016.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
OP558015.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
DQ149627.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
LC128918.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
AB330096.1	TATGGGTGGAGAAAACATTACAATTAGCGGTTTGCAAATTTGGAACAGATACTACAAAT--
DQ149618.1	TATGGGAGGAACAAATATTACAACCAGGGTTTGTAAATTTGGAACAGATACTACAAAT--
AB500123.1	CATGGGAGGCAGTAATATTTCTAAAGACGGTTTGCAGATTTGGAACGACAAAACAGCA--
DQ149638.1	TACAGGTGGTTTTAATATTACAGATCAGGGTTTGTACTTTGGAACGAGGAAAACAGCTGA
DQ149619.1	CATGGGTGGAGAAGACATTACAGAAAAGGTTTCAAAATTTGCAATTTGATGAACTAAAGA
OP558009.1	CACTGGCGGATATAACATTACAAAAGAAGGACTTCAGATTTGGAATTTGATGAACTAAAGA
OP558011.1	CACTGGCGGATATAACATTACAAAAGAAGGACTTCAGATTTGGAATTTGATGAACTAAAGA
OP558007.1	CACTGGCGGATATAACATTACAAAAGAAGGACTTCAGATTTGGAATTTGATGAACTAAAGA
AB023546.1	CACTGGCGGATATAACATTACAAAAGAAGGACTTCAGATTTGGAATTTGATGAACTAAAGA
	* ** ** * * * * * * * * * * * * * * * *
OP558008.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558010.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558013.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558012.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558014.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558016.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
OP558015.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
DQ149627.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
LC128918.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
AB330096.1	-GGCAAACAAGACCCGATATATGCTAATAAGCTGTATCAACCAGAGCCTCAAGTAGGAGA
DQ149618.1	TAATCCTCAAAGGAAATCTTTGCAGACAAAATTTCCAGCCAGAACCTCAAGTAGGAGA
AB500123.1	-AATGCTGAAAACCAATCTATGCAGACAAAATTTCCAGCCAGAACCTCAAGTTGGAGA
DQ149638.1	AAACGTTAAAAAGGATATCTATGCAGAGAAAATTTCCAGCTGAACCTCAAGTTGGTGA
DQ149619.1	AGAAAATAACAAG--ATATTTGCCAATGAAATATACCAACCAGAACCTCAAGTTGGGAGA
OP558009.1	AGATCCAAACAACAAAATTTATGCAGATAAAAACATTTCCAGCCGGAACCAAAATAGGGGA
OP558011.1	AGATCCAAACAACAAAATTTATGCAGATAAAAACATTTCCAGCCGGAACCAAAATAGGGGA
OP558007.1	AGATCCAAACAACAAAATTTATGCAGATAAAAACATTTCCAGCCGGAACCAAAATAGGGGA
AB023546.1	AGATCCAAACAACAAAATTTATGCAGATAAAAACATTTCCAGCCGGAACCAAAATAGGGGA
	* ** * * * * * * * * * * * * * * * *
OP558008.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558010.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558013.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558012.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558014.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558016.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
OP558015.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
DQ149627.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
LC128918.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
AB330096.1	AGAAAACGGCAGGAAACAGAAGCCTTCTATGGAGGAAGGGCTCTTAAAAAGGAAACCAA
DQ149618.1	GGAAAACGGCAAGACCAATGCATTTTATGGAGGCAGGGCTCTTAAAGAGGAAACTAA
AB500123.1	AGAAAACGGCAGGATAATGATGAATATTATGGCGGCAGGGCTCTTAAAAAGGATAACCAA
DQ149638.1	AGAAAACGGCAGGAAAGTGAAGCCTTTTATGGAGGAAGGGCTATTAAGAAAGACACCAA
DQ149619.1	AGAAAACGGCAGGAAACATTTGTTTTTTATGGCGGTAGAGCTCTTAAAGAGGATAACCAA
OP558009.1	AAATAATTGGCAGGACACTAATGTTTTCTATGGCGGCAGAGCTCTTAAAAAGGAAACCAA
OP558011.1	AAATAATTGGCAGGACACTAATGTTTTCTATGGCGGCAGAGCTCTTAAAAAGGAAACCAA
OP558007.1	AAATAATTGGCAGGACACTAATGTTTTCTATGGCGGCAGAGCTCTTAAAAAGGAAACCAA
AB023546.1	AAATAATTGGCAGGACACTAATGTTTTCTATGGCGGCAGAGCTCTTAAAAAGGAAACCAA
	* ** * * * * * * * * * * * * * * * *
OP558008.1	GATGAAACCATGCTATGGCTCATTGCCAGACCCACAAATGAAAAGGAGGACAGGCAA
OP558010.1	GATGAAACCATGCTATGGCTCATTGCCAGACCCACAAATGAAAAGGAGGACAGGCAA
OP558013.1	GATGAAACCATGCTATGGCTCATTGCCAGACCCACAAATGAAAAGGAGGACAGGCAA



**Table 1.** The NCBI-BLAST analysis of genetic diversity analysis among locally isolated of HAdV and NCBI-BLAST closely linked HAdV-genotype isolates:

Adenovirus isolates	Accession number	Homology sequence identity (%)		
		Identical genotype	Accession number	Identity (%)
IQC.1	OP558007.1	Type 8	AB023546.1	99.37%
IQC.2	OP558008.1	Type 56	LC128918.1	99.68%
IQC.3	OP558009.1	Type 8	AB023546.1	99.84%
IQC.4	OP558010.1	Type 56	LC128918.1	99.12%
IQC.5	OP558011.1	Type 8	AB023546.1	99.32%
IQI1	OP558012.1	Type 56	LC128918.1	99.37%
IQI2	OP558013.1	Type 56	LC128918.1	99.12%
IQI3	OP558014.1	Type 29	DQ149627.1	99.84%
IQI4	OP558015.1	Type 29	DQ149627.1	99.48%
IQI5	OP558016.1	Type 15	AB330096.1	99.52%

#### 4. Discussion

Worldwide, adenovirus infections are common and mainly cause gastroenteritis and respiratory and ocular symptoms in young children and infants [12]. The most frequent cause of viral conjunctivitis has been proposed to be HAdVs, which are considered responsible for 65–90% of cases [13]. The infectious condition known as adenoviral conjunctivitis can be contracted in the community or nosocomially. Particularly frequent nosocomial conjunctivitis infections cause serious outbreaks in ophthalmology units [14].

On the other hand, Diarrheal disease is a major cause of death and disability globally, and adenovirus gastroenteritis can result in severe illness and frequent hospitalization [15].

Worldwide, acute gastroenteritis illness (AGE) is a main cause of mortality and morbidity among young children and infants, especially in low- and middle-income nations. Gastroenteritis viruses cause more than 70% of all diarrheal episodes [16]. HAdV is a significant virus that has been identified as causing diarrhea in children is the human adenovirus (HAdV) [17].

Based on this, this study was conducted on patients with conjunctivitis and gastrointestinal to identify the most important types of adenovirus associated with these infections in Iraq.

According to the present study, adenovirus type 8 appears to be obviously associated with a severe form of conjunctivitis named as epidemic keratoconjunctivitis (EKC), which in turn is consistent with many other similar studies worldwide, such as (Akhil et al.,2016; Otta et al.,2021; Adhikary and Banik,2014; Yawta et al.,2016; Singh et al.,2015; Jonas et al.,2016; Shieh,2022) [18], [19], [20], [21], [22], [23], [24].

While, present study showed, contrary to what is known, some types of adenovirus, which are originally non-enteric but primarily their clinical impact is often seen in the eyes, such as type 15,29,56, have been isolated from patients suffering from adenovirus gastrointestinal infections that enrolled in the present study. This may be consistent with some other related studies (Ismail et al; Mundy et al; Kumthi et al; La Rosa et al; De Francesco et al.,) [25], [26], [27], [28], [29].

The presence of such types of adenovirus [29], [15] in gti patients may be due to many potential reasons, such as horizontal transmission of the virus occurring in an accidental manner, as is known, the adenovirus is highly contagious and easily transmitted between individuals by many ways, like the fecal-oral route [30], [31], [32]. Additionally, HAdV infections are usually moderate and self-limiting, but the incidence of HAdV-viraemia can cause life-threatening illnesses in immunocompromised individuals and younger children

who are more likely to acquire disseminated infections and severe multiorgan damage, including gastroenteritis [33].

Furthermore, this virus may have acquired a genetic mutation that changed its tropism towards new cells, which would explain its capacity to infect previously unidentified organs [34], [35].

## 5. Conclusions

The present study demonstrated the frequency and variation of HAdV genotypes prevalent in pediatric and young adult patients who suffer from acute gastroenteritis and keratoconjunctivitis illnesses. Phylogenetic tree analytic tool showed widely varied genotypes and species of HAdV, with regard to eye infection the the predominant type of HAdV was 8, whereas non-enteric HAdVs, including species 15,29,56, were isolated from gastroenteritis patients reported in this study. Epidemiological molecular surveillance of HAdV is crucial to be continuously performed in different countries to acquire information across many countries worldwide, enhance the knowledge about this virus, and produce of efficient vaccine or treatment to decrease the impact of this disease linked with infection of HAdV future.

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