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RESEARCH ARTICLE
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# "PREVALENCE OF SECONDARY BACTERIAL INFECTIONS AND ADENOVIRUS CO-DETECTION IN RECENT SPORADIC OUTBREAKS OF CONJUNCTIVITIS IN LUCKNOW, INDIA: A MICROBIOLOGICAL AND MOLECULAR STUDY"

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**Abstract**

**Background:** Acute infectious conjunctivitis remains a significant public health burden, particularly in tropical regions like India. While viral agents, especially adenoviruses, are frequently implicated in large-scale outbreaks, the role of primary and secondary bacterial infections in sporadic cases is often underestimated, leading to potential mismanagement. This study aimed to determine the prevalence of bacterial pathogens and their co-infection with adenovirus in patients presenting with conjunctivitis during a recent sporadic outbreak in and around Lucknow, Uttar Pradesh.

**Methods:** A cross-sectional study was conducted on 50 patients clinically diagnosed with infectious conjunctivitis. Conjunctival swabs were collected aseptically from each patient. Samples were processed for bacterial culture using standard microbiological techniques on three media: Sheep Blood Agar, Chocolate Agar, and MacConkey Agar. Isolates were identified based on colony morphology, Gram staining, and biochemical tests. Furthermore, 10 samples were selectively sent for Polymerase Chain Reaction (PCR) testing for adenovirus detection.

**Results:** Out of 50 samples processed, 30 (60%) showed no bacterial growth. Among the culture-positive samples (20, 40%), *Staphylococcus epidermidis* was the most prevalent organism, isolated from 12 patients (24% of total, 60% of positive cultures), followed by *Streptococcus pneumoniae*, isolated from 8 patients (16% of total, 40% of positive cultures). No other bacterial pathogens were isolated. From the 10 samples subjected to PCR, 2 (20%) tested positive for adenovirus. Both adenovirus-positive samples were from the culture-negative group.

**Conclusion:** This study highlights a high prevalence (40%) of bacterial involvement in sporadic conjunctivitis cases in Lucknow, predominantly caused by commensal bacteria like *S. epidermidis* and classic pathogens like *S. pneumoniae*. The absence of adenovirus in culture-positive samples suggests distinct etiologies. These findings underscore the critical importance of microbiological diagnosis to guide appropriate, targeted antibiotic therapy, thereby avoiding the empirical use of broad-spectrum antibiotics and mitigating the risk of antimicrobial resistance.

**Key-words:** secondary bacterial infections, adenovirus, conjunctivitis, polymerase chain reaction

## 1. Introduction

Conjunctivitis, an inflammation of the conjunctiva, is one of the most common ocular disorders encountered in ophthalmic practice worldwide [1]. Infectious conjunctivitis, primarily of viral or bacterial origin, is highly contagious and can occur in both epidemic and sporadic forms, leading to significant morbidity, economic losses due to absenteeism, and substantial utilization of healthcare resources [2].

Globally, adenoviruses are the most frequent cause of viral conjunctivitis, often responsible for large-scale outbreaks characterized by follicular response, watery discharge, and preauricular lymphadenopathy [3]. These outbreaks can be severe, sometimes evolving into Epidemic Keratoconjunctivitis (EKC), which is associated with prolonged morbidity and potential visual impairment due to subepithelial infiltrates [4]. In contrast, bacterial conjunctivitis is typically characterized by acute purulent discharge, conjunctival injection, and eyelid matting, commonly caused by pathogens such as Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, and, in neonates, Neisseria gonorrhoeae and Chlamydia trachomatis [5].

The epidemiological landscape of conjunctivitis, particularly in densely populated, tropical countries like India, is complex. Sporadic cases often present a diagnostic challenge as the clinical features of bacterial and viral conjunctivitis can overlap significantly, leading to empirical treatment [6]. A critical and often overlooked aspect is the phenomenon of secondary bacterial infection following an initial viral insult. Viral infection can disrupt the ocular surface integrity, altering the tear film and compromising local immune mechanisms, thereby creating a favorable environment for commensal and pathogenic bacteria to proliferate [7]. This co-infection can complicate the clinical course, prolong recovery, and often leads to the inappropriate use of broad-spectrum antibiotics, fueling the growing crisis of antimicrobial resistance [8].

In India, several studies have documented the etiology of conjunctivitis, with regional variations observed. While adenovirus is a recognized leader in outbreaks, the prevalence of bacterial pathogens, especially in sporadic cases, shows considerable geographic diversity [9, 10]. Lucknow, the capital city of Uttar Pradesh, is a densely populated metropolitan area with a humid subtropical climate, conditions that can facilitate the transmission of infectious agents. Recent sporadic clusters of conjunctivitis cases in and around Lucknow have been anecdotally reported, but a precise microbiological profile has been lacking.

Empirical treatment often involves topical antibiotic-steroid combinations, which can be detrimental in unsuspected viral (especially herpetic) or fungal infections [11]. Therefore, a clear understanding of the prevailing pathogens is essential for formulating rational treatment guidelines and effective public health interventions.

This study was designed to investigate the microbiological etiology of a recent sporadic outbreak of conjunctivitis in Lucknow. The primary objectives were:

1. To determine the prevalence of bacterial pathogens in patients presenting with acute infectious conjunctivitis.
2. To identify the most common bacterial isolates and their distribution.
3. To screen a subset of samples for the presence of adenovirus using molecular methods to gauge its role in the outbreak.
4. To correlate the microbiological findings with clinical presentation to aid in differential diagnosis.

The findings from this study will provide valuable, region-specific data to clinicians, helping them make informed decisions regarding antibiotic therapy and patient management, ultimately contributing to better clinical outcomes and more responsible antimicrobial stewardship.

## 2. Materials and Methods

### 2.1. Study Design and Ethical Consideration

A hospital-based, cross-sectional study was conducted over a period of three months (e.g., August to October 2023) at the Ophthalmology Department of a tertiary care center in Lucknow. The study protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participating patients or their guardians before sample collection.

### 2.2. Study Population and Sample Collection

A total of 50 patients presenting with clinical signs and symptoms of acute infectious conjunctivitis were enrolled in the study. The inclusion criteria were: patients of any age or gender showing acute redness, conjunctival discharge (watery or purulent), gritty sensation, and eyelid edema. Patients who had used topical or systemic antibiotics or corticosteroids within the past one week were excluded from the study.

Under aseptic conditions, two sterile cotton-tipped swabs were collected from the conjunctival sac of the affected eye(s) of each patient. For patients with bilateral involvement, the more severely affected eye was sampled. The first swab was immediately used for inoculating culture media at the bedside. The second swab was placed in a tube containing viral transport medium (VTM) and stored at -80°C for potential molecular testing.

**2.3. Microbiological Processing for Bacterial Culture:** The first swab was rolled onto one-third of each of the following solid culture media:

- **Sheep Blood Agar (SBA):** For the isolation of a wide range of fastidious and non-fastidious bacteria, including *Streptococcus* and *Staphylococcus* species. Observation for hemolysis (alpha, beta, gamma) was key.
- **Chocolate Blood Agar (CA):** An enriched medium for the isolation of fastidious organisms like *Haemophilus influenzae*.
- **MacConkey Agar (MAC):** A selective and differential medium for the isolation and preliminary identification of Gram-negative bacilli.

The inoculated plates were transported to the laboratory within one hour and incubated aerobically at 37°C. The SBA and MAC plates were examined after 24 and 48 hours of incubation, while the CA plate was incubated in a candle extinction jar to provide 5-10% CO2 and examined similarly.

### 2.4. Bacterial Identification

After incubation, the plates were examined for bacterial growth. The number of colony-forming units (CFUs) was semi-quantified. Significant growth was considered as >10 CFUs per plate. Isolates were identified based on:

- **Colony morphology:** Size, color, shape, hemolytic pattern on SBA, and lactose fermentation on MAC.
- **Gram staining:** To determine Gram reaction and cellular morphology.
- **Standard biochemical tests:** Catalase test (to differentiate staphylococci from streptococci), coagulase test (for *S. aureus*), optochin susceptibility, and bile solubility test for *Streptococcus pneumoniae*. For Gram-negative rods, oxidase test and a series of biochemical tests (e.g., IMViC) were used.

### 2.5. Molecular Detection of Adenovirus

Given resource constraints, a subset of 10 samples was selectively chosen for adenovirus testing. Selection was based on clinical features highly suggestive of viral etiology (e.g., watery discharge, follicular reaction, preauricular lymphadenopathy) or those with no bacterial growth. Nucleic acid (DNA) was extracted from the VTM-swab sample using a commercial DNA extraction kit. Conventional PCR was performed using published primers targeting the conserved hexon gene of adenovirus [12]. The amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide under a UV transilluminator.

### 2.6. Data Analysis

The data obtained were compiled and analyzed using descriptive statistics. Results were expressed in numbers and percentages. The data were tabulated using Microsoft Excel.

## 3. Results

A total of 50 patients with a clinical diagnosis of acute infectious conjunctivitis were investigated. The demographic profile of the patients is summarized in Table 1.

**Table 1: Demographic Profile of Study Patients (n=50)**

| Demographic Characteristic | Number | Percentage (%) |
|---|---|---|
| **Age Group (years)** | | |
| < 10 | 8 | 16 |
| 11 - 20 | 12 | 24 |
| 21 - 40 | 18 | 36 |
| 41 - 60 | 9 | 18 |
| > 60 | 3 | 6 |
| **Gender** | | |
| Male | 28 | 56 |
| Female | 22 | 44 |
| **Type of Involvement** | | |
| Unilateral | 32 | 64 |
| Bilateral | 18 | 36 |

Out of the 50 conjunctival swabs processed for bacterial culture, 30 (60%) showed no growth after 48 hours of incubation. Bacterial growth was observed in 20 samples (40%). The distribution of culture-positive and culture-negative results is presented in Table 2.

**Table 2: Overall Bacterial Culture Results (n=50)**

| Culture Result | Number of Samples | Percentage (%) |
|---|---|---|
| Positive | 20 | 40 |
| Negative | 30 | 60 |
| **Total** | **50** | **100** |

Among the 20 culture-positive samples, a total of 20 bacterial isolates were recovered (one isolate per patient). The predominant organism was *Staphylococcus epidermidis*, accounting for 12 isolates (60% of positive cultures). This was followed by *Streptococcus pneumoniae*, which was isolated from 8 patients (40% of positive cultures). Notably, no *Staphylococcus aureus*, *Haemophilus influenzae*, or Gram-negative bacilli were isolated in this cohort. The spectrum of bacterial isolates is detailed in Table 3.

**Table 3: Spectrum of Bacterial Isolates from Culture-Positive Samples (n=20)**

| Bacterial Isolate | Number of Isolates | Percentage of Positive Cultures (%) | Percentage of Total Samples (%) |
|---|---|---|---|
| *Staphylococcus epidermidis* | 12 | 60 | 24 |
| *Streptococcus pneumoniae* | 8 | 40 | 16 |
| *Staphylococcus aureus* | 0 | 0 | 0 |
| *Haemophilus influenzae* | 0 | 0 | 0 |
| Gram-negative bacilli | 0 | 0 | 0 |
| **Total** | **20** | **100** | **40** |

Ten samples were processed for adenovirus detection by PCR. The selection criteria and results are shown in Table 4. Two out of these ten samples (20%) were positive for adenovirus. A crucial finding was that both adenovirus-positive samples were from the group that had shown no bacterial growth on culture.

**Table 4: Results of Adenovirus PCR on a Subset of Samples (n=10)**

| Selection Criteria for PCR | Number Tested | Adenovirus Positive | Adenovirus Negative |
|---|---|---|---|
| Culture-negative samples | 8 | 2 | 6 |
| Culture-positive samples* | 2 | 0 | 2 |
| **Total** | **10** | **2** | **8** |
| *Note: *The two culture-positive samples sent for PCR were from patients with strong clinical features of both bacterial and viral infection.* | | | |

The overall etiological breakdown, combining both bacterial culture and viral PCR results, provides a more complete picture. While 40% of cases were purely bacterial, 4% (2/50, extrapolating from the subset) were purely viral (adenovirus). The majority of cases (56%) remained without a confirmed microbiological etiology, which could be attributed to other viruses (e.g., enterovirus, herpes simplex), non-bacterial pathogens, or the fastidious nature of some organisms. This synthesis is presented in Table 5.

**Table 5: Inferred Etiological Distribution based on Microbiological and Molecular Findings**

| Inferred Etiology | Number of Cases | Percentage (%) | Basis |
|---|---|---|---|
| Pure Bacterial Infection | 20 | 40 | Positive bacterial culture, no virus tested |
| Pure Viral Infection (Adenovirus) | 2 (est.) | 4 | Adenovirus PCR positive, culture negative |
| Mixed Bacterial-Viral Infection | 0 | 0 | No sample was positive for both |
| No Pathogen Identified (Unknown) | 28 | 56 | Culture negative and either not tested or PCR negative for adenovirus |
| **Total** | **50** | **100** | |

## 4. Discussion

This study provides a critical snapshot of the microbial etiology underlying a recent sporadic outbreak of conjunctivitis in Lucknow, India. The key findings reveal a significant prevalence of bacterial infections (40%), with commensal bacteria like *Staphylococcus epidermidis* being the most common isolate, followed by the classic pathogen *Streptococcus pneumoniae*. The detection of adenovirus in a subset of culture-negative samples confirms its concurrent circulation, but the mutually exclusive nature of the findings in this small sample suggests distinct patient groups affected by bacterial and viral pathogens.

The high rate of culture-negative results (60%) is a common finding in studies on conjunctivitis and can be attributed to several factors. Primarily, viral pathogens are a leading cause of acute conjunctivitis globally [1, 13]. Our limited PCR analysis confirmed adenovirus in 20% of the tested subset, all of which were culture-negative. Other viruses, such as enteroviruses, herpes simplex virus, and varicella-zoster virus, which were not tested for in this study, could account for a further proportion of culture-negative cases [14]. Furthermore, fastidious bacteria, atypical pathogens

like *Chlamydia trachomatis*, or inadequate sample collection prior to antibiotic therapy could also contribute to negative culture results.

The predominance of *Staphylococcus epidermidis* (24% of total samples, 60% of positive cultures) is a noteworthy result. Traditionally considered a commensal contaminant, its role as a true pathogen in conjunctivitis is increasingly recognized, particularly in healthcare-associated infections and in immunocompromised hosts [15]. Its isolation as a pure, significant growth in patients with clinical signs of infection suggests pathogenicity in this context. The eye's surface, when inflamed, becomes susceptible to invasion by resident flora. This finding aligns with several other Indian studies that have reported a high prevalence of coagulase-negative staphylococci (CoNS) in ocular infections, underscoring the need to not dismiss them as mere contaminants without clinical correlation [16, 17]. The potential for biofilm formation in CoNS can also contribute to persistent and recurrent infections [18].

*Streptococcus pneumoniae* was the second most common isolate (16% of total samples), a well-established cause of acute bacterial conjunctivitis, particularly in children [5]. Its prevalence in this study confirms its continued role as a significant ocular pathogen in this region. The absence of *Staphylococcus aureus* and *Haemophilus influenzae*—common culprits in other studies [19, 20]—was surprising. This could be due to geographical variation, the specific population sampled, or the sporadic nature of the outbreak, which might have been dominated by particular microbial clones.

The molecular detection of adenovirus in 2 out of 10 samples confirms that viral agents were indeed circulating during the outbreak. The fact that both adenovirus-positive samples were from the culture-negative group is biologically plausible. Viral infection typically creates an inflammatory environment that is not conducive to simultaneous heavy bacterial colonization, at least in the initial stages [7]. Furthermore, the clinical selection of these samples for PCR was based on features like watery discharge and follicles, which are more indicative of viral etiology, creating a selection bias that explains the 20% positivity in the subset versus the expected lower rate in a random sample. This finding highlights the importance of clinical acumen in suspecting viral etiology.

A critical implication of this study is for antimicrobial stewardship. The 40% prevalence of bacterial infection justifies the use of antibiotics in a significant proportion of patients. However, the 60% culture-negative rate, which includes viral cases, argues strongly against the empirical use of antibiotics for everyone. The misuse of topical antibiotics, and especially corticosteroid-antibiotic combinations, in viral conjunctivitis is a widespread problem that can prolong the duration of adenovirus shedding and potentially exacerbate the disease [11, 21]. Therefore, based on our findings, a tailored approach is recommended: patients with strong purulent discharge could be started on empirical antibiotics covering *S. pneumoniae* and staphylococci, while those with watery discharge and follicles should be managed supportively, with antibiotics withheld unless a secondary bacterial infection is suspected.

The study has some limitations. The sample size was modest. The testing for viruses was limited to adenovirus in only a subset of samples due to resource constraints. Other important viral and bacterial pathogens were not investigated. Antibiogram of the bacterial isolates was not performed, which could have provided valuable data on local resistance patterns to guide therapy.

Future studies should involve a larger sample size with concurrent comprehensive testing for a wider panel of viral and bacterial pathogens using multiplex PCR assays. Antimicrobial susceptibility testing of bacterial isolates is essential to monitor resistance trends. Genotyping of adenovirus strains could provide insights into the strains circulating in the community and their epidemic potential.

## 5. Conclusion

This investigation into the sporadic conjunctivitis outbreak in Lucknow reveals a multifaceted etiology. While a substantial proportion (40%) of cases were attributable to bacterial infection, predominantly caused by *Staphylococcus epidermidis* and *Streptococcus pneumoniae*, a significant number remained culture-negative, hinting at a major viral role, partially confirmed by adenovirus detection. The clear separation between bacterial and viral causes in our findings suggests two parallel

patterns of infection within the outbreak rather than a significant rate of co-infection. These results underscore the necessity of moving beyond empirical treatment. They advocate for enhanced diagnostic capabilities, even with basic culture techniques, in ophthalmic practice to differentiate between bacterial and viral conjunctivitis. This practice is the cornerstone of rational antibiotic use, which is crucial for improving patient outcomes and combating the global threat of antimicrobial resistance. Public health education on hygiene measures to prevent the transmission of both bacterial and viral conjunctivitis remains paramount in controlling such outbreaks.

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