

# A Multicentric Molecular Study on the Conjunctivitis Outbreak in Delhi: Keeping an Eye on “Pink Eye”

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## Abstract

**Background:** Hospitals in the national capital were staring at the rising cases of pink eye, more than 100 of which had been daily hitting the outpatient departments of major hospitals in Delhi during the month of July 2023. Outbreaks of Conjunctivitis are common globally, with people across all age and socio-economic status getting affected.

**Objectives:** The aim of the study was to do a molecular analysis on the etiology in cases of epidemic keratoconjunctivitis.

**Methods:** A multicentric prospective observational study was carried out during the outbreak of conjunctivitis in Delhi. The clinical samples were processed by polymerase chain reaction for common etiology of acute keratoconjunctivitis.

**Results:** Adenoviral etiology was witnessed in 8.3% of cases, while no other bacterial or viral etiology could be documented.

**Conclusion:** Studying the etiological pattern of any outbreak of infections can provide insights into the prevalent transmissible pathogens and help in future preparedness.

**Keywords:** Conjunctivitis, Outbreak, Delhi, Adenovirus, Multicentric

## 1. Background

With Yamuna surpassing the danger water level mark, Delhi witnessed a sudden surge of conjunctivitis since heavy rains, flooding and water logging that lashed the national capital in the last few weeks of July 2023. The hospitals in the national capital were "on alert" to deal with the rising cases of pink eye, more than 100 of which had been daily hitting the outpatient departments of major hospitals in Delhi. Conjunctivitis, one of the most common causes of red eye, affects patients of all ages and socioeconomic classes. As reported by a leading newspaper, 60% of eye OPD cases have been conjunctivitis. The monsoon led surge in the conjunctivitis cases post flood and rains has been probably due to the humidity in the air. Though a relative increase in the number of cases is witnessed every year during this time, the rate has been very high (80-100 cases a day unlike 10-20 last year) coupled with severity of symptoms and high transmission rates this year as reported by the ophthalmologists. This is attributable to the worst recorded flood in Delhi this year in the last four decades with the unprecedented Yamuna levels raising sanitation issues.

The frequency of occurrence of conjunctivitis in the population depends on various factors like age, gender, or even the time of the year. In an emergency or an outpatient department, a bimodal distribution is seen among the cases of acute conjunctivitis, the first peak being commonly observed among children, with the highest occurrence in less than five years. The second peak ranges between 22 and 28 years among men and women, though men have reported relatively higher rates than women. Seasonality too has a role to play in the presentation and diagnosis of conjunctivitis, and remains constant across geographical areas irrespective of changes in climate and weather patterns. Across all age groups, there is a peak incidence of conjunctivitis in children 0 to 4 years during the month of March, followed by other age groups during the month of May. Though allergic conjunctivitis, the most common form which affects 15% to 40% of the population, is mostly witnessed during spring and summer months. Bacterial conjunctivitis, on the other hand, are highest in occurrence from the month of December to April.<sup>1,2</sup> Conjunctivitis in the U.S. occurs in 13 of every 1,000

people every year in those under 74 years of age (overall prevalence of 0.13%).<sup>1</sup> Approximately 3% of all the visits in the emergency department are ocular related, of which, 30% is conjunctivitis (vis a vis 2% among the patients visiting primary care physicians of whom conjunctivitis is indicated in approximately 54%).<sup>2,3</sup> Several previous clinical studies suggest that the prevalence of adenoviral conjunctivitis ranges from 20% to 62% of all the cases of acute conjunctivitis and adenovirus is thought to represent 80% to 90% of all viral conjunctivitis.<sup>4</sup>

Variation in the susceptibility and tolerability of the patients to clinical disease and the clinical uncertainty or accuracy in differentiating among the various etiologies of acute conjunctivitis, could likely be the main contributors to the wide variation in prevalence estimates. The vast majority of the clinical cases reported as unspecified etiologically might also highlight the confidence of the physicians in differentiating viral from bacterial or other causes of conjunctivitis. This is reflected in literature that, though, adenoviral conjunctivitis represents 40% to 50% of all the cases of conjunctivitis, it was found to represent only 0.37% of the coded cases. While the clinical diagnosis remains uncertain, most clinicians rarely ask for conjunctival cultures and prefer to empirically treat patients with acute conjunctivitis despite the availability of a rapid diagnostic test.<sup>5</sup>

## 2. Objectives

This study was undertaken to report the molecular diagnosis of the etiology of the cases of conjunctivitis that were reported at a tertiary care center during the outbreak. Studying the etiological pattern of any outbreak of infections can provide insights into prevalent transmissible pathogens, both established or emerging, and may potentially improve the prediction, treatment and management of future epidemics.

## 3. Methods

A multicentric prospective observational study was carried out during the outbreak of conjunctivitis in the Departments of Ophthalmology and Microbiology at University College of Medical Sciences and Guru Teg Bahadur Hospital, a tertiary care center in the east part of Delhi.

Clinical samples: Conjunctivitis samples (conjunctival aspirate) from 72 clinical cases of conjunctivitis reporting to the ophthalmology OPD were included in the study. The samples were collected during the outbreak of conjunctivitis during July 2023. The inclusion criteria comprised of any age group, and any gender while the exclusion criteria were history of any previous eye surgeries, ocular trauma and any eye syndrome. The study was performed in accordance with the current biosafety guidelines. Ethical approval was not required, and hence not sought for, in view of the impending

outbreak and because no additional specimen was drawn from the patients beyond what was collected for routine examination.

### 3.1. Total Nucleic Acid Isolation

The samples were processed at (Biosafety level) BSL-2 facility at Department of Lab Sciences and Molecular Medicines, Army Hospital Research and Referral, New Delhi, following all the necessary precautions. The nucleic acid was extracted from the referred samples using Genome Diagnostic Pvt Ltd kit (India), as per the manufacturers' protocol. The extracted nucleic acid was further used for downstream process.

### 3.2. Real Time-Polymerase Chain Reaction (RT-PCR)

The presence of conjunctivitis causing Human Adenovirus (HAdV) was confirmed using RealStar® Adenovirus PCR Kit 1.0 (Germany). The assay is based on HAdV specific target sequence and specific probe labelled with FAM reporter and quencher dyes along with an internal control target, labelled with JOE dye. Four quantification standards were added with known concentrations as mentioned in Table 1. The assay was prepared in a final volume of 30 µl constituting 5 µl of Master A, 15 µl of Master mix B and 10 µl of template. The reaction was setup in Light Cycler 480II® Roche (Switzerland). The thermal profile consists of denaturation step at 95 °C for 10 min followed by amplification step, 45 cycles of 95 °C for 15 sec, and 58 °C for 1 min.

**Table 1.** List of HAdV Positive Samples with Cp Values (the point at which the fluorescence rises above the background fluorescence) and Concentration

S. No.	Sample ID	Cp Value	Concentration
1	S-14	11.47	8.53 x 10 <sup>7</sup>
2	S-19	23.48	1.21 X 10 <sup>3</sup>
3	S-23	25.26	2.32 X 10 <sup>2</sup>
4	S-29	18.06	1.87 X 10 <sup>5</sup>
5	S-46	11.95	5.50 X 10 <sup>7</sup>
6	S-52	19.98	3.14 X 10 <sup>4</sup>

### 3.3. Polymerase Chain Reaction (PCR)

Conventional PCR was adopted to diagnose the presence of other conjunctivitis causing viruses including Human Rhinovirus (RV), Rubella virus, Respiratory Syncytial Virus (RSV), HAdV, Herpes Simplex Virus (HSV) and Epstein-Barr Virus (EBV), different sets of reported primer targeting different viral genome regions as mentioned in Table 2, according to the respective protocol.<sup>6,7,8,9,10,11</sup> The RNA viruses (RV, Rubella virus, RSV) were processed in two steps. The first step included the conversion of RNA into cDNA and the second step, included the targeted amplification.

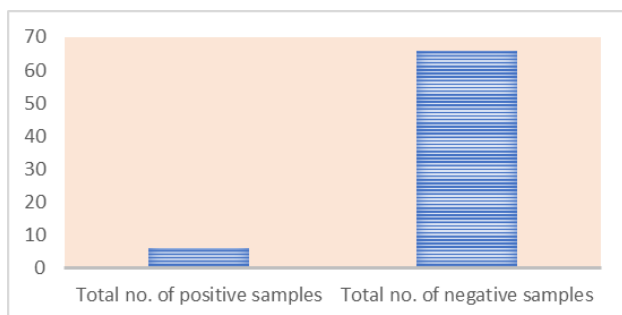
The PCR product obtained after the PCR amplification was analyzed by agarose gel electrophoresis.

**Table 2.** List of Primers

Primer Name	Sequence	No of bases	Publication
Rubella V11	CAACACGCCGACGGACAAC	20	CDC Rubella RT-PCR
Rubella V12	CCACAAGCCGCGAGCAGTCA	20	Ref 10
Rubella V13	CCACGAGCCGCGAACAGTCG	20	
HSV-1 TK3 F	AGCGTCTTGTCATTGGCGAA	20	Marshall, D S et al. (2001)
HSV-1 TK3 R	TTTTCTGCTCCAGGCGGACT	20	Ref 9
HSV-1 POL F	GTCCACCTCAGCGATCTGCCT	22	
HSV-1 POL R	CAGCAGCGAGTCCTGCACAAA	22	
HAdV Penton-F	CTATCAGAACGACCACGCAACTT	24	Wu, X., Zhang et al. (2022)
HAdV Penton-R	TCCCGTGATCTGTGAGAGCRG	21	Ref 8
HAdV HVR-F	CAGGATGCTTCGGAGTACCTGAG	23	
HAdV HVR-R	TTTCTGAAGTTCCACTCGTAGGTGA	26	
HAdVFiber-F	CCCTCTCCCAACTCTGGTA	20	
HAdVFiber-R	GGGGAGGCCAAAATAACTACTCG	22	
HAdVFiber-CR	GAGGTGGCAGGTTGAATACTAG	22	
EBV-F (TC-70)	CTTGAGACAGGCTTAACCAGACTCA	26	Nandimath, K. et al. (2018)
EBV-F (TC-72)	CCATGGCTGCACCGATGAAAGTTAT	25	Ref 11
RV cDNA F	CCCCTGAATGCTGGCTAACCT	21	Steininger C et al. (2001)
RV cDNA R	CGGACACCCAAAAGTAGTCTGGTC	23	Ref 6
RV nested F	GAATGCTGGCTAACCTTAAACCC	23	
RV nested R	CAAAGTAGTCTGGTCCCAGTCC	22	
RSVA-F	TCCAGAACCACAAGTCAA	18	
RSVA-R	CAGGACTTGATACAGCAA	18	Naeem R et al. (2023)
RSVB-F	TCTTCTAACTCTTGCTATT	20	Reference 7
RSVB-R	AGTGTACGCTGTGGAAGA	19	
Total bases		644	

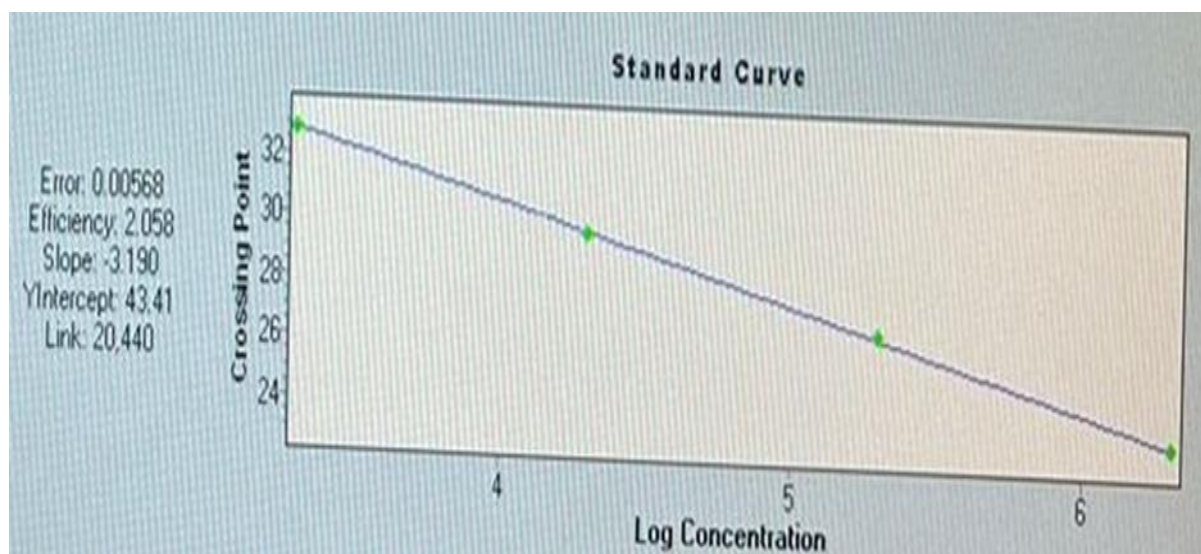
**4. Results**

Out of 72 clinical conjunctivitis samples (conjunctival aspirate), 06 samples were found positive for HAdV via Real-time PCR (Altona Kit), which exhibited the positivity



**Figure 1.** Total Number of Conjunctivitis Cases Positive for Human Adenovirus (HAdV) during July 2023.

rate of 8.3% (Figure 1). The Crossing point (Cp) value and concentration of positive samples have been listed in Table 1. The standard curve of Altona kit quantification standard and the amplification plot have been shown in Figures 2 and 3, respectively. The conventional PCR of 06 conjunctivitis-causing viruses RV, Rubella virus, RSV, HAdV, HSV and EBV was carried out in accordance with the reported protocol (1, 2, 3, 4, 5, 6). The PCR obtained product was analyzed on agarose gel electrophoresis. Out of a 72 sample, one sample was found positive for penton and fiber gene of HAdV. The product size of penton specific gene and fiber gene observed on 1% agarose gel was 1,253 bp and 1,163 bp, respectively (Figure 4). Other related viruses such as RV, Rubella virus, RSV, HSV and EBV were not observed.



**Figure 2.** Standard Curve of The RealStar® Adenovirus PCR Kit 1.0 Quantification Standards (QS).

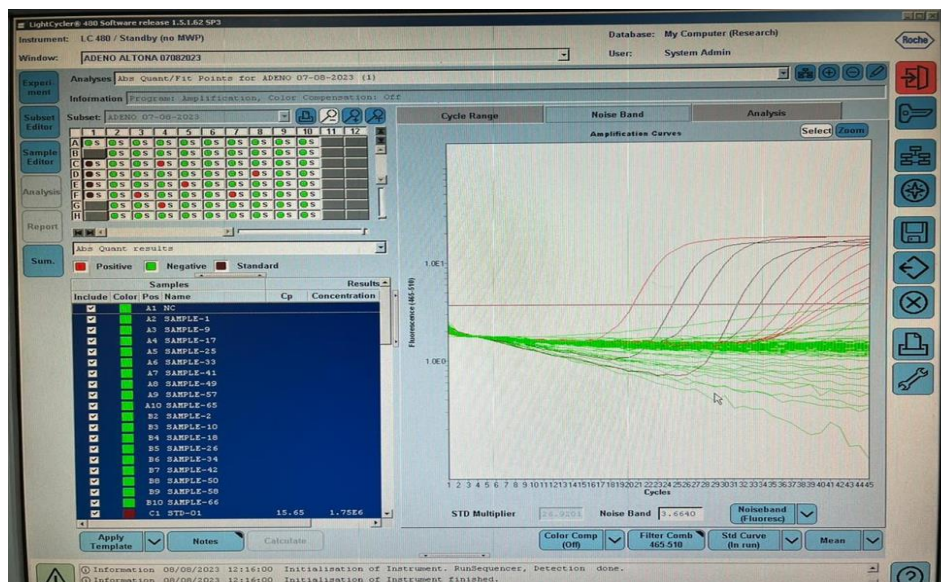


Figure 3. Showing Amplification Plot of Real Time -PCR for Human Adenovirus (HAdV).

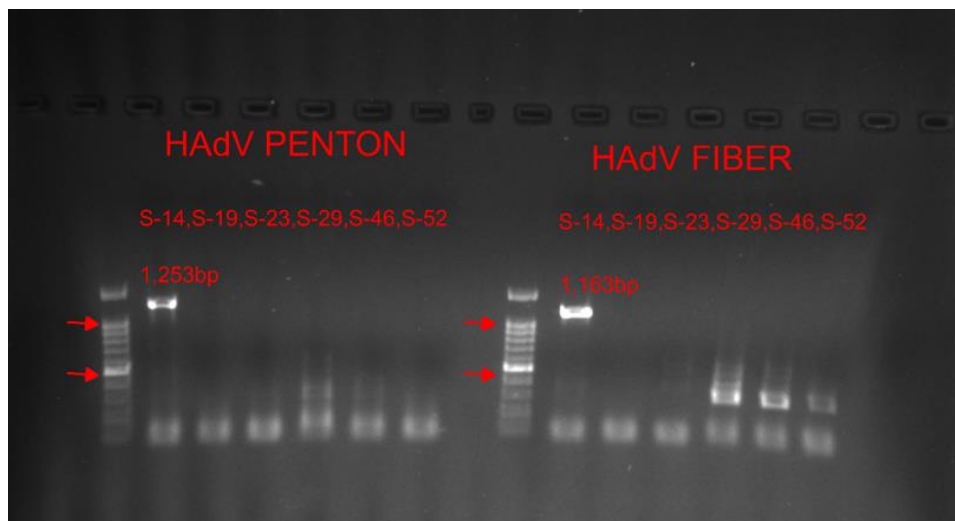


Figure 4. Image of 1% Agarose Gel with 100bp ladder: Showing HAdV specific Penton gene (1,253bp) and Fiber gene (1,163bp) Amplified Product of Sample -14.

## 5. Discussion

Acute hemorrhagic conjunctivitis, commonly known as pink eye, is an acute viral eye disease that has been prevalent in the world in the past years. Outbreaks of conjunctivitis are common globally; with people across all ages and socio-economic status, getting affected. These epidemics usually occur sporadically spatiotemporally, with predictable seasonal patterns.<sup>12</sup> Experts confirm that epidemics or outbreaks of conjunctivitis are prevalent globally, but the suspected causes are largely unknown or variable.<sup>12</sup> The etiologic identification for conjunctivitis is not typically sought for, assuming adenoviral etiology mostly; though well documented viral, fungal, bacterial or other non-infectious causes have been established. When specific etiologies were indicated, there was no agreement about exclusively attributing the conjunctivitis epidemics to adenoviral origin. This is consistent with

prior reports indicating a lack of consensus on outbreak patterns and etiologies.<sup>13</sup> The findings in our study is also in agreement, as only 8.33% positivity was seen among the cases for adenovirus by real time PCR in the present study.

The uncertainty in the diagnostic aetiology of conjunctivitis may lead to misdiagnosis, which will not only contribute to the costs of unnecessary medication, but also endanger the very principle of antimicrobial stewardship. Antibiotic treatment for presumptive viral conjunctivitis or conjunctivitis of non-infectious origin will fuel the already existing drug resistance menace.<sup>14,15</sup> Unless we know the regional aetiologies of outbreaks without assuming that their self-limited nature, we may not be able to prevent antibiotic resistance or any impending systemic implications. Hence, a comprehensive systematic surveillance guideline for the confirmation of

the microbial aetiology of conjunctivitis in routine clinical practice as well as during epidemics, is the need of the hour.

The patients of acute hemorrhagic conjunctivitis mainly manifest symptoms such as pain in the eye, redness, and increased secretion from the eye or congestion of the conjunctiva. These findings are consistent with the features reported in our study.<sup>16-18</sup> The condition, characterized by rapid onset and strong infectivity, can cause some degree of visual impairment due to the spread to the cornea. Local epidemiological analysis and targeted intervention can reduce the occurrence of acute hemorrhagic conjunctivitis and hence prevent local outbreaks and global pandemics.<sup>16</sup> Poor hygiene and breach in sanitation have been reported to be the main cause of acute hemorrhagic conjunctivitis.<sup>19</sup> The outbreak that we report here, occurred during the time when Delhi witnessed its worst flood in decades, with the Yamuna swelling and resulting into water logging in various areas. The hospital that reported the outbreak is located in an area adjoining a river and cases were seen to be witnessed in higher frequency and escalated more rapidly from the areas that are adjoining the banks of the Yamuna River. Though monsoons are the perfect time for germs to breed and multiply due to the humidity and compromised hygiene in the relief camps post flood, this year the increase in the cases of conjunctivitis was unprecedented, and it had almost doubled in frequency compared to previous years. Previous studies have reported the concentration of cases mainly from April to August, with the highest incidence in May.<sup>16,20</sup> Many studies have reported that acute conjunctivitis is a seasonal infectious disease that occurs mostly in summer.<sup>21</sup> The age of the presenting cases of acute haemorrhagic conjunctivitis reportedly concentrates mainly in the 0-20 years old and 60 years old, consistent with the age of the patients presenting in our study; indicating that the younger and the older age groups are more vulnerable mainly due to the decreased resistance of the body to fight infections or due to the relatively poorer hygiene or lifestyle in these age groups.<sup>16,22,23</sup>

Acute hemorrhagic conjunctivitis is a self-limited infection caused by a plethora of organisms. Commonly encountered aetiology include enterovirus 70 (EV70), coxsackievirus A24 variant (CVA24v), and adenovirus (Adv) among several other viruses or bacteria. HEV70 and CVA24v have been positivity reported in higher frequency than adenovirus, though some serotypes of adenovirus have also been frequently reported to be the causative agents.<sup>16,24-26</sup> In the present study, 8.3% positivity was reported for adenovirus by real time PCR. In order to diagnose the presence of other conjunctivitis-causing viruses, different sets of reported primer targeting different viral genome regions were selected and synthesised and PCR was carried out. The viruses that we targeted were RV, RSV, HAAdV, HSV, Rubella virus, and

EBV. We did not detect any other virus besides adenovirus. The lower sensitivity of the conventional PCR over real time PCR may account for this. Previous literature has reported viral conjunctivitis as the main aetiology of infectious conjunctivitis (accounting for almost 75%), and that the viral type is highly contagious and severe. Adenovirus (serotypes Ad 5, 8, 11, 13, 19, and 37) have been usually implicated in viral conjunctivitis.<sup>27</sup> Though few cases in this outbreak showed a secondary bacterial infection with staphylococcus, the overwhelming majority of cases were associated with adenovirus from different Delhi hospitals, as per media reports. In various parts of India, apart from this outbreak, a mix of bacterial and viral conjunctivitis outbreaks have been reported, with adenoviruses being the predominant pathogen responsible for the inflammation in the eyes. Adenovirus 8 was the most common known cause of epidemic outbreaks of acute viral conjunctivitis and keratoconjunctivitis prior to 1970, Enteroviruses; either Enterovirus 70 (EV 70) or Coxsackie Virus A 24 or their variants/in combinations have been reported after 1970.<sup>28</sup>

Our patients predominantly complained of itching, watery discharge with redness in the eyes, sticky discharge in the mornings with swollen eyelids and a crust of the discharge on the eyelashes during the nights. Matting of eyelashes, photophobia, constant irritation, blepharospasm, and mucopurulent discharge were other reported complaints. Patients presented with a variable degree of severity ranging from mild to moderate discomfort in the eyes. Minutely following up each patient was not possible in the context of an outbreak with almost more than 50 cases reporting in each OPD every day. None of the patients reported any severe complication. Literature also reports that the majority of patients of acute keratoconjunctivitis experience a self-limited illness, typically sudden onset of painful, swollen, red eyes with conjunctival haemorrhaging and excessive tearing.<sup>29</sup>

## 6. Conclusion

In the absence of a routine disease surveillance system, outbreaks of conjunctivitis often go unreported, unless they draw the local media attention especially from congregation settings.<sup>29</sup> Identifying the outbreak and controlling the spread is vital to further prevent an impending larger community outbreak. As Delhi was staring at the epidemic with the cases spiking, there were recommendation from the National Centre of Disease Control, Delhi, to identify the type of prevailing conjunctivitis. A recent Indian study on the trend analysis and correlation of temporal pattern of presentation of presumed adenoviral epidemic keratoconjunctivitis with meteorological parameters concluded that, despite popular perception of seasonal correlation, an increase in

rainfall, wind speed, and humidity contributed to a lower prevalence and no statistically significant correlation was found with temperature. The study highlighted the significance of routine and regular surveillance of such outbreaks for better preparedness.<sup>30</sup> The need for identification of the type of conjunctivitis and the responsible strain prevalent in the population can help in understanding the epidemiology and also guide further preventive measures.

### Research Highlights

#### What Is Already Known?

Conjunctivitis is one of the most common causes of red eye and affects patients of all ages and socioeconomic classes. The monsoon led surge in the conjunctivitis cases post flood and rains is witnessed every year during this time, the rate has been high coupled with severity of symptoms and high transmission rates this year.

#### What Does This Study Add?

With Yamuna surpassing the danger water level mark, Delhi witnessed sudden surge of conjunctivitis in the last few weeks of July 2023. Adenoviral etiology was witnessed in 8.3% of the cases, while no other bacterial or viral etiology could be documented. The study points to the significance of routine and regular surveillance of such outbreaks for better preparedness.

### Author Contributions

GKD, BK and AB have contributed towards the conception and design, the drafting of the article or critical revision for important intellectual content and the final approval of the version to be published. PY, BS, NS, and S have contributed towards the acquisition, analysis, or interpretation of the data and the drafting of the article or critical revision for important intellectual content.

### Conflict of Interest Disclosures

All authors declared that they have no conflict of interest.

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