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Microbiology for optometrists

ABDO has awarded this article 2 CET credits (GD).

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Optometrists need to understand the bacteria, viruses and amoebae which can infect the lids, conjunctiva and the cornea and how they may be influenced by wear of contact lenses. This article will consider these organisms by the tissue that they infect and the methods used to treat the infection they cause.

BASIC MICROBIOLOGY

Bacteria

Bacteria are self-replicating (possessing both DNA and RNA) and are able to grow on a cell free medium. In the laboratory, bacteria are cultured on agar (extracted from seaweed), to which nutrients are added (such as 5% horse blood or oatmeal). This latter medium is nutritious but non-selective, allowing the growth of most bacteria. The bacteria grow as colonies on the agar and have a characteristic appearance in size, shape or colour. For example, *Staphylococcus aureus* has a golden yellow colour and exhibits a shiny well-formed colony whereas, *Staphylococcus epidermidis* has a white colour. Certain bacteria can be recognised by clear zones of haemolysis around the colonies such as that caused by *Streptococcus pyogenes* – a virulent bacterium producing copious pus at the site of infection. Other agars are clear and have sugars and/or other indicators added such as MacConkey's medium (used to identify the coliforms – abundant in the faeces of warm blooded animals, *Escherichia coli* being particularly common in the human species) as lactose fermenting (with red colonies) or non-lactose fermenting (with opaque colourless colonies).

In order to grow on agar within 18 hours, bacteria needs to be incubated at 37°C in order to gain their maximum multiplication rate. Certain bacteria grow in air (aerobic bacteria), others require the presence of at least 4% carbon dioxide (capnophilic bacteria), and some will only grow in the absence of oxygen (anaerobic bacteria). The atmosphere required for bacterial growth is used for initial classification and identification.

Bacteria are further identified from their colonial appearance by making a smear on a glass slide (one drop of saline and a small pick of the colony with a bacteriological "loop"). The bacteria can be stained with a simple stain such as methylene blue **(Figure 1)**, when all the bacteria on the slide will be seen under the microscope with a x 10 eyepiece and a x 100 objective lens (x 1000 magnification required). However, a much more useful stain is Gram's stain, devised by Gram over 100 years ago and still practised daily in hospital microbiology laboratories. It is practised as follows:

- Heat fix colony smear on glass slide
- Apply methylene blue for 30 seconds
- Wash with tap water for five seconds
- Apply iodine for 30 seconds
- Rinse with acetone for five seconds
- Apply a counter-stain such as neutral red or carbol fuchsin for one minute
- Wash with tap water for five seconds
- Leave to dry

Gram's stain is a very useful method to identify bacteria causing infection. Certain bacteria, such as *staphylococci* **(Figure 2)** and *streptococci* **(Figure 3)**, appear as black cocci. This is because they retain the methyl violet and iodine stain (being resistant to the decolourising effect of the acetone). Such an appearance is termed "Gram-positive". Other bacteria such as the coliforms appear as red rods since they are decolourised by the acetone and then take up the red counter stain, termed "Gram-negative". The bacteria cannot be seen without stain unless they are viewed against a dark background, when they are seen as refractile and often mobile objects. Bacteria are approximately 1 to 5μ (micron) in size.

Bacteria are subdivided into round forms or cocci, rectangular forms or rods, Gram-positive or Gram-negative, and aerobic or anaerobic in their atmospheric requirement. This basic classification of those bacteria infecting man has remained in place for the last 100 years.

Antibiotics

Antibiotics act to prevent bacterial growth in tissues in four different ways:

1. by preventing development of the cell wall of the organism which results in elongation of the bacterial cell until it bursts – this effect kills the bacteria (bactericidal), e.g. penicillins and cephalosporins;
2. by binding to the ribosomes to prevent cell protein synthesis from messenger RNA – this effect can inhibit the growth of the bacteria (bacteriostatic) but not kill it (relying on polymorphonuclear cells instead) e.g. the aminoglycosides (gentamicin, tobramycin), tetracycline, fucidin, azithromycin and chloramphenicol. However, if the antibiotic is prescribed in a high enough dosage the effect can be bactericidal;
3. by interfering with bacterial DNA polymerase so preventing replication of the cell's DNA (bactericidal) e.g. quinolone drugs (ciprofloxacin, ofloxacin); *and*
4. by inhibiting the enzyme dihydrofolate reductase, which interferes with the folic acid cycle (bacteria constructs tetrahydrofolate which is needed as a co-factor in the production of purines for nucleic acid synthesis in the bacterial cell) – this effect is also known as bacteriostatic, e.g. sulphonamides and trimethoprim.

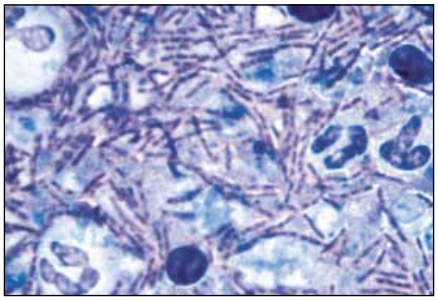

Figure 1
Methylene blue stain of anthrax bacilli

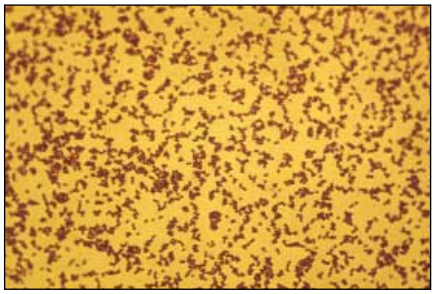

Figure 2
Gram's stain of *staphylococci* in grape-like clusters

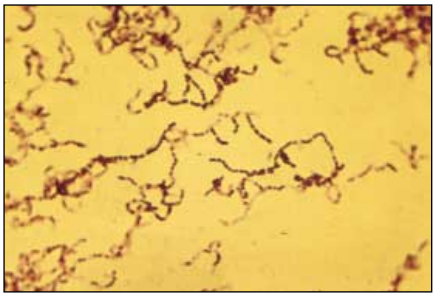

Figure 3
Gram's stain of *streptococci* in chains

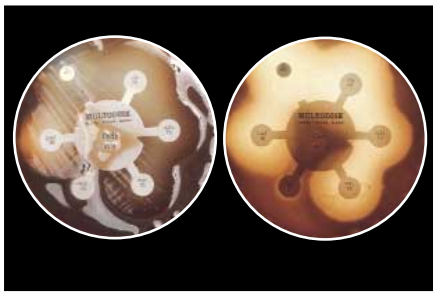

Figure 4
Antibiotic sensitivity plate

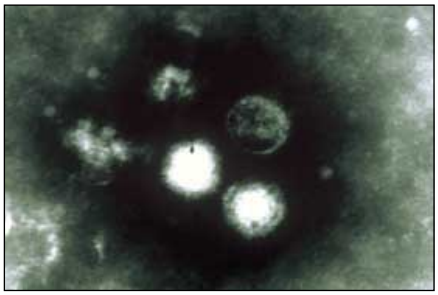

Figure 5
Electron micrograph of *Herpes Simplex* Virus

Figure 6
Trophozoite of *Acanthamoeba* showing spiky projections (Acanthopodia)

Antiseptics or disinfectants such as polyhexamethylene biguanide (PHMB), which contain highly charged molecules, penetrate through the bacterial cell wall, or the ostiole (communication arm – see later) of *Acanthamoeba*, and bind to the cell membrane. This results in damage with leakage of electrolytes from the cytoplasm resulting in cell death.

Once a bacteria is isolated, sensitivity tests are conducted in order to establish the most effective antibiotic to prescribe **(Figure 4)**. A colony is picked off the agar plate culture, emulsified in a small volume of saline, and then spread on a sensitivity test agar plate. A paper disc, which has been impregnated with various antibiotics, is placed on top of the culture on the agar sensitivity plate which is then incubated at 37˚C for 18 hours. "Zones of diameter of inhibition" are read around each antibiotic and compared with standard values to establish whether the zone size represents a sensitive, intermediate or resistant strain.

Resistance to antibiotics can develop within a bacterium by either mutation (following prolonged therapy) or acquisition of transferable resistance plasmids from another bacterium. Mutation gives rise to resistance to the selective antibiotic and affects a single group (such as the amonioglycosides), whilst plasmids can transfer multiple resistance mechanisms to different types of antibiotics. There is a strong association between overuse of an antibiotic (in humans or animals), and the selective pressure leading to resistance.

Viruses

Viruses are not able to self-replicate in a cell free medium and possess either DNA or RNA but not both. Therefore, viruses must be cultured within cells, using monocell layers in test tubes or flasks. Viruses must integrate themselves within the cells' genomic structure and reprogram the production of their own nucleic acid and protein coat to produce new viral particles. New virions are thus shed out of the cell that has been parasitised by the virus.

Viruses are much smaller than bacteria being between 50 and 150nm in size (1000 nanometres = 1 micron) and can only be visualised by the electron microscope **(Figure 5)**. A sample has to be prepared on a special grid which is placed in a vacuum within an electron chamber. Although this is a time consuming and expensive process, it results in an excellent image of the virus.

Viruses are classified initially by the type of nucleic acid that they contain – DNA or RNA, their size, and appearance under the electron microscope. This is followed by biochemical parameters related to their protein coat.

Viruses are cultured in the laboratory in various different tissue culture cell lines in which their presence can be recognised by a "cytopathic effect" (such as rounding up or lysis of the cells). Alternatively, there may be no cytopathic effect and so the presence of the replicating virus needs to be identified indirectly by alternative tests such as the absorption of red cells to the surface of the virus-infected cell. Another method is immuno-fluorescence in order to identify the presence of the virus antigen on the surface of the cell by use of an antibody in serum labelled to fluorescein, which glows when used in conjunction with an ultra-violet light source in the microscope.

Treatment regimes for viruses are detailed later.

Amoebae *(protozoa)*

The most frequent amoeba to infect the eye is *Acanthamoeba,* which is a free-living protozoan found in soil and water. *Acanthamoeba* replicates in a cell free environment and feeds on bacteria in its natural state. Its size is 12 x 10μ and it can contain up to 3000 bacteria. Amoeba move by producing pseudopodia (into which cytoplasm flows), which allows it to surround phagocytose bacteria.

Amoebae can exist in two forms – an active motile form or trophozoite **(Figure 6)**, which ingests the food source and multiplies, or as the stationary cyst form containing the internalised amoeba within a double-walled cyst **(Figure 7)**. Both forms exist naturally although the cyst form tends to occur when the food source, such as bacteria, becomes deficient. Both forms can also exist within a contact lens (CL) storage case contaminated with tap water and within the cornea; in the latter site, the amoeba is thought to feed on the cell nuclei.

For culture, scrapes should be inoculated directly onto non-nutrient agar, made up in Page's amoebal saline. If non-nutrient agar without Page's saline is used, then the plate should be inoculated again with a turbid suspension (on a swab) of heat-killed *Klebsiella aerogenes*, or other coliforms, as a nutrient source for the amoebae. If facilities for culture are not available, corneal scrape specimens may be mailed in saline to a suitable laboratory. Further information regarding suitable laboratory procedures can be obtained from Seal *et al* (1998). Following culturing, amoebae will usually be visible by low power light microscopy as trophozoites after one week, and after two weeks, the whole plate is covered by the typical double walled, star-shaped cysts. Each point of the star is the ostiole through which the internalised amoeba communicates with the outside world; it is normally plugged with mucopolysaccharide.

Domestic tap water is the main source of *Acanthamoeba* and hence tap water should not be used to wash or store CLs or storage cases **(Figures 8 and 9)**. Similarly, CL wearers who have been exposed excessively to hot tubs or natural springs and have developed unusual corneal disease may have *Acanthamoeba* keratitis.

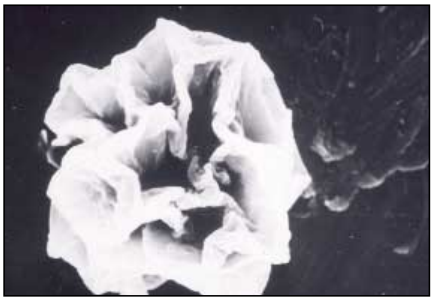

Figure 7
Cyst of *Acanthamoeba*

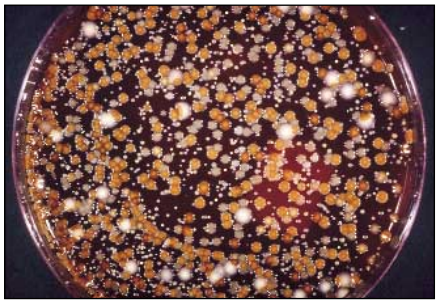

Figure 8
Heavy bacterial growth from a storage case

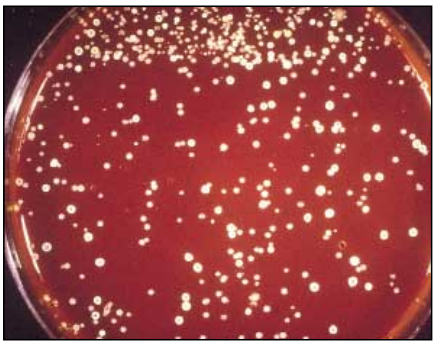

Figure 9
Contact lens as worn from contaminated storage case

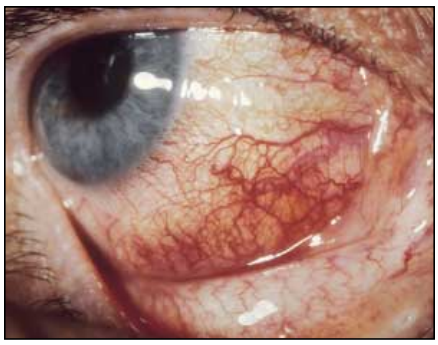

Figure 10
Adult with bacterial conjunctivitis

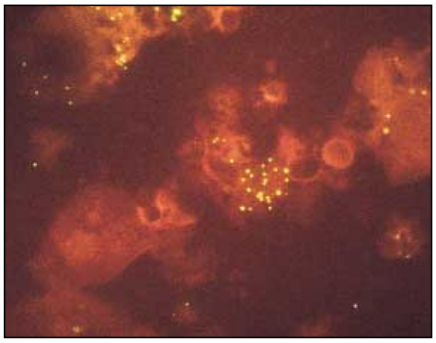

Figure 11
Immunofluorescence of *chlamydia* within conjunctival cells

Hartmannella vermiformis[1] has also been isolated from the cornea of two soft CL wearers with "amoebic-type" keratitis, in Dublin and Glasgow, and hence should now be considered a potential ocular pathogen.

LIDS

There are only two types of bacteria that infect the lids – *Staphylococcus aureus* and *Staphylococcus epidermidis* (otherwise called coagulase-negative *staphylococci* – CNS). These bacteria are Gram positive cocci and are distinguished by the coagulase test – *S. aureus* produces coagulase and clots serum – this can also be coated onto latex particles to produce a simpler agglutination (Staphurex) test. Atopic subjects have a high frequency (up to 60%) of lid colonisation with *S. aureus* whilst the normal lid has a frequency rate of 10%. *S. aureus* can be a pathogenic bacterium causing suppurative infection or it can colonise the lids and cause a cell-mediated delayed-type immune inflammatory reaction to the presence of its antigens. Both normal and inflamed lids have a colonisation rate of approximately 80% with the commensal CNS.

Marginal blepharitis is an inflammation of the lid margin which may be anterior or posterior. Anterior blepharitis involves the lash line whilst posterior blepharitis involves dysfunction of the meibomian glands. Both are strongly associated with skin disease, chiefly seborrheic and atopic dermatitis and rosacea.

The term staphylococcal blepharitis describes anterior blepharitis with lash collarettes, crusting lid ulceration and folliculitis with isolation of *S. aureus*.

Anterior and posterior blepharitis often occur together because of their mutual association with skin disease. Posterior blepharitis takes the form of obstructive meibomian gland disease (MGD) which can be cicatricial or seborrheic. MGD is not an infective condition and any role which bacteria may play is probably indirect through the action of its lipases producing toxic fatty acids.

Blepharitis is diagnosed by clinical examination. The lid margin is examined using the slit lamp for evidence of folliculitis and collarettes. In the acute condition, there may be beads of pus and an ulcerated margin. With chronicity, there is a loss or misdirection of the lashes, telangiectasia and a swollen lid margin. This may be associated with recurrent marginal ulceration.

In chronic blepharitis, regular lid hygiene should be performed with lid scrubs and baby lotion and misdirected lashes removed. Intermittent therapy should be given with topical fucidin gel, "Fucithalmic", or by dibromopropamidine ointment (available as a pharmacy only "over-the-counter" product) to suppress the presence of *S. aureus* on the lids. Patients should be encouraged to wash with antiseptic soaps to suppress carriage of *S. aureus* to other skins sites, especially axillary and perineal; chlorhexidine (Hibiscrub) is the most efficacious product and gives a persistent anti-staphylococcal effect to skin. Topical corticosteroids may be required to suppress the associated marginal keratitis.

CONJUNCTIVA

Conjunctivitis can be due to bacteria, chlamydia (a bacteria that will only grow within cells), viruses, helminths (worms), fungi and protozoa. Non-infective forms due to allergy or toxicity also exist. Toxicity may be due to preservatives, such as thiomersal, associated with CL wear, or occasionally to circulating bacterial toxins, as in Toxic Shock Syndrome due to *S. aureus* (TSST-1) and *Streptococcus pyogenes* (exotoxin A).

Clinical presentation

Bacterial conjunctivitis presents with an acute purulent discharge which frequently becomes bilateral **(Figure 10)**. There may be mild lid swelling. The presentation may be hyperacute with *N. gonorrhoeae* (the gonococcus) or meningococcus with massive lid swelling and a characteristic, profuse yellow-green discharge. In the neonate, this infection can progress rapidly to keratitis and perforation leading to blindness; it requires immediate treatment with frequent penicillin drops, combined with systemic penicillin therapy. Bacterial conjunctivitis should be treated with antibacterial drops such as the quinolones (0.3% ofloxacin or ciprofloxacin) or Polytrim (polymyxin/trimethoprim). Topical chloramphenicol may also be used (despite some scare-mongering five years ago that topical chloramphenicol could be toxic for bone marrow, later work has shown it to be quite safe) as it is effective against most bacteria (although *Ps. aeruginosa* is always resistant).

Bacterial conjunctivitis may also be watery and only mildly purulent. It is then often confused with viral or chlamydial disease, the latter beginning with a watery discharge that later becomes more purulent. Chlamydial infection can be treated with topical tetracycline ointment for six weeks or with a single dose of oral azithromycin which penetrates well into the conjunctival cells and kills the chlamydia within them **(Figure 11)**. Systemic therapy may be needed to treat chlamydial infection at other sites (genito-urinary). In hot countries, repeated conjunctival infection with chlamydia results in chronic conjunctival scarring of trachoma with severe corneal damage as well from lid entropion and recurrent microbial keratitis.

In viral, and to a lesser degree chlamydial conjunctivitis, there is often a non-specific follicular conjunctival response, which is usually absent in bacterial infection **(Figure 12)**. Multiple serotypes of adenovirus can cause conjunctivitis which may begin unilaterally, but commonly becomes bilateral, and, depending on the causative type, may cause a disabling punctate keratitis. In its acute form, it lasts up to 21 days; full recovery may take 28 days or longer. Serotypes 3, 7, 11 and 21 commonly cause "swimming pool" outbreaks of conjunctivitis in young adults in the Summer months as well as pharyngo-conjunctival fever. Infection by serotypes 8, 9 and 19 may be

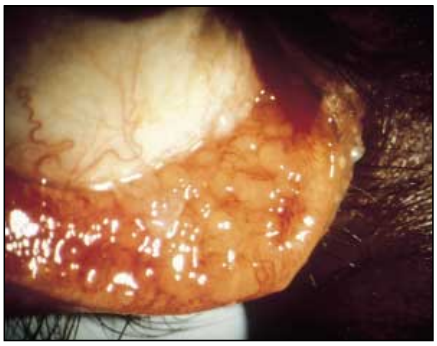

Figure 12
Follicular (viral) conjunctivitis

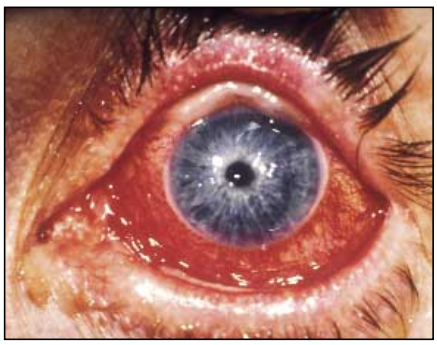

Figure 13
Acute haemorrhagic conjunctivitis due to adenovirus

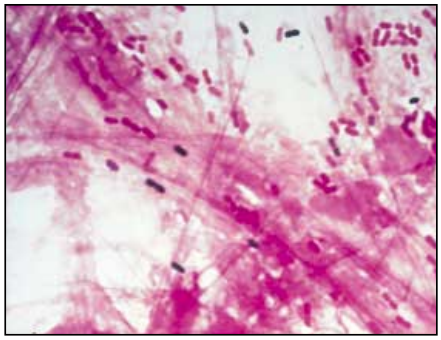

Figure 14
Gram's stain of *Moraxella* - brick shaped rods

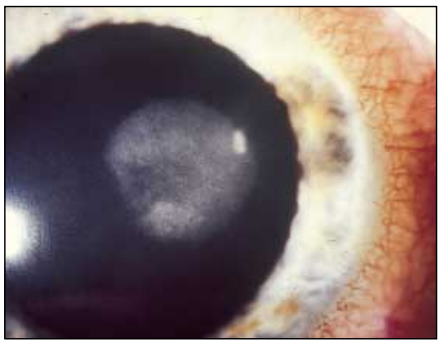

Figure 15
Keratitis in a contact lens wearer due to *Pseudomonas aeruginosa*

complicated by corneal ulceration. Management is palliative only as there are no effective drugs. Adenoviruses are highly contagious and may be transmitted within eye clinics by staff and their equipment.

Acute haemorrhagic conjunctivitis (AHC) is due to Enterovirus 70 or the Coxsackie A24 variant virus and is associated with epidemics especially in South East Asia and India **(Figure 13)**. The infection involves an incubation period of 18 to 36 hours with a sudden onset in one eye, followed by the other the same day. There is lid swelling, photophobia, irritation and a sero-mucous discharge, becoming watery on the second day, with pre-auricular lymphadenopathy in 65% of patients. The tarsal conjunctiva is hyperaemic with small petechiae (bleeding into the skin in small dots) and is oedematous in the lower fornix. Small follicles develop on the second day in the lower, temporal conjunctiva and last up to 10 days. The bulbar conjunctiva is oedematous and shows subconjunctival haemorrhage, which often starts in the upper temporal portion one day after onset, and is the characteristic sign. Bleeding can vary in size from a pinpoint to the whole of the bulbar conjunctiva, and is exacerbated by clinical manipulation on examination. Bleeding decreases after the second day and absorbs gradually over one week. The cornea shows fine punctate epithelial keratitis but nummular opacities, as seen with adenovirus after eight days, do not occur. The infection is highly contagious with rapid spread amongst people in homes and work-places including hospitals. Spread is mainly person-to-person. Diagnosis is usually clinical and treatment is symptomatic.

Figure 16
Colonies of *Pseudomonas aeruginosa* on an agar plate

Summary of causes of conjunctivitis

Bacteria

Gram-positive cocci

- *Staphylococcus aureus*, associated with blepharitis
- *Streptococcus pneumoniae*, associated with sinus disease
- *Streptococcus pyogenes*, associated with throat infections

Gram-positive bacilli

- *Corynebacterium diphtheriae*, associated with a pseudomembrane
- *Listeria monocytogenes*, associated with rural and farmyard dust (Farmer's eye)

Gram-negative cocci

- *Neisseria meningitidis/gonorrhoeae,* associated with throat/genital infection

Gram-negative bacilli

- *Haemophilus influenzae*, associated with intrinsic throat flora
- *Klebsiella aerogenes* and coliforms, associated with CL wear
- *Moraxella sp.* **(Figure 14)**, associated with damaged ocular surface
- *Proteus sp.*, associated with old age (more in men than women)
- *Pseudomonas aeruginosa*, associated with CL wear **(Figures 15 and 16)**

Chlamydia

- *Chlamydia trachomatis*, associated with overcrowding in houses and flies (encouraged by cattle dung) in the Near and Middle East and tropics
- **TRIC** - (Trachoma/Inclusion Conjunctivitis Syndrome), associated with genital infection in Western countries and may be transmitted via towels, and swimming pools

Viruses

- Adenovirus, associated with epidemics from shipyards, close living quarters, and eye clinics (via tonometers and staff handling of patients). Early diagnosis is required to bring outbreaks to a quick halt
- Acute haemorrhagic conjunctivitis (AHC) may occur in epidemics and is due to Enterovirus 70 and Coxsackie virus A 24 variant
- *Herpes simplex* virus (HSV), associated with corneal disease
- *Herpes zoster* virus (HZV), associated with shingles of the Vth nerve.

Presumed bacterial conjunctivitis laboratory protocol

If bacterial infection is presumed, culture swabs should be collected plus two smears on glass slides for Gram and acridine orange stains. Conjunctival swabs should be cultured on blood and chocolate agars (and a selected gonococcal agar if thought relevant). Culture should take place in CO_2 at 37°C for 48 hours. Pathogens should be identified and sensitivity tests performed.

CORNEA

Microbial keratitis

Suppurative bacterial keratitis presents clinically as a corneal stromal infiltrate or abscess with an overlying epithelial defect. It is usually central, except in cases of trauma. Because corneal thickness is only about 0.5mm, such an ulcer may rapidly progress to perforation within 24 hours of onset. There is an urgent need to treat bacterial keratitis with high doses of effective antibiotic.

Bacteria account for over 80% of ulcerative keratitis occurring in Northern climates (see overleaf) and 60% in Southern climates where fungal keratitis is more common. Mixed bacterial and fungal infection frequently occur in the tropics and occasionally in Northern climates when associated with rural injuries. In temperate climates, Gram-positive bacteria predominate; the yeast *Candida sp.* **(Figure 17)** is an occasional pathogen, and *Acanthamoeba* is associated with CL wear. This compares with *Pseudomonas aeruginosa* and filamentous mycelial fungi which appear to predominate in

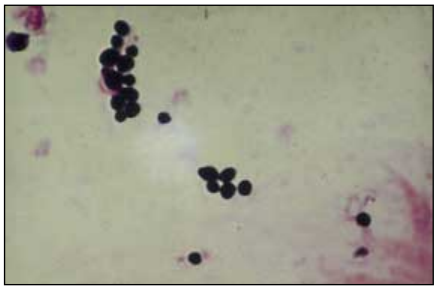

Figure 17
Gram's stain of *Candida sp.* yeast cells

tropical and sub-tropical climates. In the latter situation, *Acanthamoeba* is usually a non-CL associated infection or can be detected as a chronic microbial keratitis.

Summary of bacteria causing suppurative keratitis

Gram-positive cocci
- *Staphylococcus aureus*
- Coagulase-negative *staphylococci*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- Anaerobic *streptococci* (rare)

Gram-negative diplobacilli
- *Moraxella sp.*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*

Gram-positive rods
- *Corynebacterium diphtheriae* (rare)

Gram negative rods
- *Acinetobacter sp.*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Morganella morganii*
- *Proteus sp.*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*

Acid-fast bacteria
- *Mycobacterium chelonei*
- *Nocardia asteroides*

In the past, suppurative keratitis was due chiefly to trauma, or occurred in compromised eyes with existing corneal disease. With the growth of CL use in recent years, there has been a rapid increase in contact-lens-associated keratitis most of which has been due to *Acanthamoeba* in the UK. In general, the risk is much less for hard than soft lens wearers and is greater with extended wear lenses than daily wear lenses. In a multicentre case-controlled study, the overall risk for ulcerative keratitis with extended wear lenses was four times greater than that for daily wear. In addition, overnight wear of CLs increased the risk of keratitis to 10 to 15 times that occurring with daily wear alone[1].

Soft CL wear also increases the risk of microbial keratitis in corneal graft patients. This can be due to "crystalline" keratopathy, an infection associated with corneal grafts, in which there are sheets of streptococci deposited between the stromal lamellae giving the macroscopic appearance of ice crystals; the condition responds slowly to antibiotics, perhaps because there is a unique lack of polymorphonuclear cell infiltration within the cornea or the streptococci are surrounded by a biofilm.

The incidence of CL-associated microbial keratitis (presumed infection) has been estimated to be 20.9/100,000 (one in 500) for extended-wear patients and 4.1/100,000 (one in 2,500) in daily wear patients in the USA. However, a lower figure for daily wear of one in 6,700 (confirmed infection) or one in 5,100 (presumed infection) has recently been reported in Scotland.

The bacteria responsible for CL-associated keratitis include most of those usually associated with suppurative keratitis – Gram-negative bacteria are more commonly encountered than Gram-positive and *Ps. aeruginosa* is more frequent than other Gram-negative bacteria. Contamination of CL care solutions is an important potential source of keratitis. The most common infection in CL wearers in the UK has been due to *Acanthamoeba sp.* but this figure has now declined due to the introduction of sterile multipurpose solutions.

Fungi can also cause infection (mycotic keratitis). This is unusual in the temperate climates and when it does occur is usually due to *Candida sp.* or *Aspergillus sp.* (a mycelial fungus). Mycotic keratitis is much more common in hot humid climates where it can be responsible for up to 50% of cases.

Diagnosis

Diagnosis depends on smears and cultures from direct scrapes of the corneal ulcer. A blade is best but calcium alginate swabs can be used except for deep stromal infection. One drop of *unpreserved* topical anaesthetic (preservatives inhibit bacterial replication) is instilled to achieve anaesthesia. The surface material from the ulcer should be debrided using a swab. This may be plated on to blood or chocolate agar but is less helpful than later scrapes since it usually contains only cellular debris and mucus. The base and edge of the ulcer are most likely to yield organisms. The second scrape should be taken for microscopy. Using a platinum Kimura spatula, a large gauge sterile needle or a disposable surgical blade, the base and edge of the ulcer is firmly scraped. A freshly sterile instrument is used for each sample.

The material gathered should be firmly spread on to a clean glass slide to create a thin smear. This is then air dried. The second scrape should be similarly used for a second smear. The third scrape should be plated on to blood, chocolate and Sabouraud agars then fluid media, preferably brain heart infusion for anaerobes and fungi, should be inoculated with the same blade. In addition, a Lowenstein-Jensen slope (used for culturing mycobacteria) should be inoculated if the keratitis is chronic, although the atypical *M. chelonae* will grow on blood agar incubated for one week at 37˚C. For the chronic ulcer, blood agar should be incubated for one week in 4% CO_2 in order to facilitate culture of *Nocardia sp.* When *Acanthamoeba* keratitis is suspected, a specimen should also be taken for culture on appropriate media.

For each patient, all media should be inoculated directly at the slit lamp. If possible, duplicate specimens should be taken to allow for culture at different temperatures. Transport medium should not be necessary. Culture of agar plates should always take place at 37˚C for one week. The fluid media should be incubated at 30˚C in 4% CO_2 for at least three weeks. Anaerobic cultures should be considered when there is an unsatisfactory response to therapy.

Material collected should be stained for bacteria according to the clinical presentation. Stains include: Gram's stain and acridine orange for common bacteria; the modified Ziehl-Nielsen stain (decolourising with 5% acetic acid only) for *Nocardia* and mycobacteria; the full Ziehl-Nielsen stain for mycobacteria and methenamine silver (Grocott) stains for fungi and protozoan cysts. Selective stains include the use of labelled polyclonal or monoclonal antibodies. In general terms, the acridine orange and Gram's stain together will identify organisms in 80% of cases. It is also possible to maximise the available material and decolourise and restain the same smear slide with a further intermediate stain and finally an end stain.

Treatment of suppurative keratitis

A frequent approach is to use hourly combination drop therapy with fortified preparations produced in the hospital pharmacy, whose combined antibacterial spectra cover most infective possibilities caused by the Gram-positive and Gram-negative (non-acid fast) bacteria listed above (acid-fast bacteria, such as *Mycobacterium tuberculosis*, do not decolourise in the Ziehl-Neilsoen stain with strong acid). A common and effective empirical combination is gentamicin (or tobramycin) forte 1.5% [15mgs/ml] with cefuroxime (or cephazolin) 5% [50mgs/ml]. Recently, equal success has been reported using commercial preparations of either ciprofloxacin (Ciloxan, Alcon) 0.3% [3mgs/ml] or ofloxacin (Exocin, Allergan) 0.3% [3mgs/ml] at the same frequency. Ofloxacin treatment causes less irritation. Topical ciprofloxacin may leave microcrystalline deposits on the corneal surface, which take up to six months to dissolve, but has two to four-fold greater activity against *Ps. aeruginosa*.

Drops should be given hourly day and night for the first three days, then two hourly by day. Successful eradication of bacterial infection is reported in about 90% of patients treated in this way. Inclusion of adrenaline 0.3ml (of 1:1000) in 1ml of solution prolongs the effective concentration of antibiotic in the cornea and aqueous from about six to 24 hours. Antibiotic ointment may be given at night in the later stages of therapy once infection is under control, but in the acute stages it may interfere with absorption from drop therapy. It is important to note that systemic antibiotics have no place in the management of bacterial keratitis in the absence of limbal involvement or perforation.

Antibiotics are modified according to the results of cultures and the evaluation of the clinical response to initial therapy. If there is a clear clinical response, the same regime should be continued. Microbiological sensitivities may be misleading because they are performed on lower tissue antibiotic levels than can be achieved in the cornea during topical therapy. Therapy should be reduced by increasing the

interval between drops every three to four days and not by reducing their concentration. The decision to terminate therapy is based on clinical response and the virulence of the causative organism.

If there is no response, all topical therapy should be stopped in order to allow the various drugs and preservatives to leach from the tissues. After 24 or 48 hours, the clinical condition is reappraised, and the cornea is scraped again. On this occasion, a full search must be made for more exotic or fastidious organisms which may be unusual or have special cultural requirements, such as nocardia, mycobacteria and microaerophilic or anaerobic bacteria. A corneal biopsy may be required to identify the organism in the deeper stroma and has been successfully used for *streptococci*, *Fusarium sp.* and *Acanthamoeba*.

If no organism is identified, a second-line, broad-spectrum empirical antibiotic regime should be started to include antimicrobial action against resistant *streptococci*, nocardia and mycobacteria. This may include topical vancomycin 50mg/ml (5%) plus amikacin 50mgs/ml (5%) and trimethoprim 0.5% (given as POLYTRIM ointment) [or ciprofloxacin or ofloxacin at 3mgs/ml (0.3%) or erythromycin 0.5% ointment or rifampicin 2.5% ointment instead].

Acanthamoeba keratitis

A high index of suspicion must be maintained for all CL-related keratopathies presenting with epithelial disturbances or infiltrations with a "snowstorm" appearance on slit lamp microscopy, multiple superficial abscesses or dendritiform ulcers. Pain out of all proportion to the physical signs together with photophobia are among the first presenting symptoms. There may also be lid swelling and a conjunctival reaction.

A keratoneuritis (infiltration around the corneal nerve) seen on slit lamp microscopy is diagnostic for the condition, which gives rise to the excessive pain and photophobia. The initial diagnosis may be confused with adenovirus punctate keratopathy and be missed. In adenovirus infection, the nummular stromal infiltrates appear at least nine days *after* the punctate keratopathy while in *Acanthamoeba* infection, they present within the first eight days. Denritiform ulceration due to *Herpes simplex* Virus (HSV) **(Figure 18)** is rare in the young CL wearer and *Acanthamoeba* should always be considered in the first instance **(Figure 19)**.

If unrecognised, the infection progresses and at four to eight weeks, there is anterior stromal infiltration; this may remain in the central cornea or give rise to the classic ring abscess. There will be an accompanying limbitis, episcleritis and occasionally scleritis. Epithelial scrapings will reveal the amoeba, but if missed, the infection proceeds to either a large, deep infiltrated ulcer, that may be secondarily infected with *streptococci* as for a "crystalline keratopathy", or a deep ring abscess. At this late stage, corneal biopsy may be required to find the amoeba. Tissue sections can be examined by electron microscopy when trophozoites or cysts are easily seen. A severe scleritis develops in a few patients, which can be particularly difficult to treat.

Treatment

Treatment should start with 0.02% (200μg/ml) chlorhexidine digluconate in physiological saline and Brolene (propamidine isethionate) 0.1% (1000μg/ml) in physiological saline. If chlorhexidine is unavailable, PHMB 0.02% can be used instead but it is not licensed for use as a drug and requires the practitioner to have indemnity insurance. Hexamidine (Desmodine) can be used as an alternative, commercially-available diamidine drug instead of propamidine.

These drugs are given every hour, day and night for the first three days, reducing to two hourly by day only. This requires the patient's admission to hospital. Adjunctive therapy includes oral flurbiprofen, for both non-steroidal anti-inflammatory and analgesic effects, and topical mydriatic. Thereafter, combination therapy is given three hourly by day for two months and then four hourly by day for two months more. Control is rapidly gained but treatment is needed for two to six months in some patients, partly because drop therapy is not an ideal vehicle with which to treat this infection.

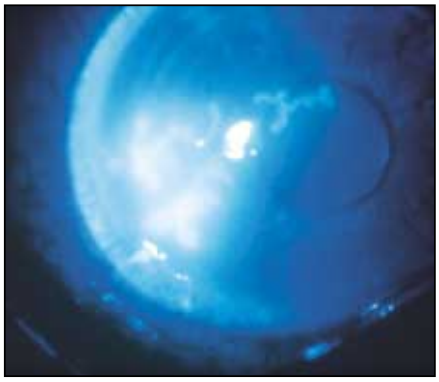

Figure 18
Herpes dendritic ulcer (dendrite)

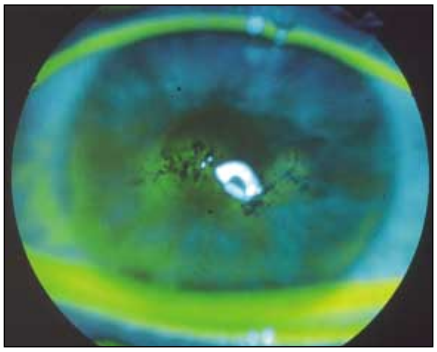

Figure 19
Pseudo-dendrite in a young contact lens wearer due to *Acanthamoeba sp.*

Virus infection

HSV and adenovirus account for 1% of all acute conjunctivitis in an ophthalmic casualty department. Antiviral agents are available for the treatment of HSV and *Herpes Zoster* Virus (HZV) infections but not for adenovirus infection. A number of other ocular viral infections occur for which there is no specific antiviral therapy but topical antibiotics are often prescribed to reduce the risk of secondary bacterial infection. Treatment of other viruses that infect the eye such as Human Immunodeficiency Virus (HIV) and Cytomegalovirus (CMV) causing disease of the retina is given in Seal *et al* (1998).

Herpes Simplex eye disease

Primary HSV infection of the eye is a self-limiting disease which may be expressed as blepharitis, conjunctivitis or punctate keratitis. It may be followed by zosteriform spread along the axons of the Vth cranial nerve with the establishment of latency in the trigeminal ganglion. This may also follow asymptomatic infections within the territory of the first division of the Vth nerve dermatome, and probably inoculations in the second and third divisions. All subsequent ocular disease results from reactivation of virus, associated with peripheral shedding, and is termed recurrent disease. Recurrent eye disease includes epithelial keratitis (dendritic and geographic ulcers), stromal keratitis (disciform and necrotising), limbitis, keratouveitis, secondary glaucoma and rarely, acute retinal necrosis. Antiviral therapy is effective in the treatment of epithelial keratitis but its role in the management of other forms of recurrent disease is less clear. No form of therapy affects the incidence of recurrences.

Treatment of herpetic infection

Antivirals used (usually acyclovir or penciclovir and others) are activated by virus-induced enzymes within the cell (e.g. thymidine kinase) and therefore exert their action chiefly in infected cells. Thus, acyclovir is phosphorylated by viral thymidine kinase and converted to the active triphophosphate form by host cell enzymes. The triphosphate is a potent inhibitor of DNA polymerase and acts as a chain terminator to the growing viral DNA strand. These drugs are more inhibitory to herpetic DNA polymerase than cellular polymerase, and preferentially inhibit viral DNA synthesis.

Prophylactic antivirals are used in patients receiving topical steroids to suppress the inflammatory features of herpetic keratouveitis or stromal herpetic disease. Similar considerations to those in corneal graft prophylaxis apply and risks of antiviral drug toxicity again arise because of the prolonged nature (weeks or months) of the immunosuppressive therapy. A controlled trial of oral acyclovir in patients receiving topical steroids and trifluridine for stromal keratitis has showed no benefit from this additional

treatment. No form of treatment will reduce the frequency of clinical recurrences of herpetic disease.

Corticosteroids may provoke the appearance of dendritic ulcers in some subjects, convert dendritic ulcers to geographic ulcers and worsen the overall prognosis of HSV keratitis. Steroid use predisposes the conversion of untreated dendritic ulcer into the more aggressive, geographic ulcer. F3T, acyclovir and ARA-A can all be effective for treatment. Where prolonged use occurs, acyclovir would be preferable because of its lower level of toxicity.

It has been suggested that the use of corticosteroids in stromal keratitis may predispose to recurrences and that the use of antivirals alone may be preferable in mild disease. Corneal perforation due to HSV rarely occurred before the steroid era. For these reasons, the use of steroids in the treatment of herpetic disease is controversial. Their use, however, is indicated in selected cases and always requires ophthalmic supervision. Steroids are capable of suppressing the inflammatory response in disciform and other forms of stromal keratitis and in uveitis, but rebound inflammation may occur on stopping therapy. In this setting, it is important to give prophylactic antiviral therapy to patients receiving steroids in all but very low dosages due to the risk of activation of epithelial HSV keratitis.

Conclusion

Optometrists need to understand the basics of microbiology to appreciate how to prevent infection in their clinics and how to assist in early identification of microbial keratitis and other important ocular infections. Optometrists have a special role in primary care practice to identify *Acanthamoeba* keratitis at an early stage in a contact lens wearer to save considerable morbidity in the patient. Early recognition by optometrists and subsequent early therapy with chlorhexidine in hospital has shown that *Acanthamoeba* keratitis is readily treatable within days; if this does not happen, this very painful infection is prolonged for weeks or months. Optometrists should also be aware of cross-infection between patients in their clinics and be especially careful of the red eye patient infected with adenovirus.

About the author

Dr David V. Seal is an ophthalmologist and microbiologist. He has pursued the many causes of ocular infection for 20 years and instigated the use of chlorhexidine for the treatment of *Acanthamoeba* keratitis. He has a special interest in the epidemiology and prevention of *Acanthamoeba* keratitis and the prevention of endophthalmitis following cataract surgery.

Further Reading

1. Seal DV, Bron AJ, Hay J. Ocular infection - investigation and treatment in practice. London: Martin Dunitz, 1998, pp 1-275.
2. Seal DV, Dalton A, Doris D. Disinfection of contact lenses without tap water rinsing - is it effective? *Eye* 1999: 13; 226-230.
3. Houang E, Lam D, Fan D, Seal D. Microbial Keratitis in Hong Kong - relationship to climate, environment and contact lens disinfection. *Trans Roy Soc Trop Med & Hyg*, 2001; 95: 361-67.

Multiple choice questions
Microbiology for optometrists

Please note there is only one correct answer

1. Which one of the following statements is correct? Bacteria from lid swabs will not grow on:
a. tissue culture cells
b. blood agar
c. nutrient agar
d. oatmeal agar

2. Which one of the following statements is correct? Bacteria are classified by:
a. their sensitivity to penicillin
b. shape and colour on Gram's stain
c. susceptibility to PHMB (polyhexamethylene biguanide)
d. requirement for co-factors

3. Which one of the following statements is correct? Bacteria fail to take up stain with:
a. methylene blue
b. iodine
c. neutral red
d. acetone

4. Which one of the following statements is correct? Quinolone antibiotic is bactericidal by:
a. preventing cell wall formation
b. acting on ribosomes
c. damage cell membranes
d. interfering with DNA polymerase

5. Which one of the following statements is correct? The PHMB disinfectant binds to:
a. DNA
b. RNA
c. cell membrane
d. cell wall

6. Which one of the following statements is correct? Bacterial resistance is not due to:
a. selective pressure
b. mutation
c. transmissible plasmids
d. atmospheric pressure

7. Which one of the following statements is correct? Viruses fail to grow in:
a. cell free mediums
b. monocell layers
c. egg membranes
d. mice

8. Which one of the following statements is correct? *Herpes Simplex* Virus keratitis is generally treated with:
a. ganciclovir
b. acyclovir
c. azidothymidine (AZT)
d. cidofovir

9. Which one of the following statements is correct? *Acanthamoeba* is most likely to be found in:
a. contact lens storage cases containing 3% hydrogen peroxide
b. fresh distilled water
c. home tap water
d. bottled mineral water

10. Which one of the following statements is correct? *Acanthamoeba* should be cultured on:
a. non-nutrient saline agar
b. 1% PHMB agar
c. 1% chlorhexidine agar
d. egg membranes

11. Which one of the following does not cause microbial keratitis in contact lens wearers in the UK:
a. *Pseudomonas aeruginosa*
b. *Acanthamoeba*
c. *Vibrio vulnificus*
d. *Candida sp.*

12. Which one of the following statements is correct? *Acanthamoeba* keratitis can be treated with:
a. tetracycline
b. polyquad
c. chlorhexidine
d. thiomersal

An answer return form is included in this issue. It should be completed and returned to: CPD Initiatives (c4082b), OT, Victoria House, 178–180 Fleet Road, Fleet, Hampshire, GU51 4DA by April 3, 2002.