

The Prevalence of Bacteria, Fungi, Viruses, and *Acanthamoeba* From 3,004 Cases of Keratitis, Endophthalmitis, and Conjunctivitis

Regis P. Kowalski, M.S., M.(ASCP), Shannon V. Nayyar, B.S., M.(ASCP), Eric G. Romanowski, M.S., Robert M.Q. Shanks, Ph.D., Alex Mammen, M.D., Deepinder K. Dhaliwal, M.D., and Vishal Jhanji, M.D.

Purpose: The definitive identification of ocular pathogens optimizes effective treatment. Although the types of ocular pathogens are known; there is less definitive information on the prevalence of causative infections including viruses, fungi, and protozoa, which is the focus of this retrospective laboratory review.

Methods: Data used for laboratory certification were reviewed for the detection of bacteria, viruses, fungi, and protozoa, from patients with infectious keratitis, endophthalmitis, and conjunctivitis. The main outcome parameter was laboratory-positive ocular infection.

Results: The distribution of infectious agents for keratitis (n=1,387) (2004–2018) was bacteria 72.1% (*Staphylococcus aureus* 20.3%, *Pseudomonas aeruginosa* 18%, *Streptococcus spp.* 8.5%, other gram-positives 12.4%, and other gram-negatives 12.9%), *Herpes simplex virus* 16%, fungi 6.7%, and *Acanthamoeba* 5.2%. For endophthalmitis, (n=770) (1993–2018), the bacterial distribution was coagulase-negative *Staphylococcus* 54%, *Streptococcus spp.* 21%, *S. aureus* 10%, other gram-positives 8%, and gram-negatives 7%. The distribution for conjunctivitis (n=847) (2004–2018) was *Adenovirus* 34%, *S. aureus* 25.5%, *Streptococcus pneumoniae* 9%, *Haemophilus* 9%, other gram-negatives 8.8%, other gram-positives 6%, coagulase-negative *Staphylococcus* 4.5% and *Chlamydia* 3.2%.

Conclusion: An updated monitoring of ocular pathogens creates an awareness of the different infectious etiologies and the importance of laboratory studies. This information can determine treatment needs for infectious ocular diseases.

Key Words: Keratitis—Endophthalmitis—Conjunctivitis—Ocular pathogens—Infectious ocular infections.

(*Eye & Contact Lens* 2020;46: 265–268)

From the The Charles T. Campbell Eye Microbiology Laboratory, Department of Ophthalmology, University of Pittsburgh Medical Center (UPMC), University of Pittsburgh, Pittsburgh, PA.

The authors have no funding or conflicts of interest to disclose.

We are grateful to the Pennsylvania Lions Club, The Charles T. Campbell Foundation, Eye and Ear Foundation of Pittsburgh, PA, National Institutes of Health Core Grant P30 EY008098, and Unrestricted Grant from Research to Prevent Blindness, New York, NY, for continued financial support.

Address correspondence to Regis P. Kowalski, M.S., M.(ASCP), Department of Ophthalmology/Microbiology, University of Pittsburgh School of Medicine, The Eye and Ear Institute Bldg., Ophthalmic Microbiology, Room 642, 203 Lothrop Street, Pittsburgh, PA 15213; e-mail: kowalskip@upmc.edu

Accepted June 30, 2019.

DOI: 10.1097/ICL.0000000000000642

Ocular infections can cause significant ocular morbidity and most fortunately will resolve in most instances if properly detected and appropriately treated. Although the etiologic agents of keratitis, endophthalmitis, and conjunctivitis have been described,^{1–3} an accurate prevalence including viral, fungal, and protozoal infection has not been reported. Our specialized ophthalmic laboratory was able to describe the number of laboratory-positive agents responsible for ocular infections.

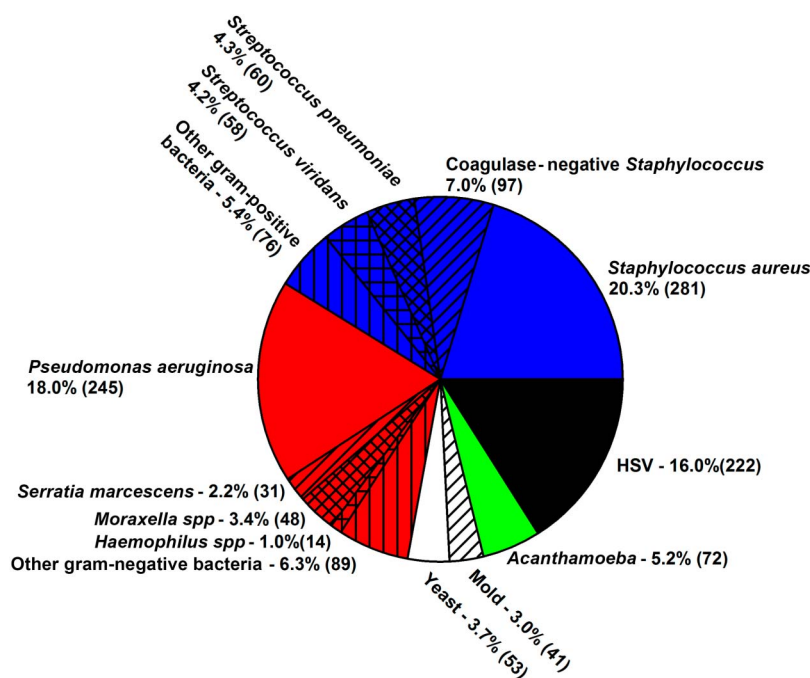
Bacterial conjunctivitis is generally self-limiting, but adenovirus can spread to others and chlamydia can be prolonged without treatment. The incidence of ocular adenoviral infection among conjunctivitis patients in the United States has not been completely determined, and there is an interest in developing a topical treatment for this “unmet need.”^{4–6} Bacterial keratitis may be troublesome if not treated appropriately with the correct antibiotic. The prognoses of *Acanthamoeba* and fungal infections can be devastating and sight threatening with prolonged and incorrect treatment if identified in an untimely fashion. There is a need for a consistent treatment for *Acanthamoeba* keratitis, and although there are several remedies for fungal keratitis, differentiation from other infectious agents can be an advantage. *Herpes simplex virus* (HSV) keratitis needs to be correctly diagnosed to properly treat without exacerbating with anti-inflammatory agents. The incidence of bacterial endophthalmitis is important to monitor surgical prophylaxis and assure that intravitreal therapy is appropriate.

Our study, based on monitored data, provides insight on the occurrence of ocular pathogens that are causative agents of keratitis, endophthalmitis, and conjunctivitis/blepharitis.

METHODS

The data used for the certification of our clinical ophthalmic microbiology laboratory was reviewed for pathogens that are implicated in keratitis, endophthalmitis, and conjunctivitis/blepharitis from 1993 to 2018. These cases represent all positive cultures from our outpatient clinics, in-house private practices, the emergency department, the surgical centers, satellite offices, the community ophthalmologist, and patients sent to our ophthalmic microbiology laboratory for cultures and laboratory studies. The data in part are used to provide antibiotic susceptibility patterns to treat ocular bacterial infections (not part of this study). Bacterial susceptibility patterns of the isolates in part are found in a previous manuscript.⁷ Laboratory certification mandates that yearly changes in antibiotic

FIG. 1. The prevalence of 1,387 laboratory-positive pathogens of infectious keratitis from 2004 to 2018. Blue represents gram-positive bacteria and red represents gram-negative bacteria.



susceptibility, testing, and laboratory procedures be shared with the ophthalmic practicing community through direct mail communication or in our case a website (<http://eyemicrobiology.upmc.com>). This website details the laboratory tests for identifying infectious agents of keratitis, endophthalmitis, and conjunctivitis.

Since 1993, pathogens and patient samples isolated from keratitis, conjunctivitis, and endophthalmitis have been stocked for validation of new testing and patient treatment. These isolates represented an Ocular Clinical Tissue Bank in which the isolates were de-identified to comply with IRB protection of patient identity. Clinical presentation data, patient identity, and demographics were not tabulated for the isolates. No patient contact was involved in this study (University of Pittsburgh, Institutional Review Board, IRB# 1904003).

It must be noted that testing changed to the better during the study period (1993–2018). The patient-positive results were included into the database as saved stocks, but the original patient requisitions were not available before 2004. Because it was an infrequent cause of endophthalmitis, we could not accurately tabulate fungal infection before 2004, and subsequently we only included *Acanthamoeba* infections after 2004.

RESULTS

Figure 1 depicts the prevalence of 1,387 laboratory-positive pathogens of infectious keratitis from 2004 to 2018. The distribution of infectious agents for keratitis ($n=1,387$) was bacteria 72.1% (*Staphylococcus aureus* 20.3%, *Pseudomonas aeruginosa* 18%, *Streptococcus spp.* 8.5%, other gram-positives 12.4%, and other gram-negatives 12.9%), *H. simplex virus* 16%, fungi 6.7%, and *Acanthamoeba* 5.2%. These data do not include three cases of microsporidial keratitis.

Figure 2 depicts the prevalence of 770 laboratory-positive bacterial pathogens of endophthalmitis from 1993 to 2018. For endophthalmitis, ($n=770$), the bacterial distribution was coagulase-negative *Staphylococcus* 54%, *Streptococcus spp.* 21%, *S. aureus* 10%, other gram-positives 8%, and gram-negatives 7%. We did not have a complete data-set to add accurate fungal incidence. From 2015 to 2018, 11 of 34 (32.3%) of endogenous endophthalmitis were due to fungal infection (Amarasekera S et al. Epidemiology, clinical features, and outcomes of bacterial and fungal endogenous endophthalmitis at a tertiary care hospital. *Invest Ophthalmol Vis Sci.* 2018 (suppl):2024).

Figure 3 depicts the prevalence of 847 laboratory-positive pathogens of infectious conjunctivitis/blepharitis from 2004 to 2018. The distribution for conjunctivitis ($n=847$) was *Adenovirus* 34%, *S. aureus* 25.5%, *Streptococcus pneumoniae* 9%, *Haemophilus* 9%, other gram-negatives 8.8%, other gram-positives 6%, coagulase-negative *Staphylococcus* 4.5%, and *Chlamydia* 3.2%.

DISCUSSION

Do the data detailed by the three pie charts represent a realistic snapshot of ocular infections? The data may be a function of “culturing bias” with three apparent scenarios: (1) There are people who have infections and do not seek help for many reasons, (2) There are people who have infections and physicians empirically treat based on training intuition; and (3) There are people who have serious eye infections that require laboratory studies and treatment.

The literature is sparse for reports of microbial prevalence in conjunctivitis. Woodland reported a prevalence of bacteria 18.3%, *Adenovirus* 75%, HSV 2.3%, and *Chlamydia trachomatis* 1.8%.⁸ In general, bacterial conjunctivitis is self-limiting and physicians do not seek culture, but physicians may treat topically especially in the school age population. If the conjunctivitis does not resolve

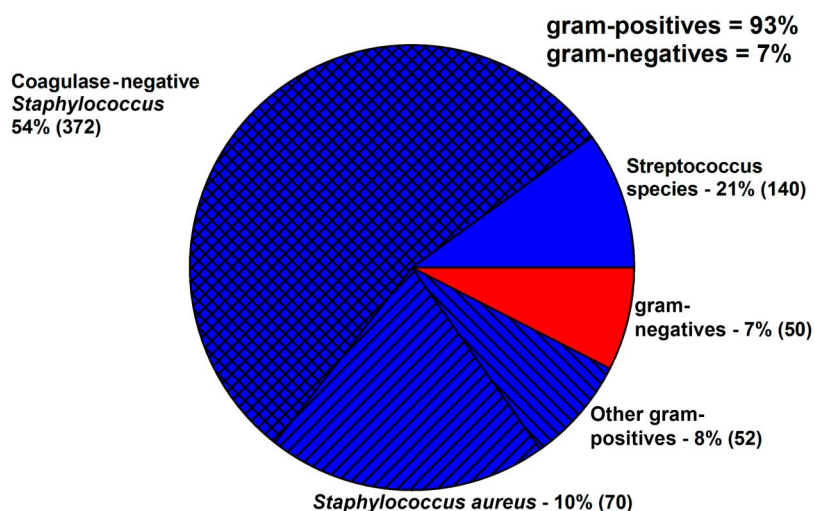


FIG. 2. The prevalence of 770 laboratory-positive bacterial pathogens of endophthalmitis from 2004 to 2018. Blue represents gram-positive bacteria and red represents gram-negative bacteria.

within a few days, adenovirus infection is suspected and the physician may treat the symptoms and perform laboratory studies to confirm infection. In a previous study from our laboratory, actual prevalence with other infections was not shown, but seasonal changes of prevalence with adenovirus infection were demonstrated, whereas most adenovirus infections appear from July to September and the least from April to June.⁹ A wide range of adenovirus prevalence has been reported from 0.08% to 75%.^{10–13} Chronic conjunctivitis supported with classic symptoms may support chlamydia infection, and the social ramifications may require laboratory studies to confirm and adequately treat the patients and their significant others for chlamydia infection. The prevalence of laboratory-positive isolation of conjunctivitis from this study probably represent those patients with serious conjunctivitis that were deemed necessary to culture. The accuracy of the data can be reasonably debated.

The culturing of presumed bacterial keratitis is dictated by the size of ulceration, clinical presentation, and the physicians' training. Small infiltrates with staining (less than 2 mm) due to contact lens are generally not cultured, and the patients are administered topical empiric antibiotics. Large infiltrates with ulceration (greater

than 2 mm) are cultured, placed on fortified topical antibiotics, and the patients are followed carefully until the keratitis resolves. Microbial keratitis with a differential diagnosis of fungus, *Acanthamoeba*, *Mycobacteria*, *Nocardia*, etc. have samples obtained for laboratory studies. HSV and *Varicella zoster virus* (VZV) infections have classic presentations that do not require culture, but suspicion in patients with nonclassical presentations may have need for laboratory studies to rule-out viral infection. The prevalence of laboratory-positive isolation of keratitis may be a better sampling method for the general population, but the prevalence of HSV infection is probably understated in this study.

Endophthalmitis is generally a serious bacterial infection that is, confirmed with laboratory studies. The vitreous and anterior chamber of patients with endophthalmitis are tapped for culture confirmation of infection and to assure susceptibility to the antibiotics that are intravitreally injected. Our data are comparative to the Endophthalmitis Vitrectomy Study, a multicenter study, with the predominance of gram-positive bacteria (93%–94%), coagulase-negative *Staphylococcus* (54%–70%), *S. aureus* (10%–10%), *S. spp* (21%–14%), other Gram-positives (8%–3%), and gram-negatives (7%–6%).¹⁴ The prevalence of laboratory-

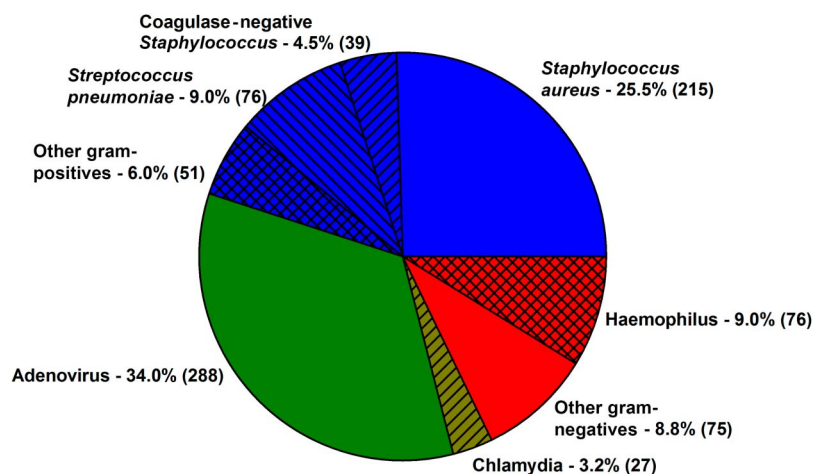


FIG. 3. The prevalence of 847 laboratory-positive pathogens of infectious conjunctivitis from 2004 to 2018. Blue represents gram-positive bacteria and red represents gram-negative bacteria.

positive isolation of endophthalmitis from our study may represent a closer sampling of the general findings from the total endophthalmitis population set.

We must emphasize that this is a single-center study in the United States and may not reflect prevalence of ocular infections worldwide. The data from our study definitely could be used to focus on new antimicrobials for future and seasonal infections. It can be reasonably debated that our data do not completely represent the precise prevalence of infection in the total population, but may represent the total population of ocular infections from patients with severe ocular infections that sought and received antimicrobial treatment. Finally, our study demonstrates the importance of laboratory studies of ocular infections and the monitoring of these infections to provide updated patient care.

REFERENCES

1. Truong DT, Bui MT, Cavanagh D. Epidemiology and outcome of microbial keratitis: Private university versus public hospital care. *Eye Contact Lens* 2018;44:S82–S86.
2. Callegan MC, Engelbert M, Parke DW, et al. Bacterial endophthalmitis: Epidemiology, therapeutics, and bacterium-host interactions. *Clin Microbiol Rev* 2002;15:111–124.
3. Azari AA, Barney NP. Conjunctivitis: A systemic review of diagnosis and treatment. *JAMA* 2013;310:1721–1729.
4. Yates KA, Shanks RM, Kowalski RP, et al. The in vitro evaluation of povidone-iodine against multiple ocular adenoviral types. *J Ocul Pharmacol Ther* 2019;35:132–136.
5. Romanowski EG, Yates KA, Shanks RMQ, et al. Benzalkonium chloride demonstrates concentration dependent antiviral activity against adenovirus *in vitro*. *J Ocul Pharm Ther* 2019;35:1–5.
6. Pepose JS, Narvekar A, Liu W, et al. A randomized controlled trial of povidone-iodine/dexamethasone ophthalmic suspension for acute viral conjunctivitis. *Clin Ophthalmol* 2019;13:535–544.
7. Kowalski RP. Perspective: Is antibiotic resistance a problem in the treatment of ophthalmic infections? *Exp Rev Ophthalmol* 2013;8:119–226.
8. Woodland RM, Darougar S, Thaker U, et al. Causes of conjunctivitis and keratoconjunctivitis in Karachi, Pakistan. *Trans R Soc Trop Med Hyg* 1992;86:317–320.
9. Lee J, Bilonick R, Romanowski EG, et al. Seasonal variation in human adenovirus conjunctivitis: A 34-year study. *Ophthalmic Epidemiol* 2018;25:451–456.
10. Butt AL, Chodosh J. Adenoviral keratoconjunctivitis in a tertiary care eye clinic. *Cornea* 2006;25:199–202.
11. O'Donnell B, McCrudden EA, Desselberger U. Molecular epidemiology of adenovirus conjunctivitis in Glasgow 1981–1991. *Eye* 1993;7:8–14.
12. Pinto RD, Lira RP, Arieta CE, et al. The prevalence of adenoviral conjunctivitis at the clinical hospital of the State University of Campinas, Brazil. *Clinics (Sao Paulo)* 2015;70:748–750.
13. Heindl LM, Augustin AJ, Messmer EM. Adenovirus initiative study in epidemiology (ADVISE)—Results of a multicenter epidemiology study in Germany. *Graefes Arch Clin Exp Ophthalmol* 2019:249–251.
14. Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the endophthalmitis vitrectomy study. *Am J Ophthalmol* 1996;122:1–17.