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SHORT COMMUNICATION

Deep sequencing analysis of acute conjunctivitis in Burkina Faso, Africa

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Background: Seasonal and epidemic conjunctivitis (pink eye) infections are highly contagious and impose a significant economic burden worldwide. Long-term visual impairment can occur.

Methods: This study used metagenomic deep sequencing to evaluate pathogens causing acute infectious conjunctivitis in Burkina Faso.

Results: We found that pathogens causing conjunctivitis in Burkina Faso are diverse, with human adenoviruses responsible for a small fraction of the samples tested.

Conclusions: These results are unexpected and suggest the importance of regional surveillance.

Keywords: acute conjunctivitis, Burkina Faso, epidemics, human adenovirus, SCORPIO Study Group.

Introduction

With worldwide interest in emerging infectious disease, our ability to rapidly and accurately survey for etiologies of ocular infection has wide-reaching implications. Burkina Faso, in West Africa, is one of many countries that experience frequent seasonal outbreaks of presumed infectious conjunctivitis.[1] Globally, most cases of epidemic conjunctivitis are suspected to be viral, with experts assuming that human adenoviruses are the most likely pathogen.[2]

SCORPIO (Seasonal Conjunctivitis Outbreak Reporting for Prevention and Improved Outcomes) is an international study aiming to determine the causes of infectious conjunctivitis, worldwide. This study leverages RNA deep sequencing (RNA-seq) as an unbiased diagnostic tool. The Health Research Center in Nouna is one participating site.

Methods

Patient selection

Inclusion criteria required presumed acute infectious conjunctivitis symptoms for <14 days. Exclusion criteria were presumed allergic conjunctivitis or medicamentosa. Conjunctival and nasal swabs were obtained from patients who presented during 10 March–2 April 2021 at the Centre de Santé et de Promotion Sosiale in Nouna, Burkina Faso with ocular symptoms.

Samples

Sterile polyester applicators (Puritan, Guilford, ME, USA) were used to swab the lower conjunctival fornix of each eye and the anterior nares. All swabs were immediately placed in DNA/RNA-Shield (Zymo Research) and transferred to a −20°C freezer for storage until shipment to the University of California, San Francisco (UCSF). Details of sequencing, library preparation and bioinformatics analyses have been previously described.[3] Only conjunctival samples were used for pathogen identification. Water samples (no template controls) processed in the same sequencing run were used for background subtraction.

Results

Nineteen participants were included in this study; 47% were female. The average age was 9 (range 0–47) years. The average

Table 1. Patient demographics and pathogen identification

Patient #	Age (y)	M/F	Eye(s) affected	Duration of symptoms (d)	Presenting clinical details	Topical medication on presentation	Pathogens identified
1	1	M	Both eyes	8	Coughing, purulent discharge	No	EBV, *Moraxella catarrhalis*
2	10	M	Both eyes	3	Coughing, purulent discharge	No	*Corynebacterium mastiditis*
3	1 m	M	Both eyes	10	Runny nose, purulent discharge	Ofloxacin	Rhinovirus C
4	1	M	Both eyes	2	Purulent discharge	No	CMV, HSV-1
5	2	F	Both eyes	7	Purulent discharge	No	HAdV-C
6	1	F	Both eyes	3	Purulent discharge	No	Mamastrovirus 6
7	46	M	Both eyes	2	Runny nose, coughing, purulent discharge	Ofloxacin and Diclofenac	Negative
8	19	M	Both eyes	3	Runny nose, coughing, purulent discharge	No	Negative
9	4	F	Both eyes	4	Purulent discharge, sick contacts, corneal involvement	No	*Streptococcus pneumoniae,* EBV
10	3	F	Both eyes	6	Runny nose, sick contacts, corneal involvement	No	EBV
11	4 m	F	Both eyes	10	Runny nose, purulent discharge	No	HAdV-C
12	47	M	Left eye	7	Purulent discharge	No	HSV-1
13	2	F	Both eyes	2	Purulent discharge	No	*Neisseria meningitidis*
14	1	F	Both eyes	2	Purulent discharge	No	*Haemophilus influenzae*
15	4 m	F	Both eyes	8	Purulent discharge	No	Negative
16	8	M	Left eye	1	Runny nose, purulent discharge, corneal involvement	No	Negative
17	4	M	Both eyes	3	Purulent discharge	No	Negative
18	16	M	Right eye	2	Purulent discharge	Tetracycline and Gentamycin	VZV
19	20	F	Left eye	7	Itching	No	Negative

Abbreviations: CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAdV, human adenovirus; HSV-1, herpes simplex virus type 1; VZV, Varicella–Zoster virus.

length of ocular symptoms was 5 (2–10) days. Both eyes were affected in 15/19 cases (79%). The predominant ocular presenting symptom was purulent discharge, present in 17/19 (89%) cases. Corneal involvement was noted in three (16%) cases. Systemic symptoms of rhinorrhea were reported in 6/19 (32%) and coughing was reported in 4/19 (21%). Diarrhea, vomiting or sore throat was not reported in any participant. Two participants (10%) reported contact with similarly symptomatic persons. Three participants (16%) reported the use of topical medications on presentation.

RNA-seq identified a virus as the pathogen in 53% (10/19) of cases (Table 1). Bacterial pathogens were identified in 26% (5/19). A mixed bacterial and viral infection was noted in 2/19 (11%). No pathogens were identified in 32% (6/19) of the samples.

Human adenovirus was identified in two patients. Other viruses identified included rhinovirus, influenza A and herpesviridae, including herpes simplex virus (HSV), Varicella–Zoster virus (VZV), Epstein–Barr virus (EBV) and cytomegalovirus (CMV). The five bacteria identified were *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Neisseria meningitidis*, *Haemophilus influenzae* and *Corynebacterium mastiditis.*

Discussion

In a small sample of predominantly young patients in Burkina Faso, Africa, the pathogens identified in patients presenting with acute, mostly purulent, clinically presumed infectious conjunctivitis were surprising. Human adenoviruses, commonly associated with acute conjunctivitis in developed countries, were identified in only two cases (11%). Here, human adenovirus (HAdV-C) was detected on the conjunctiva of a 2-y-old and a 4-mo-old. Although purulent discharge was reported in 89% of participants, a bacterial etiology was identified in only 26% of the cases. These

results suggest that the clinical signs of purulent discharge may not always predict pathogen (viral vs bacteria) in this patient population.[4]

This study confirms that conjunctivitis etiology is likely to vary depending on geography and climate.[2] Whereas most cases of viral conjunctivitis should be allowed to 'run their course´ without treatment, identified etiologies such as HSV, CMV and VZV may suggest otherwise.[5] Indeed, some of these patients may benefit from medical treatment. Clinical practices commonly recommended for acute conjunctivitis in developed countries, such as observation and reassurance, may not be appropriate for the pink eye patient population in Burkina Faso, as some conjunctivitis may be early signs of treatable systemic infections.

The limitations of this study include the small sample size and the lack of traditional microbiological evaluations for these patients. However, even in high-income countries, microbiologic investigation for infectious conjunctivitis is not routinely performed. In addition, sequencing results may vary depending on sample collection techniques, sequencing methods (DNA-seq vs RNA-seq), and bioinformatics pipelines.

Local surveillance and pathogen identification of seasonal and epidemic conjunctivitis outbreaks are necessary to determine definitive etiologies. The unbiased nature of high-throughput sequencing allows for the identification of all known pathogens of acute infectious conjunctivitis, including unexpected etiologies. Such a surveillance strategy can guide appropriate treatment strategies, inform region-specific public health initiatives and has the potential to improve ocular and socioeconomical burdens.

Authors' contributions: TD, GDS and TML designed and supervised the study. MB, AS, BC, TD, KR, EL and EC oversaw the fieldwork and sample collection. TD supervised all laboratory work. LZ, CC and KR performed laboratory-related experiments. TD, AH, MD and GDS performed the bioinformatics analyses. TD and GDS 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 study was approved by The Institutional Review Board of the University of California, San Francisco (UCSF) and Comité National d'éthique in Burkina Faso and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from patients and/or guardians.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author. Limitations apply to variables that may compromise participant privacy or consent.

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