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Heterogenous pathogen profile associated with acute conjunctivitis in Nepal

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Background: Infectious conjunctivitis is common in Nepal.

Materials and Methods: This prospective study recruited 60 patients with presumed acute infectious conjunctivitis from the B.P. Koirala Lions Center for Ophthalmic Studies in Kathmandu, Nepal. Swabs from the conjunctiva and anterior nares were processed for metagenomic RNA deep sequencing (RNA-seq).

Results: Pathogens were identified in 55% of cases. RNA viruses were the most common pathogen class identified. Severe acute respiratory syndrome coronavirus 2 was the most common RNA virus identified.

Conclusions: Acute infectious conjunctivitis varies by location. Contrary to expectations, RNA viruses predominated. Repeat surveillance may be useful and RNA-seq allows for detection of the unexpected pathogen including RNA viruses.

Keywords: infectious conjunctivitis, RNA deep-sequencing, SCORPIO.

Introduction

Conjunctivitis is a common health problem in Nepal. In 2003, Nepal experienced a nationwide outbreak of acute hemorrhagic conjunctivitis, affecting nearly one-half of the entire population. Both human adenovirus and coxsackievirus viruses were identified,¹ suggesting that epidemics can be caused by more than one pathogen and that DNA and RNA viruses can commingle. A 2011 prospective Kathmandu-based study identified *Streptococcus pneumoniae* as the most common isolated organism in young patients presenting with acute conjunctivitis.² A 2013 prospective study in Pokhara identified *Staphylococcus aureus* as the most common. Viruses were not queried in either the 2011 or 2013 studies. SCORPIO—Seasonal Conjunctivitis Outbreak Reporting for Prevention and Improved Outcomes—is a University of California San Francisco (UCSF)-based international study aiming to determine worldwide causes of infectious conjunctivitis. B.P. Koirala Lions Center for Ophthalmic Studies (BPKLCOS) in Kathmandu, Nepal, is one participating site.

Materials and Methods

This research was approved by the Institutional Review Boards of both BPKLCOS in Kathmandu, Nepal, and the UCSF, and adhered to the tenets of the Declaration of Helsinki. Samples were obtained from August 2021 through September 2022 at the BPKLCOS eye clinic. Inclusion criteria required signs and symptoms suggestive of acute infectious conjunctivitis for a duration of <14 d in any age group. Sterile polyester applicators (Puritan) were used to swab the lower fornix of each eye and each anterior nasal passage. Swabs were placed in a DNA/RNA-Shield (Zymo Research) and stored in a -20°C freezer prior to shipping to UCSF for processing. Sample processing, library preparation, sequencing and bioinformatic algorithm for pathogen identification have been previously described.³ The prespecified criteria for pathogen identification were: (1) organism known to be a human pathogen and representing the most abundant matched reads after water background subtraction; or for viral pathogens (2) ≥ 2 unique reads covering separate regions in DNA virus genomes; or (3) ≥ 1 unique reads matching RNA virus genomes.

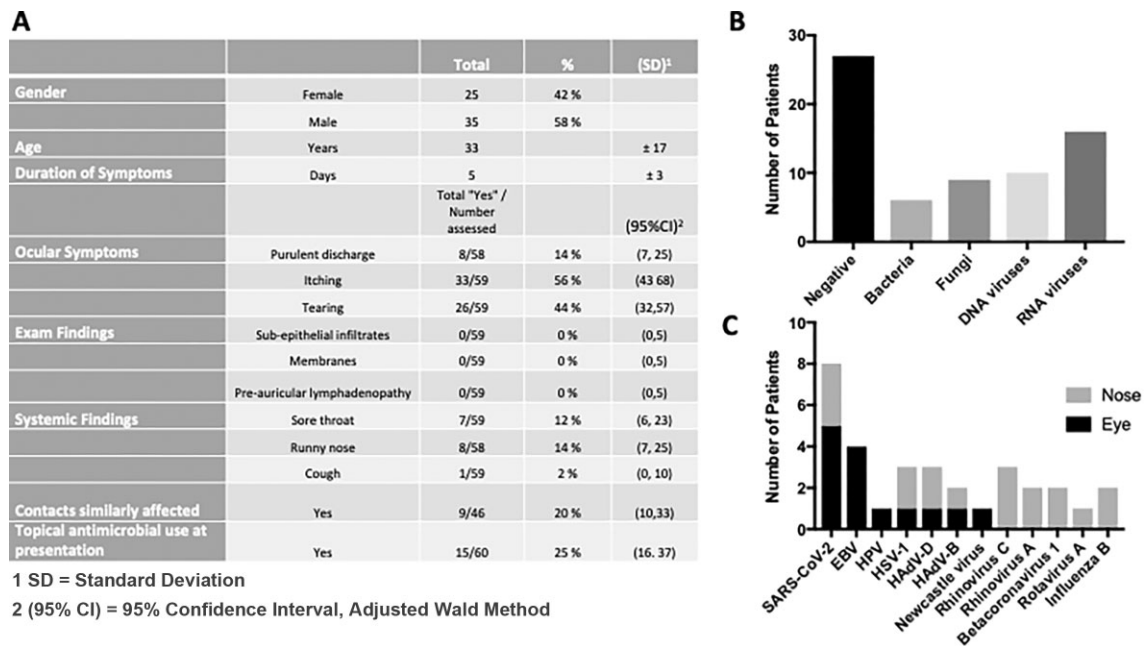


Figure 1. (A) Demographics and clinical signs and symptoms of study participants. (B) Pathogen class identified by RNA-seq. (C) Specific pathogens identified in conjunctiva and nasal swabs.

Results

Sixty patients were enrolled. Forty-two percent were female. Demographics, clinical signs and symptoms are documented in Figure 1A. The mean age was 33 (SD±17) y. Bilateral eye involvement occurred in 30% (95% CI 20 to 43%). Itching was the most reported eye symptom present in 56% (95% CI 43 to 68%) followed by tearing in 44% (95% CI 32 to 57%), then purulent discharge in 14% (95% CI 7 to 25%). On clinical examination, subepithelial infiltrates were present in 19% (95% CI 11 to 31%), membranes or pseudo-membranes in 3% (95% CI 0 to 12%) and preauricular lymphadenopathy in 2% (95% CI 0 to 10%). Comorbid systemic symptoms were not common with rhinorrhoea in 14% (95% CI 7 to 25%), sore throat in 12% (95% CI 6 to 23%) and cough in 2% (95% CI 0 to 10%). Twenty percent (95% CI 10 to 33%) reported similarly affected close contacts. Twenty-eight percent (95% CI 18 to 41%) presented for care on topical antibiotics. Metagenomic sequencing identified candidate pathogens in 55% (95% CI 42 to 67%). Polymicrobial infections were suspected in 13% (95% CI 7 to 24%). Of the pathogens identified (Figures 1B and 1C), RNA viruses were the most common class of pathogens identified in 16 patients (including conjunctival and nasal swabs). Genomic reads corresponding to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were the most frequently isolated RNA virus, identified in five of the ocular swabs and three of the nasal swabs. Notably, two cases of common circulating betacoronavirus 1 and one case of Newcastle virus,⁴ were identified. Bacterial pathogens were associated with nine participants, with *Streptococcus pneumoniae* representing the most frequent isolate and *Haemophilus* spp. identified as the other bacterial pathogen. DNA viruses were identified in 17% of the patients. Interestingly, adenoviruses were only identified in

three cases representing only 5% (95% CI 1 to 14%) of the overall case series. One fungal pathogen, *Vittaforma corneae*, was identified in six of the 60 samples, representing 10% (95% CI 4 to 20%) overall.

Discussion

In this case series, pathogens associated with acute conjunctivitis in Katmandu Nepal are diverse. Interestingly, SARS-CoV-2 was the most frequent pathogen associated with acute infectious conjunctivitis. Even although three of the five patients in whom SARS-CoV-2 was identified from the conjunctival swabs also had this virus present in their nasal swabs, none of these participants reported any systemic symptoms. This study is limited by a lack of normal controls and no follow-up data. Like many sites in the SCORPIO study, roughly one-third of the patients presented using topical antimicrobial drops, which limit bacterial and some fungal pathogen identification. This study highlights the importance of location in disease surveillance, as different pathogens appear to predominate in different areas of the world. Repeat surveillance, especially with seasonal variation, is important. Lastly, the use of RNA-deep sequencing emphasises the importance of RNA virus detection in disease surveillance.

Authors' contributions: TD, GDS and TML designed and supervised the study. MC and SS oversaw the fieldwork and sample collection. TD supervised all laboratory work. KR, CC, LZ, DL, TA and DY performed laboratory-related experiments. TD, AH and GDS performed the bioinformatics

analyses. GDS and TD wrote the initial draft and all coauthors reviewed the manuscript and agreed to publication.

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Competing interests: None declared.

Ethical approval: This research was approved by the Institutional Review Boards of both BPKLCOS in Kathmandu, Nepal, and University of California, UCSF, and adhered to the tenets of the Declaration of Helsinki.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

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References

- 1 Gurung R, Rai SK, Kurokawa M, et al. Acute hemorrhagic conjunctivitis epidemic—2003 in Nepal. *Nepal Med Coll J.* 2003;5(2):59–60.
- 2 Sthapit PR, Tuladhar NR, Marasini S, et al. Bacterial conjunctivitis and of antibiotics in Dhulikhel Hospital-Kathmandu University Hospital. *Kathmandu Univ Med J.* 2011;9(34):69–72.
- 3 Lalitha P, Seitzman GD, Kotecha R, et al. Unbiased pathogen detection and host gene profiling for conjunctivitis. *Ophthalmology.* 2019;126(8):1090–4.
- 4 Prajna NV, Lalitha P, Chen C, et al. Acute keratoconjunctivitis resulting from coinfection with avian Newcastle virus and human adenovirus. *Cornea.* 2022;41(5):630–1.