Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects

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SUMMARY Although the bacterial flora of the conjunctiva in children and adults has been studied, there has been no previous comparison between these two age groups of anaerobic as well as aerobic bacteria. Conjunctival cultures from 229 eyes of 144 uninfected subjects were analysed for aerobic, microaerophilic, and anaerobic bacteria. Adults showed a greater number of species per eye than did younger subjects (1.47 versus 1.13; $p < 0.05$). Anaerobic species, predominantly *Propionibacterium*, were obtained from 27.1% of all eyes, but from a significantly greater percentage (30.2% versus 12.8%) of adults' than children's eyes ($p < 0.04$). *Streptococcus* spp. were cultured from 14.9% of the children's eyes as opposed to only 2.2% from adults ($p < 0.005$). Understanding the differences in conjunctival flora between normal adults and children aids the interpretation of culture results and leads to properly defining and treating potential pathogens.

The presence of micro-organisms in the normal human conjunctiva was established in the 19th century.¹ Since that time the normal aerobic flora of the adult human conjunctiva has been studied by numerous authors from various countries.²⁻⁴

More recently, improved microbiological techniques have allowed the isolation and delineation of anaerobic bacteria whether normal residents of the ocular surface or potential ocular pathogens.⁵⁻⁸ The relatively few reports on the frequency of anaerobic colonisation of the normal conjunctiva have shown conflicting results. While some investigators⁵⁻⁷ have reported as many as 80% of normal human conjunctivae to be colonised by anaerobes, others have found few or none.^{3,9,10} The only modern series to compare the bacterial flora of children with that of adults studied only aerobic bacteria.³ To study these issues we have compiled the first series to compare both aerobic and anaerobic conjunctival bacterial floras in children and adults.

Materials and methods

One hundred and forty-four patients undergoing

ophthalmic surgery were studied. Their ages ranged from 6 months to 95 years. Children were defined as those aged 17 years or less. No patient had an active infection or obstructed lacrimal outflow system at the time of study. No antibiotics or other medications were applied to the eye prior to study for at least four weeks. A sterile, anaerobic transport, dry swab was applied to the inferior conjunctival fornix of each eye.

Each culture swab was washed three times in 0.5 ml of Schaedler's broth, and manually vortexed repeatedly after being wrung out by pressing it along the sides of the tube. The swab was cultured in 10 ml of Schaedler's broth. Blood and chocolate agar plates were inoculated with 0.1 ml of eluant, which was spread on the surface of the agar with a glass rod. The blood agar plates which contained brain-heart infusion agar base (BBL, Baltimore, MD), 5% sheep blood supplemented with vitamin K, and Fildes's enrichment were incubated for at least seven days at 35°C in an anaerobic jar with a gas mixture of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. Chocolate agar plates (Scott, Carson, CA) were incubated with 5% to 10% carbon dioxide at 35°C for at least seven days. After incubation the colonies were differentiated and enumerated by standard bacteriological techniques, including the VPI technique for anaerobic bacteria.¹⁰

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Results

The mean numbers ($\pm$ SD) of colonies and species per eye are presented in Table 1. The number of colonies isolated per eye was not significantly different statistically between children and adults. The number of different species isolated per eye was significantly higher in adults than in children (1.47 versus 1.13; $p<0.05$). Anaerobic species, predominantly *Propionibacterium acnes*, were obtained from 27.1% of eyes overall, but from a significantly higher percentage of the adults' than the children's eyes (30.2% versus 12.8%; $p<0.04$ by Fisher's exact test).

Positive culture results delineated by species are shown in Table 2. Sterile eyes (with no growth of bacteria from the conjunctiva) are not included in Table 2. The percentage of sterile eyes was similar in both groups (23% in younger subjects and 21% in adults). Streptococcus species were cultured from 14.9% of children's conjunctivae as opposed to only 2.2% of adults' ($p<0.005$ by χ^2 test). Culture results were otherwise similar between the two groups.

Table 1 Results of cultures (mean $\pm$ SD)

	Children	Adults	Total
No. of patients	25	119	144
No. of eyes	47	182	229
No. of sterile eyes	11	39	50
No. of aerobic cultures	47	212	259
No. of anaerobic cultures	6	56	62
No. of colonies per eye	322 $\pm$ 824	439 $\pm$ 1329	
No. of species per eye*	1.13 $\pm$ 0.89	1.47 $\pm$ 1.08	

* $p<0.05$ by t test.

Table 2 Species of bacteria isolated and fraction of total isolates

	Children	Adults	Total
<i>Bacillus subtilis</i>	1 (2%)	0	1
<i>Corynebacterium</i> species	1 (2%)	14 (5%)	15
Diphtheroids	16 (30%)	68 (25%)	84
<i>Haemophilus influenzae</i>	0	5 (2%)	5
<i>Micrococcus</i> species	0	2 (1%)	2
<i>Penicillium</i>	0	1 (0.4%)	1
<i>Proteus</i> species	0	2 (1%)	2
<i>Serratia marcescens</i>	0	1 (0.4%)	1
<i>Staphylococcus aureus</i>	3 (6%)	7 (3%)	10
<i>Staphylococcus epidermidis</i>	19 (36%)	108 (40%)	127
<i>Streptococcus</i> species*	7 (13%)	4 (1%)	11
True anaerobes:			
<i>Peptococcus</i>	0	1 (0.4%)	1
<i>Propionibacterium acnes</i> **	6 (11%)	55 (21%)	61
Total no. of isolates	53	268	321
Total no. of eyes	47	182	229

* $p<0.005$ by Fisher's exact test.

** $p<0.04$ by Fisher's exact test.

Discussion

The aerobic bacterial flora of the normal adult human conjunctiva has been studied by numerous authors during the past 50 years.^{2-4,9,11-13} These reports have shown remarkable consistency across time and geographic space. From these studies certain conclusions can be drawn. Most eyes harbour *Staphylococcus epidermidis*, with diphtheroids only somewhat less common. While other species are less usual, there are a few noteworthy exceptions. Locatcher-Khorazo and Seegal³ reporting on cultures obtained from 10271 individuals between 1952 and 1968 noted a 42% frequency of *Staphylococcus aureus* colonisation. Orfila and Courden² reported a prevalence of 11.6% Gram-negative rods in an Algerian population. Cason and Winkler¹² cultured streptococcus species from less than 1% of their patients in Birmingham, Alabama. Soudakoff⁴ in 1954 cultured diphtheroids from only 2.8% of eyes in his Los Angeles based series. The type of aerobic flora of adult conjunctivae in our study is consistent with the majority of published reports.

Two modern studies have addressed the question of conjunctival flora variation with age. Khorazo and Thompson¹⁴ reported no differences other than an increasing frequency of diphtheroids after age 50. The larger series reported by Locatcher-Khorazo and Segal³ showed a lower incidence of diphtheroids in subjects below 18 years of age. Our data show no difference in the prevalence of diphtheroids between children and adults. However, since we studied a smaller population and did not distinguish between younger and older adults, we may have missed a trend toward increased numbers of diphtheroids in the elderly.

In a study of conjunctivitis in paediatric patients Brook studied the normal aerobic and anaerobic flora in some paediatric patients as controls.¹⁵ There are some striking similarities between his data and ours. As in our study, Brook found *Propionibacterium* and *Peptococcus* to be the most common anaerobic bacteria and *Staphylococcus epidermidis* to be the most common aerobic. From his data one can calculate that the average number of species isolated per paediatric patient was 1.1, which is virtually identical to our findings. The two studies differed in Brook's finding *Corynebacterium*, and not diphtheroids as we found, to be the second most common aerobe. Brook did not compare his data with any from an adult population.

Our study found a highly significant difference ($p<0.002$) in the prevalence of streptococcus species found in children's and adults' conjunctivae (14.9% versus 2.2%). Streptococcal colonisation of both skin¹⁶ and upper respiratory tract¹⁷ is known to be

more frequent in children than in adults. Since these sites are believed to be an important source of conjunctival flora,³ one might expect more streptococci in the conjunctivae of children as well.

Anaerobic bacteria were first reported in the normal conjunctiva by Haenel *et al.*¹⁸ Nagata¹⁹ in 1963 isolated and identified *Propionibacterium* species. Bachrach and colleagues⁹ were unable to culture any anaerobic organisms in a series of 277 young adults. This inability may have resulted from failure to use enriched, solid-culture media and to incubate for at least seven days in order to maximise the yield of these often fastidious, slow-growing species.

Anaerobic bacteria are proved aetiological agents in all major forms of ocular and periocular infections.⁸ Anaerobes such as *Actinomyces*, *Arachnia*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Lactobacillus*, *Peptococcus*, *Peptostreptococcus*, and *Propionibacterium* have been cultured from uninfected ocular tissue. These anaerobic bacteria have also been reported to be ophthalmic pathogens.⁸ Some anaerobic ocular pathogens such as *Fusobacterium*, *Butyrovibrio*, and *Veillonella* have never been reported as normal flora. We now report normal anaerobic flora, primarily *Propionibacterium*, from 27.1% of all eyes in our study.

One of the main sources of conjunctival bacteria is the adjacent skin. Since *Propionibacterium* is the predominant anaerobic organism of the skin, it is understandable that it should play the same role in the conjunctiva.⁸ Leyden and colleagues showed *Propionibacterium* to be present on the facial skin of every one of the 342 individuals tested and to be in much greater concentration after 20 years of age.²⁰ They attributed the age related changes in *Propionibacterium* concentration on skin to the greater production of sebum, as androgen secretion increases in adulthood. Sebum would supply nutrients such as triglycerides and free fatty acids to the resident bacteria. This is consistent with our finding of *Propionibacterium* in a significantly higher percentage of the conjunctivae of adults than children.

The differences in the conjunctival flora between adults and children may be explained by several potential mechanisms. These include age-related changes in general immune responsiveness, tear composition and dynamics, patterns of exposure to bacteria, past antibiotic utilisation, and the flora of adjacent areas such as skin and upper respiratory tract. Our study demonstrates that adults' eyes harbour more different species per eye than children's eyes (1.47 versus 1.13; $p < 0.05$). Our data suggest that there are also more total microbial colonies per eye in adults than in children; however, the difference did not achieve statistical significance

because of the large standard deviations encountered.

Studies on the frequency of normal conjunctival sacs which are sterile have shown marked disparity. At the low end of the spectrum, Perkins and colleagues,⁷ Chang,¹¹ Matuura,⁶ and Locatcher-Khorazo and Seegal³ respectively reported 9.4%, 9%, 2.5%, and 0% of conjunctivae to be sterile. At the upper end Bachrach *et al.*,⁹ Debnath,²¹ McNatt *et al.*,⁵ and Smith¹³ reported 33%, 30%, 39%, and 47% respectively to be sterile. Our series reports 22% of conjunctivae sterile to both aerobic and anaerobic culture, with no significant difference between children and adults. This is of particular concern to the ophthalmologist when planning surgery, since sterility at the time of surgery presumably decreases the frequency of postoperative infections. It has been shown that with aggressive preoperative preparation, consisting of broad-spectrum topical antibiotics and skin and conjunctival application of diluted povidone-iodine solution, the percentage of sterile conjunctival sacs can be increased to 83%.²² Other frequently used methods of preoperative antimicrobial preparation are less effective.^{23,24}

We believe that a thorough understanding of the differences in conjunctival flora between paediatric and adult subjects is essential for the ophthalmologist. This knowledge has an important role both in the interpretation of clinical culture results and in the management of potential pathogens colonising the ocular surface.

References

- 1 Lawson A. The bacteriology of the normal conjunctival sac, and its practical bearing on the utility of antiseptics in ophthalmic surgery. *Br Med J* 1898; ii: 486-7.
- 2 Orfila J, Courden B. Contribution à l'étude de la flore conjunctivale normale. *Ann Oculist (Paris)* 1961; **194**: 892-9.
- 3 Locatcher-Khorazo D, Seegal BC. *Microbiology of the eye*. St Louis: Mosby, 1972: 13-7.
- 4 Soudakoff PS. Bacteriologic examination of the conjunctiva. *Am J Ophthalmol* 1954; **38**: 374-6.
- 5 McNatt J, Allen SD, Wilson LA, Dowell VR. Anaerobic flora of the normal human conjunctival sac. *Arch Ophthalmol* 1978; **96**: 1448-50.
- 6 Matuura H. Anaerobes in the bacterial flora of the conjunctival sac. *Jpn J Ophthalmol* 1971; **15**: 116-24.
- 7 Perkins RE, Kundsins RB, Pratt MV, Abrahamsen I, Leibowitz HM. Bacteriology of normal and infected conjunctiva. *J Clin Microbiol* 1975; **1**: 147-9.
- 8 Jones DB, Robinson NM. Anaerobic ocular infections. *Ophthalmology* 1977; **83**: OP309-31.
- 9 Bachrach U, Gurevitch J, Landau J, Birnbaum D. The flora of the normal conjunctiva of healthy people in Israel. *Acta Med Oriental* 1953; **12**: 10-3.
- 10 Holdeman LV, Cato EP, Moore WEC. *Anaerobe Laboratory Manual*. Blacksburg: VPI Anaerobe Laboratory, 1977.
- 11 Chang WL. Bacteria flora of the normal conjunctiva. *Chin Med J* 1957; **75**: 233-5.
- 12 Cason L, Winkler CH. Bacteriology of the eye. *Arch Ophthalmol* 1954; **51**: 196-9.

- 13 Smith CH. Bacteriology of the healthy conjunctiva. *Br J Ophthalmol* 1954; **38**: 719–26.
- 14 Khorazo D, Thompson R. The bacterial flora of the normal conjunctiva. *Am J Ophthalmol* 1935; **18**: 1114–6.
- 15 Brook I. Anaerobic and aerobic bacterial flora of acute conjunctivitis in children. *Arch Ophthalmol* 1980; **98**: 833–7.
- 16 Somerville DA. The effect of age on the normal bacterial flora of the skin. *Br J Dermatol* 1969; **81** (suppl 1): 14–22.
- 17 Masters PL, Brumfitt W, Mendez RL. Bacterial flora of the upper respiratory tract in Paddington families, 1952–4. *Br Med J* 1958; **iv**: 1200–5.
- 18 Haenel H, Feldheim G, Muller-Beuthow W. Zur mikrobiologischen Ökologie des Menochen. *Zentralbl Bakteriol Mikrobiol* 1958; **172**: 73–92.
- 19 Nagata M. Corynebacterium in conjunctival sac. *Nitsuseki Igaku* 1963; **16**: 115–27.
- 20 Leyden JL, McGinley KJ, Mills OH, Kligman AM. Age-related changes in the resident bacterial flora of the human face. *J Invest Dermatol* 1975; **65**: 379–82.
- 21 Debnath SC. Effect of preoperative antibiotics on bacterial colony counts of the conjunctival sac. *J Ocul Ther Surg* 1985; **4**: 122–4.
- 22 Isenberg SJ, Apt L, Yoshimori R, Khwarg S. Chemical preparation of the eye in ophthalmic surgery. IV. Comparison of povidone-iodine on the conjunctiva with a prophylactic antibiotic. *Arch Ophthalmol* 1985; **103**: 1340–2.
- 23 Isenberg SJ, Apt L, Yoshimori R. Chemical preparation of the eye in ophthalmic surgery. I. Effect of conjunctival irrigation. *Arch Ophthalmol* 1983; **101**: 761–3.
- 24 Apt L, Isenberg SJ, Yoshimori R, Pacz JH. Chemical preparation of the eye in ophthalmic surgery. II. Effectiveness of mild silver protein solution. *Arch Ophthalmol* 1983; **101**: 764–5.

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