Antibiotics and Resistance in Ocular Infections—Indian Perspective

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Abstract
As in many parts of the world, infections of the eye are common in India and a wide variety of antibacterial as well as antifungal antibiotics are available in the market. Antibiotics can be administered in the eye by a number of routes; topical, subconjunctival, subtenon and intraocular. Peculiar to the treatment of eye infections, fortified eye drops from parenteral formulations are often used. These preparations achieve high concentrations; usually much above the minimum inhibitory concentration (MIC). Fair amount of research on antibiotic resistance in India deals with bacterial infections, however, studies on antibiotic susceptibility of fungal isolates are limited. The resistance in bacteria seems to be increasing in parallel with the increase seen over the years in bacteria associated with systemic infections. Although it is believed that the rise in resistant ocular bacterial isolates is linked to the rise in resistant systemic pathogens, recent evidence has correlated the emergence of resistant bacteria in the eye to prior topical antibiotic therapy. Probably, either of these contributes to the emergence of resistance among bacteria and fungi associated with eye infections. This review describes the current scenario of antifungal and antibacterial susceptibility of fungi and bacteria associated with eye infections with special reference to the Indian subcontinent.

Introduction
Micro-organisms are closely associated with the eye forming the microbial flora of the external ocular surface at birth while the inner parts of the eye remain sterile. Several mechanisms in the extraocular surface protect the eye and only a breach in surface epithelium due to trauma or lowering of local or systemic immunity may predispose the eye to infections. In addition, the number and virulence of the invading organisms play an important role in launching an infection. The resident bacteria of the conjunctival sac or the environmental bacteria or fungi can establish infection and need to be treated with antibiotics. Occasionally, endogenous infections arising from other parts of the body may affect the eye. Ophthalmologists have at their disposal a large repertoire of antibacterial and antifungal antibiotics (eye drops, ointments, tablets and parenteral). These are in use since the beginning of the antibiotic era and similar to other infections. Antibiotic resistance in eye infections is a matter of concern to the ophthalmologists and microbiologists. Until recently it was thought that the source of resistant bacteria or fungi in eye infections is an outcome of the organisms acquiring resistance during treatment of systemic diseases. Although not yet demonstrated for fungi, evidence in literature suggests emergence of resistant bacteria in the eye owing to prior antibiotic therapy of the eye.[1] This review presents the Indian perspective of antibiotic resistance among bacteria and fungi causing eye infections.
Barriers to entry of drugs into the eye

In a normal eye, transfer of antibiotics from ocular surface to the inside of the eye and from blood to ocular tissues is hindered at various levels (Figure 1). The surface epithelia of the bulbar conjunctiva and the cornea are relatively impermeable, especially to water soluble agents.

A breach in surface epithelium allows the entry of these drugs more effectively in the anterior segment of the eye. However, because of the diffusion barrier across the lens zonule compartment and anterior vitreous, entry of drugs through cornea and conjunctiva does not reach the posterior segment of the eye. Inability of the drugs to reach the posterior segment from the anterior segment is also due to movement of the aqueous from the posterior chamber through the pupil and its drainage into the venous circulation. Barriers of the surface epithelium may be overcome by subconjunctival and subtenon injections. While a blood-aqueous barrier inhibits the entry of water soluble drugs across the ciliary body epithelium, the blood-retinal barrier limits the entry of drugs into the retina. Diffusion across the outer retina is blocked by cells of retinal pigment epithelium which constitute the outer blood-retinal barrier. Resistance across the retinal capillaries by endothelial tight junctions is known as inner blood-retinal barrier. These barriers are partially broken down in presence of inflammation. Understandably, direct intraocular injections always achieve higher concentration compared to systemic administration of drugs.

Antibacterial and antifungal drugs used for the treatment of eye infections

Unlike many other organs in the body the eyes are amenable to antibiotic therapy by a number of routes such as topical, subconjunctival, subtenon, intraocular etc. Several commercial eye drops are available in the required concentration. However, eye is probably the only structure for which fortified drops at higher concentrations are used that may achieve bio-availability of the drugs higher than minimum inhibitory concentrations (MIC) for the offending organisms. Antifungal as well as antibacterial fortified topical drops are generally prepared aseptically from parenteral drugs. Using distilled water as solvent to make fortified drops runs the risk of contamination. Therefore, they are preferably dissolved and diluted in artificial tear preparations to avoid contamination. Table 1 lists the antibacterial drugs that are currently in use, and their mode of action. [2]

The major groups of antifungal drugs are azoles (ketoconazole, fluconazole, itraconazole, voriconazole, imidazole, miconazole) and polyenes (amphotericin B, natamycin, nystatin) that are respectively fungistatic with interference in protein synthesis and fungicidal with action on cell wall function. Antifungal activity of cationic antiseptics such as chlorhexidine and polyhexamethylene biguanide, which are amoebicidal and function by creating pores in the cell membrane, is also well known.

For most eye infections the therapy is topical instillation of antimicrobial eye drops. In bacterial and fungal keratitis the patient is given a topical commercially available eye drop or fortified eye drop, with or without systemic treatment. Frequency of instillation varies from disease to disease. In contrast, intravitreal therapy is preferred for many intraocular infections with or without systemic therapy. Subconjunctival and subtenon injections may be preferred under special circumstances.
Resistance among ocular pathogens seems to be increasing in consonance with the increase of resistance among bacteria and fungi associated with systemic infections. The factors contributing to development of drug resistance among ocular bacterial isolates include overuse of antibiotics for systemic infection as well as overuse of topical antibiotics in the eye. [1] Other factors that may contribute are improper dosing regimen, misuse of antibiotics for viral infections, extended duration of therapy and not to a small extent current globalization and migration of populations.

Topical antibiotics that are in common usage for the treatment of bacterial conjunctivitis include aminoglycosides, polymyxin B combinations, macrolides and fluoroquinolones. Although not used in the United States for its side effects, chloramphenicol is commonly used in India. Streptococcus pneumoniae is a common cause of conjunctivitis and usually a sensitive organism to a large repertoire of antibiotics. However, resistance to gentamicin, tobramycin and polymyxin B has been reported among Streptococcus pneumoniae isolates from acute conjunctivitis. [3] No resistance to gentamicin was reported in S. pneumoniae between 1989 and 1992, however, resistance to gentamicin was reportedly 42.3% in 1997 which increased to 56% in the year 2000.[4] Similarly, resistance to tobramycin rose from 43.6% in 1997 to 46% in 2000. Azithromycin is a recently recommended broad spectrum drug for the treatment of bacterial conjunctivitis, however, moderate to very high resistance to azithromycin has been reported for H. influenzae, S. pneumoniae, S. aureus and S. epidermidis isolates from bacterial conjunctivitis. Fortunately, Haemophilus influenzae, a common cause of bacterial conjunctivitis, remains sensitive to aminoglycosides and polymyxin B.

Methicillin resistant S. aureus (MRSA) has emerged as a dreaded organism for its wide range of resistance to several groups of antibiotics. Its prevalence in conjunctivitis is highly variable. One study has shown an increase in MRSA in bacterial conjunctivitis from 4.4% (1994-5) to 42.9% (2002-3).[5] Situation in coagulase negative staphylococci (CoNS), a common cause of keratitis and endophthalmitis, is no less precarious. Until 2003 approximately 19% of CoNS were reported to be resistant to gentamicin and 2% were resistant to gatifloxacin. [6,7] However, by the year 2006, nearly 11% of CoNS from normal ocular surface and 53% of CoNS from endophthalmitis were reported to be resistant to gatifloxacin. [8] All ciprofloxacin resistant MRSA and methicillin resistant S. epidermidis (MRSE) demonstrate resistance to 4th generation fluoroquinolones such as gatifloxacin and moxifloxacin but not to besifloxacin, the latest among the fluoroquinolones. [9] Currently not available in India, besifloxacin is the first fluoroquinolone that has been developed only for ophthalmic use. In vitro activity of besifloxacin shows lower minimum inhibitory concentration (MIC) compared to all other fluoroquinolones and azithromycin. [10] Reduced susceptibility of S. aureus to vancomycin was first noted in Japan in 1997 in systemic infections.[11] Using disc diffusion susceptibility testing method there are some reports of vancomycin resistant S. aureus (VRSA) ocular infections [12-14] however, till date there are no confirmed VRSA ocular isolates.

Pseudomonas comes next to staphylococci in its importance as a causative agent of eye infections. It tops the list of challenging organisms to treat because of high prevalence of resistant strains. Most strains of P. aeruginosa isolated from contact lens associated corneal ulcers were resistant to ampicillin, cephalothin, neomycin and tetracyclins.[15] In the last decade topical ciprofloxacin replaced aminoglycosides and became the best drug to treat P. aeruginosa keratitis. It probably resulted in over use as the preferred antibiotic for preoperative prophylaxis in eye surgeries. Multidrug resistant P. aeruginosa have been reported from keratitis and endophthalmitis patients leaving no choice but to use piperacillin/tazobactum or imipenem for the treatment of such cases.[16,17] The landmark multicentric study from the United States – Endophthalmitis Vitrectomy Study reported 11% of gram negative organisms

<table>
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<tr>
<th>CLASS OF DRUG</th>
<th>TYPE OF DRUG</th>
<th>MODE OF ACTION</th>
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<tbody>
<tr>
<td>Penicillins/cephalosporins</td>
<td>Bactericidal</td>
<td>Cell wall inhibitor</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Bactericidal</td>
<td>DNA gyrase inhibitor</td>
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<tr>
<td>Tetracyclines</td>
<td>Bacteriostatic</td>
<td>Inhibitor of protein synthesis</td>
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<tr>
<td>Erythromycin/azithromycin</td>
<td>Bacteriostatic/Bactericidal</td>
<td>Inhibitor of protein synthesis</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Bacteriostatic</td>
<td>Inhibitor of protein synthesis</td>
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<tr>
<td>Aminoglycosides</td>
<td>Bactericidal</td>
<td>Inhibitor of protein synthesis</td>
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Table 1: Common antibacterial drugs used to treat eye infections and their mode of action
to be resistant to amikacin and ceftazidime. [18] In contrast, the level of resistance in gram negative organisms associated with endophthalmitis was higher in a study from India at 39% to ceftazidime, 18% to amikacin and 13% to ciprofloxacin. [19] The same group has recently reported with serious concern a high level of multidrug resistance (MDR) among gram negative bacteria causing endophthalmitis in Indian patients. [20] This study also reported MDR enteric bacteria such as Escherichia coli and Klebsiella pneumoniae from patients with endophthalmitis with poor visual outcome. Among Enterobacteriaceae isolated from eye infections in another study, the resistance to gatifloxacin was 3.4%, to ofloxacin and ciprofloxacin was 5.1% and to gentamicin was 8.5%. [21]

Information about use of linezolid to treat ocular infections is limited. In a patient with vancomycin resistant Enterococcus faecium endophthalmitis, intravenous and oral linezolid led to resolution of infection. [22] Reports of vancomycin resistant Enterococcus (VRE) ocular infections are rare. In a series of 26 cases of E. faecalis endophthalmitis, between 1995-2007 from India, there was one VRE. [23]

The drug of choice for the treatment of infections caused by non-tuberculous mycobacteria and Nocardia species is generally amikacin and all ocular isolates are reported to be susceptible to this drug. [24,25] Closely classified with these organisms are non-diphtherial Corynebactium species which have recently been recognized as ocular pathogens. C. macginleyi is said to be associated with conjunctivitis and keratitis and reported to be sensitive to a large number of antibiotics including fluoroquinolones. However, what may be an alarming signal, 11 out of 16 C. macginleyi isolates from normal conjunctiva were found to be resistant to three fluoroquinolones (ciprofloxacin, norfloxacin, levofloxacin) by E test. [26]

Susceptibility test results for fungal isolates from the eye against antifungal antibiotics are limited. A recent study from L V Prasad Eye Institute in India, tested 60 isolates from fungal keratitis against natamycin and found low MIC of natamycin (<16 μg/ml) for all isolates except Aspergillus flavus. [27] Sometime back, another study from India reported the susceptibility of fungal keratitis isolates to ketoconazole and fluconazole. While two thirds of the isolates were resistant to fluconazole, variable sensitivity ranging from MIC of 0.5–10 mg/ml was found among others. Most of the isolates were Aspergillus species. [28] The authors however, did not comment on the breakpoint they considered for determining the susceptibility level of the isolates. An interesting study from Aravind Eye Hospital in India showed antifungal activity of antibacterial antibiotics such as tobramycin, moxifloxacin and chloramphenicol against Fusarium and Aspergillus species isolated from fungal keratitis patients. [29] This may explain the anecdotal reports of fungal keratitis responding to inadvertent treatment with antibacterial antibiotics. However, the MIC90 of these drugs was much higher (500–1000μg/ml) than what is achieved with antifungal agents and surely they are not recommended for the treatment of fungal keratitis. The same study showed a low MIC90 (16–32 μg/ml) of benzylkonium chloride against these fungal isolates. Benzylkonium chloride is a common preservative used in commercial eye drops and this may further explain the occasional response to antibacterial antibiotics. Over 90% isolates of Fusarium and Aspergillus were found to be sensitive to natamycin and itraconazole respectively in a study on large number of fungal keratitis isolates from China. [30] Currently, natamycin remains the drug of choice for fungal keratitis caused by filamentous fungi.

Testing of bacterial and fungal isolates for susceptibility to Drugs

Emergence of antifungal drug resistance has made it important to test for susceptibility although the in vitro results may not correlate with the treatment outcome. Techniques for performing antifungal susceptibility testing have usually been difficult which has led to dearth of studies on antifungal susceptibility, especially in ocular microbiology. Antibiotic concentration in ocular tissues during topical therapy is difficult to measure, therefore, ocular tissue-specific breakpoints are not yet available to use for determining the susceptibility of ocular fungal or bacterial isolates to antibiotics. Clinical and Laboratory Standards Institute (CLSI) guidelines based on breakpoints derived from serum/plasma/cerebrospinal fluid levels of antibiotics are used for determining susceptibility of bacterial and fungal isolates from the eye. These systemic breakpoints, however, may have limited predictive value for ocular isolates. Studies are needed to resolve the dynamics of breakpoint versus antibiotic resistance of ocular isolates and their relationship to clinical response. Nevertheless, in absence of a better alternative, the current systemic therapy based breakpoints to determine susceptibility of ocular isolates remains useful. User friendly, well standardized procedure yields consistent results and helps to track trends of susceptibility and compare data. [6] Parmar et al found comparable results between ketorolac urea rates and in vitro susceptibility results by disc diffusion method in patients treated with topical gatifloxacin and ciprofloxacin, thus justifying the use of CLSI standards for testing ocular isolates. [31] A good correlation between results obtained with MIC and disc diffusion tests vis-à-vis clinical outcome has been shown in Pseudomonas isolates against ciprofloxacin. [32]

A notable publication by Prajna et al has convincingly shown that eye drop preparations are an alternative to pharmaceutical grade natamycin (not available to most laboratories) for testing antifungal susceptibility. [33] Susceptibility breakpoints for natamycin have not been described so far in CLSI guidelines, however, MIC of 16 μg/ml or less is considered to indicate susceptibility of a fungal isolate. [33]

In conclusion, the treatment of ocular infections is challenging in the face of antibiotic resistance among ocular pathogens. Resistance to most groups of antibiotics is increasing throughout the world including India. There are not many newer antibiotics on the horizon except for few antibacterials such as besifloxacin and antifungals such as voriconazole and posaconazole. Judicious use of antibiotics along with development of new products is the only way forward.
References


About the Author

Dr. Savitri Sharma is the Director of Laboratory Services of L.V. Prasad Eye Institute (LVPEI) network at Hyderabad, Bhubaneswar, Vishakhapatnam and Vijayawada in India. An alumnus of JIPMER, Pondicherry (MD-1982) her field of microbiology focused on the eye when she joined Aravind Eye Care System, Madurai in 1986 where she diagnosed the first case of Acanthamoeba keratitis in India. She moved to the LV. Prasad Eye Institute, Hyderabad in 1991, and reported the first case of ocular microsporidiosis in India in 2003. She is the recipient of several grants and awards from DBT, CSIR, DST, Bausch & Lomb International Research Program, British Contact Lens Association, American Academy of Ophthalmology, All India Ophthalmological Society, Indian Association of Medical Microbiologists etc. Several randomized clinical trials involving contact lenses and treatment of bacterial and fungal keratitis have been monitored by her. She was involved in the development of the Vision Chip (Xcyton) under CSIR (NMITLI) grant. She has been the Editor-in-chief of Indian Journal of Medical Microbiology (IJMM) for eight years and the President of Indian Association of Medical Microbiologists (IAMM) from 2008-9. She has authored a book on "Ocular Microbiology" and has published 12 book chapters and 176 papers (National- 63, International-113) in peer reviewed journals. Her research interests are ocular microsporidiosis, molecular diagnosis, virulence factors, antibiotic susceptibility and pathogenesis of Staphylococcus, Fusarium, Aspergillus, Candida, Pseudomonas associated with eye infections.