

Microbial spectrum of ocular infections and antibiotic resistance pattern in bacterial isolates: A study in a tertiary care hospital

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Abstract

The eye and its associated structures are uniquely predisposed to infection by the various microorganisms. The detection of infectious agents depends on the knowledge of the site of infection and the severity of the process because a variety of organisms cause infections of the eye. The present study was done to determine the bacterial, fungal and parasitic infections of the eye and also to assess the antibiotic susceptibility pattern of bacterial isolates in a tertiary care hospital over a period of 1 year. A total of 609 samples from clinically suspected ocular infections were collected and processed in the Microbiology department. Implicating pathogens were identified and isolated based on standard laboratory procedures. Antibiotic susceptibility was done for bacterial isolates using kirby bauer disk diffusion method. A total of 609 ocular samples were collected and processed, of which 247(40.5%) were positive either by culture and microscopy or microscopy alone. Bacteria were the commonest implicating pathogen accounting up to 215 (87%), followed by fungal and parasitic pathogens. Bacterial isolates were predominantly isolated from conjunctival swab and corneal scrapings. Maximum fungal and parasitic isolates were from corneal scrapings. Coagulase negative Staphylococci were the predominant isolate. Gram positive organisms were sensitive to vancomycin 100%, gatifloxacin 91% and ofloxacin 87%, Gram negative organisms were sensitive to gatifloxacin 78.5% and ciprofloxacin 69%. Early access to clinical and microbiological diagnosis with appropriate treatment can prevent the ocular morbidity and mortality.

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Introduction

Eye is the most important sensory organ concerned with the perception of vision. It is an unique organ that is impermeable to almost all external organisms and is also aided with a number of defense mechanisms, if these barriers are broken, infection may occur.

The eye may be infected from a external sources or through intra ocular invasion of micro-organism by blood stream. While the anterior segment is infected by direct invasion from the anterior route, blood-borne infections may reach the posterior segment of the eye. Even what may be considered a minor infection elsewhere in the body can be "fatal" to the eye in terms of visual compromise. (McClellan KA, 1997; S Sharma, 2010)

Pathogenic micro-organisms cause ocular disease, frequently affecting parts conjunctiva, lid and cornea. Any part of the eye may be infected by bacteria, fungi, parasites, or viruses however bacteria are major causative agents that frequently cause infections in eye and possible loss of vision worldwide. (Ramesh S, 2010; Tesfaye T, 2013)

Infection can be mono or poly-microbial and is also associated with many factors including contact lenses, trauma, surgery, age, dry eye state, chronic nasolacrimal duct obstruction and previous ocular infections (Galvis V, 2014; Choudhury R, 2012). Conjunctivitis, keratitis, endophthalmitis, blepharitis, orbital cellulitis and dacryocystitis are the commonest bacterial manifestations. Conjunctivitis, inflammation of the mucosa of conjunctiva, is the most frequent ocular case with noticeable economic and social burdens. During chronicity, the disease can affect not only the conjunctiva but also adjacent structures including the eye lid and can be a potential risk for other extra or intraocular infections. Conjunctivitis may be caused by several groups of organisms including bacteria, Chlamydia, viruses, fungi, helminths, and protozoa (Buznach N, 2005).

Keratitis, the most serious eye infection is the leading cause of corneal blindness, second to cataract. Ocular trauma is the commonest predisposing factor of infectious keratitis in developing countries. Microbial keratitis may be caused by bacteria, fungi, viruses, or parasites. The relative frequency of different bacteria as causative agents in keratitis may vary geographically. (Henry CR, 2012; Cao J, 2014)

Pre-existing ocular disease and wearing contact lens are the common risk factors in developed countries. Moreover, the disease can also progress to endophthalmitis if not diagnosed early. (Michael Osita Emina, 2011)

Endophthalmitis may be exogenous, involving intraocular surgery or following penetrating injury to the eye or from endogenous systemic infections. Postoperative endophthalmitis can occur after any intraocular surgery, though numerically most infections are seen following cataract surgery, for the obvious reason that cataract surgeries outnumber other intraocular surgeries. Endogenous endophthalmitis can arise from systemic dissemination of the pathogens or from colonized intravenous lines or contaminated syringes used by drug addicts. (S Sharma, 2010).

Both keratitis and endophthalmitis are potentially devastating. Blepharitis is an inflammation of the eyelid margins which can result in patient discomfort and decline in visual function. Dacryocystitis is an inflammation of the lacrimal sac and duct. During chronicity the disease is associated with infection, inflammation of the conjunctiva, accumulation of fluid and chronic tearing. This can be potentially dangerous to ocular tissues such as the cornea; leading to post surgery endophthalmitis. (Maheshwari R, 2009; Amin RM, 2013).

The presence of the contact lens influences the development of the infection as the lens biomaterial acts as a vector for adherence of microorganisms with subsequent transfer to the ocular surface.

Although the presence of microorganisms is necessary, it is not necessarily sufficient for the development of infection. Other factors associated with infection include disruption to the ocular surface, often caused by the wearing of contact lens. Nevertheless, the presence of bacteria, protozoa and fungi on contact lens clearly predispose a patient to the development of infection. (Michael Osita Emina, 2011)

Bacteria are the commonest cause of ocular Infection followed by fungal and viral etiology. The etiology and resistant patterns may vary with geographical location according to the local population. (Benz MS, 2004)

Ocular infections, if left untreated, can damage the structures of the eye leading to visual impairments and blindness. Even though the eye is hard and protected by the continuous flow of tear which contains antibacterial compounds, inflammation and scarring once occurred may not be easily resolved and requires immediate management. (Ubani, 2009)

Effective management of such infections demands knowledge of the specific etiology. However, ocular infections are mostly managed empirically with broad spectrum antibiotics. The indiscriminate use of antibiotics has led to the development of resistance to many commonly used antimicrobial medications jeopardizing the treatment, with potentially serious consequences. Hence it is necessary to study and identify the dominant pathogens and map the susceptibility patterns in a hospital setting so as to enable the clinician to select the appropriate drug regimens.

With this background this study was undertaken to detect the bacterial, fungal and parasitic profile of the different forms of ocular infections and also assess the antibiotic susceptibility pattern of bacterial isolates at our institute in order to come up with concrete information for physicians and policy makers who deal with ocular infections to know the microbial profile and antibiotic susceptibility.

Materials and methods

Study design

The present study was a prospective study, conducted in a tertiary care eye hospital in Bangalore, India. A total of 609 samples from clinically suspected ocular infections over a period of one year were included in the study.

Ethical and institutional issues

The study has been approved by institutional ethics committee.

Sample collection

Samples like conjunctival swabs, corneal scrapping, corneal button, vitreous humour, aqueous humour and contact lens were collected aseptically and sent immediately to Microbiology department for the processing.

Microbiological processing

Direct Gram's staining and KOH mount was performed for all samples. All the samples were inoculated on to Brain heart infusion broth, Blood agar, Macconkey agar and Sabouraud's dextrose agar. Multiple C shaped streaks were performed on solid media for the corneal scrapings. The growth was identified based on standard laboratory procedures. (Cheesbrough M, 2006)

Antibiotic sensitivity testing

Antibiotic sensitivity was done for bacterial isolates using kirby bauer disk diffusion method using discs of standard potency. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. (Clinical and Laboratory Standards Institute (CLSI), M100-S22)

Special stains

Giemsa staining, acid fast staining and modified acid fast staining were done where ever required.

Results

In this study a total of 609 ocular samples were collected and processed, such as conjunctival swab (n=319), corneal scrapings (n=136), aqueous fluid (n=28), vitreous fluid (n=44), Corneal button (n=69), Contact lens (n=12) and

eviscerated material (n=1). Among the 609 samples, 247(40.5%) were positive either by culture and microscopy or microscopy alone.

Bacteria were the commonest implicating pathogen accounting up to 215 (87%), followed by fungal and parasitic pathogens, 27 (11%) and 5 (2%) respectively. (Table 1)

Table 1. Prevalence of pathogens from the ocular samples.

No of samples	Culture/ microscopy positive	Bacterial isolates	Fungal isolates	Parasitic isolates
609	247(40.5%)	215 (87%)	27 (11%)	5 (2%)

Of the 319 conjunctival swabs processed 156 (49%) were culture positive. 155 (99.3%) were bacterial isolates and 1(0.6%) was a fungal isolate (Table 2). A total of 136 corneal scrapings were processed of which 55 (40.4%) harbored pathogens demonstrated by culture and microscopy or microscopy alone, 35(63.6%) bacterial isolates were isolated, 15(27.27%) fungal isolates and 5(8.8%) parasitic isolates were isolated -4(7%) microsporidia & 1(1.8%) *Acanthamoeba* (Table 2). *Microsporidia* and *Acanthamoeba* were identified by modified acid fast staining and wet mount respectively. Culture was not performed. Of the 28 aqueous fluid samples 5 (18%) were culture positive and all of them were bacterial isolates (Table 2). Out of 44 vitreous fluid samples collected 5(11%) were culture positive. 3(60%) were bacterial isolates and 2(40%) were fungal isolates (Table 2). Of the 69 corneal buttons 17(25%) were culture positive. 8 (47%) were bacterial isolates and 9 (53%) were fungal isolates (Table 2). Among 12 contact lens samples 9 (75) were culture positive all yielding bacterial isolates. (Table 2)

Bacterial isolates were predominantly isolated from conjunctival swab and corneal scrapings. Maximum fungal and parasitic isolates were from corneal scrapings. Of the 215 bacterial isolates, majority of

the isolates (173, 80.4 %) were Gram positive and 42 (19.5 %) were Gram negative bacteria.

Table 2. Distribution of pathogens from the various ocular samples.

Specimen	Total no of samples	Culture/ microscopy positive	Bacterial isolates	Fungal isolates	Parasitic Isolates
Conjunctival swab	319	156	155	1	0
Corneal scrapping	136	55	35	15	5
Aqueous fluid	28	5	5	0	0
Vitreous fluid	44	5	3	2	0
Corneal button	69	17	8	9	0
Contact lens	12	9	9	0	0
Eviscerated material	1	0	0	0	0
Total	609	247	215	27	5

Coagulase negative Staphylococci was the predominant isolate in Gram positive bacterial isolates accounting up to 51 % (n=88), followed by *Staphylococcus aureus* 41.6% (n=72), *Streptococcus pneumoniae* and *Streptococcus pyogenes*, 5.7% (n=10) and 0.6% (n=1) respectively. Higher filamentous bacteria *Nocardia* and *Actinomycetes* were isolated from 1 sample (0.6%) each. Coagulase negative Staphylococci and *Staphylococcus aureus* were more frequently isolated from conjunctival swab and corneal scrapings (Table 3). *Pseudomonas aeruginosa* was more commonly isolated among the Gram negative bacteria accounting up to 66.6% (n =28), followed by *Klebsiella pneumoniae* 14% (n =6), *Serratia* spp. 9.5% (n =4), *Citrobacter* spp and *Acinetobacter baumannii* accounted up to 5% (n =2) each. Gram negative bacteria were isolated from most of the ocular samples (Table 3).

Of the 27 fungal isolates, *Fusarium* spp. was more commonly isolated 48% (n=13) followed by *Aspergillus flavus* 22% (n=6), *Aspergillus fumigatus* 11% (n=3). *Curvularia* and *Candida* were isolated in 7.4% (n=2) of the samples. While *Cladosporium* was isolated from 3.7% (n=1) of the samples only (Table 4). 55.5 % (n=15) fungal isolates were from corneal scrapings, followed by corneal button 33.3% (n=9), vitreous fluid and conjunctival swab 7.4% (n=2) and 3.7% (n=1) respectively (Table 4).

Table 3. Distribution of bacterial isolates from the various ocular samples.

Bacterial isolates	Conjunctival swab	Corneal scrapings	Aqueous fluid	Vitreous Fluid	Corneal button	Contact lens	Eviscerated Material
CONS	88						
<i>S. aureus</i>	59	11	2				
<i>S. pyogenes</i>						1	
<i>S.pneumoniae</i>		6			4		
<i>Nocardia</i>		1					
<i>Actinomyces</i>		1					
<i>Pseudo aeruginosa</i>	6	9	3	3	3	4	
<i>Kleb pneumoniae</i>	2	3			1		
<i>Citrobacter spp</i>		2					
<i>A.baumannii</i>		2					
<i>Serratia spp</i>						4	

Table 4. Distribution of fungal isolates from the various ocular samples.

Fungal isolates	Conjunctival swab	Corneal scrapings	Vitreous fluid	Corneal button
Fusarium		9	1	3
<i>A.flavus</i>	1	2	1	2
<i>A.fumigatus</i>		1		2
<i>Curvularia</i>		1		1
<i>Candida</i>		1		1
<i>Cladosporium</i>		1		
Total	1	15	2	9

Antibiotic susceptibility was done for 214 isolates. The drugs tested were Penicillin, Amoxicillin, erythromycin, ciprofloxacin, ofloxacin, gatifloxacin, tetracycline, chloramphenicol, amikacin, moxifloxacin, levofloxacin, vancomycin, ceftazidime, tobramycin, gentamycin.

The antimicrobial sensitivity pattern, all the gram positive organisms were sensitive to vancomycin 100%, the sensitivity pattern in *Staphylococcus* species was gatifloxacin 91%, ofloxacin 87%, levofloxacin 85%, while for chloramphenicol, moxifloxacin and tobramycin the sensitivity was 84%. High resistance was observed for the other antibiotics tested. All the isolates of *Streptococcus pneumoniae* were sensitive to almost all the antibiotics tested except gentamycin. In case of gram negative organisms the sensitivity pattern was gatifloxacin 78.5%, ciprofloxacin 69%, moxifloxacin 67%, ofloxacin 64%, tobramycin 62% and levofloxacin 59.5%. Other drugs tested showed higher resistance.

Discussion

The present study was conducted in a tertiary care eye hospital over a period of 1 year. A total of 609 samples from clinically suspected ocular

infections were collected and processed in the Microbiology department.

Any part of the eye may be infected by bacteria, fungi, parasites or viruses that frequently cause infections in the eye and possible loss of vision, hence identification of causative pathogens and knowledge on the prevalent susceptibility pattern along with desired interventions are important in the clinical practice.

In the present study 609 samples from clinically suspected ocular infections were collected, these were subjected to direct microscopy and culture, bacteria were the commonest implicating pathogen accounting up to 215(87%), followed by fungal and parasitic pathogens, 27(11%) and 5(2%) respectively, such a finding has been documented in literature and other authors (Mariotti SP *et al.*, 2009; Nan Wang *et al.*, 2015). In the present study conjunctivitis was the predominant infection accounting up to 63% of the total cases. Conjunctivitis is the commonest infection of the eye, caused by several groups of organisms including bacteria, Chlamydia, viruses, fungi, helminths, and protozoa.

The principal routes of inoculation are airborne droplets, hand-to-eye contact and spread from the ocular adnexa, including the lacrimal system, nose and paranasal sinuses.

Of the 316 conjunctival swabs processed 156 (49.3%) were culture positive. 155 (99.3%) were bacterial isolates and 1 (0.6%) was a fungal isolate. Both *Staphylococcus aureus* and CONS (Coagulase-negative *Staphylococci*) took the highest proportion of the isolates (38% and 57% respectively) which is in comparison with the studies done by Iwalokun A *et al.*, 2011; Summaiya M *et al.*, 2012 and Reddy GP *et al.*, 2010. *Staphylococci* are associated with any type of eye infections. *Staphylococcus aureus* is a potential threat of eye infection and has been showing significantly increasing trends over time, especially in conjunctivitis cases. Despite their normal existence, CONS are the most frequent cause of ocular infections with increasing frequencies over time. Other isolates were *Pseudomonas aeruginosa* (4%) and *Klebsiella pneumoniae* (1%) the pattern of isolation being similar to other studies. (Iwalokun A *et al.*, 2011; Dias C *et al.*, 2013; Ramesh S *et al.*, 2010)

Microbial keratitis is a leading and potentially sight-threatening ocular infection in developing countries, posing a major public health problem. Of the 136 corneal scrapings, 55 (40.4%) harbored pathogens of which 35 (63.6%) were bacterial isolates, followed by 15 (27.27%) fungal isolates and 5 (8.8%) parasitic isolates; 4 (7%) microsporidia & 1 (1.8%) *Acanthamoeba* respectively. Fungi are the most common etiological agents followed by bacteria in cases of suppurative keratitis however this varies by geographical area. *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* account for the maximum number in cases of bacterial keratitis, in the present study bacterial keratitis was predominated by gram positive bacteria, *Staphylococcus aureus* being the most common, similar to the studies by other authors (Das S *et al.*, 2013; Gopinathan U *et al.*, 2009).

Other bacterial isolates were *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter* spp and *Acinetobacter baumannii*. Higher filamentous bacteria *Nocardia* and *Actinomycetes* were also isolated. Fungal isolates causing *keratitis* include the following: filamentous fungi *Fusarium* and *Aspergillus* species, dematiaceous fungi- *Curvularia* and *Lasiodiplodia* and *Candida* species. (Klotz SA *et al.*, 2000)

Among the 15 fungal isolates, filamentous type was more common than the yeast and *Fusarium* spp was more frequently isolated, followed by *Aspergillus* spp (Table 4) these findings are in comparison with the other authors. (Alkatan H *et al.*, 2012; Idiculla T *et al.*, 2009)

Studies in south India has shown more preponderance for *Fusarium keratitis* than *Aspergillus* in compared to the other parts of the country, *Fusarium keratitis* has a more aggressive course and is less responsive to the treatment than *Aspergillus*, reinforcing the concept of early detection and treatment (S Sharma, 2010). Among the 5 parasitic isolates causing keratitis 4 were Microsporidia and 1 was *Acanthamoeba* as identified by modified acid fast staining and wet mount respectively. Culture could not be performed.

Free living protozoa such as *Acanthamoeba* spp. have been described to be associated with microbial keratitis in India since 1987 and there is occurrence of the disease in association with trauma rather than contact lens wear. Microsporidia group of organisms, that reside somewhere between fungi and parasites phylogenetically, have considerable importance in ocular infections in India and large case series have been published, these mimic viral keratitis clinically and are often misdiagnosed. Diagnosis using conventional microbiological staining procedures and PCR can be performed. (Sharma S *et al.*, 2000; Vemuganti GK *et al.*, 2005).

Exogenous endophthalmitis is an infective complication of primary cataract, intraocular surgery and ocular trauma due to the introduction of infectious pathogens whereas the endogenous one is commonly due to systemic dissemination of the pathogens. The incidence of acute endophthalmitis following cataract surgery range from 0.04-0.5%. (Ramesh S *et al.*, 2010). 16 bacterial isolates were recovered from aqueous tap, vitreous tap and corneal button, *Pseudomonas aeruginosa* was the predominant isolate followed by streptococcus pneumonia and *Staphylococcus aureus*. Fungal endophthalmitis was seen in 11 cases filamentous fungi like *Fusarium*, *Aspergillus flavus*, *Aspergillus fumigates* being the commonest and these findings are in concordance with other authors. (S Sharma *et al.*, 2010)

The unique structure of the human eye, the use of contact lenses and the constant exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by parasites, and bacteria. Some of these infectious eye diseases, prior to the invention of contact lenses were rare; in the present study bacteria were isolated from the contact lens wearers as described above. (Michael Osita Emina *et al.*, 2011).

Drug resistance was encountered in the bacterial isolates except for vancomycin, fluroquinolones, chloramphenicol and tobramycin. Resistance trend was evidently seen in other antibiotics tested. Genotypic methods such as Quantitative PCR using real-time PCR and loop-mediated isothermal amplification (LAMP) assays can be used as the final confirmatory test for detection of intraocular infections. The lack of a confirmatory test is a limitation of the present study.

Conclusion

To mitigate the burden of ocular infections, routine microbiological examination is necessary, which helps to access the changing trends in etiology and sensitivity pattern aiding the

physician to prescribe the most appropriate antibiotic, in addition to this better patient health education is mandatory which can prevent the ocular morbidity and mortality. Antimicrobial stewardship programmes are important to address excessive or inappropriate antimicrobial usage. It is important to look beyond the usage of empirical antimicrobial agents and to adopt a better access to effective and safe topical antibiotics that has been cited as the primary factor in improving patients outcome and quality of life.

Conflict of interest

The authors declare that there are no conflicts of interest.

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None.

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