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CHARACTERIZATION OF CLINICAL ISOLATES LIKE BACTERIA AND FUNGI FROM OCULAR INFECTION

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Abstract-

Introduction- Ocular infections are an important public health problem. The knowledge of the etiologic agents causing these infections is crucial in proper management of the cases.

Aims- Present study was conducted to know the epidemiology, prevalence, aetiology and assessment of *in vitro* susceptibility of ocular bacterial isolates.

Materials and Methods- Prospective analysis of patients with clinically diagnosed ocular infections presenting between march-2010 and feb-2011 was analyzed. After slit lamp examination, samples collected by aseptic techniques and processed for gram stain and 10% KOH wet mount preparation, culture, biochemical tests and ABST by Kirby-Baur method as per CLSI. Analysis was done statistically.

Results- From 130 processed ocular samples, rate of culture-positivity was 34%, of which 28% showed bacterial growth, 5% had fungal growth and 66% no growth. The bacterial spp. isolated from keratitis 60% f/b conjunctivitis 34%, endophthalmitis 3% and dacryocystitis 3%. *CONS* was M/C isolate 37% followed by *P.aeruginosa* 21%. Fungus isolates include *Aspergillus flavus* (67%) and *Candida spp* (25%) followed by *Curvularia spp.* (8%).

Majority of gram-positive organisms were susceptible to vancomycin, gatifloxacin and cefazolin, 80% gram-negative-cocco-bacilli to amikacin, tobramycin and fluoroquinolones and 96% of gram-negative-bacilli to gatifloxacin.

Most common predisposing factor was trauma 42% in case of keratitis and exogenous infections 97% in conjunctivitis.

Conclusions- Ocular infections associated with gram-positive isolates are more in comparison to Gram-negative-isolates. Gram-positive are susceptible to vancomycin, cefazolin, while Gram-negative to amikacin and gatifloxacin. Fungal etiological agents are comparatively less.

Keywords- Ocular infections, bacteria & fungi, predisposing factor, sensitivity and specificity of gram stain and KOH, antibiotic susceptibility

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Introduction

Ophthalmic infections are prevalent in the world since the ancient time, which range from mild self limiting illness to the more severe and sight threatening conditions. These include conjunctivitis, keratitis, blephritis, endophthalmitis, dacryocystitis and other infections of eye. They are responsible for increased incidence of morbidity and blindness worldwide [1,2].

Bacteria, fungi, virus and protozoa all play a prominent role in the pathogenesis of ocular disease. The bacterial isolates commonly associated with ocular infection include *Coagulase negative staphylococci, Staphylococci spp., Propionibacterium acene, Streptococcus spp., Pseudomonas aeruginosa* and *Enterobacteriacea*. These organisms can adhere to the epithelial surface due to molecular interactions. Pilli or fimbria of gram negative bacteria plays an important role for adhesion to the cell surface. After attaching to the surface it produces certain enzymes or toxins causing damage to ocular structure [3] *Staphylococcus aureus, Pseudomonas aeruginosa* and *Coagulase negative staphylococci* may persist in ocular tissue or biomaterial in a biofilm, which protect them from host defense, high level of antibiotic and eradication, which lead to ocular morbidity. Timely institution of appropriate therapy must be initiated to control the infections and thereby minimize ocular morbidity. If they are not treated as soon as, it may lead to sight threatening condition. For specific treatment, culture and identification of bacterial pathogens its antibiotic susceptibility pattern is essential. The bacterial etiology and their susceptibility as well as resistance patterns may vary with geographic location according to the local population [4,5]. *Streptococcus pneumoniae* was reported to be the predominant corneal pathogen in the study done by Leck, et al. in Tiruchirapalli [5] and in the study done by M. Srinivasan, et al. in Madurai [6] whereas in Coimbatore it was *Pseudomonas aeruginosa* [7] *Pseudomonas aeruginosa* was reported to be the most common bacterial pathogen causing postoperative endophthalmitis in the study done by KL Therese, et al. in Chennai [8] whereas in study done by P Lalitha, et al. in Madurai it was *Nocardia* sp. [9]. Similarly, there was a variation in the *in vitro* efficacy of antibacterial agents against bacterial pathogens causing ocular infections according to the local population. For instance, ciprofloxacin showed higher sensitivity against keratitis pathogens in study done by R Ramkrishnan, et al. from Tirunelveli (90%) [10] than the study done by S Sharma in Hyderabad (69.3%) [11] Thus, the current trends in the etiology of bacterial ocular infections and their susceptibilities must be updated to make a rational choice of empirical antibiotic therapy. Different types of fungi are also important etiological agents affecting cornea, orbit and other ocular structures.

In the study done by Srinivasan, et al. [6] from Bangladesh, Leck, et al. from Ghana and India and Duanlop, et al., Garg, et al. [14], Kunimoto, et al. [15], Sherwal, et al. [12] reported fungus as the major etiological agent in patients with keratitis. Fungal infection is a life threatening condition which needs early diagnosis and treatment to save the patients' eye. The purpose of this study was to identify the etiology, prevalence of ocular infections, distribution of different isolates amongst various ocular infections, associated risk factors and to assess the *in vitro* susceptibility of ocular bacterial isolates to commonly used antibiotics.

Material and Methods

130 samples were collected from patients having ophthalmic infections attending ophthalmic outpatient department (OPD) and patient admitted in ophthalmic ward in tertiary care hospital during the study period from March 2010 to Feb 2011. They were examined clinically by the slit-lamp biomicroscopic examination. After detailed ocular examinations using standard techniques specimens for culture and smear were obtained after informed consent [6,12]. Swabbing the lid margins with sterile broth-moistened cotton swabs in cases of eyelid infections, corneal swab and corneal scraping in corneal ulcer pt, conjunctival swab by wiping a broth-moistened swab across the lower conjunctival cul-de-sac in case of conjunctivitis, purulent material in cases of dacryocystitis was collected from everted puncta by applying pressure over the lacrimal sac area and vitreous fluids were collected in case of endophthalmitis.

Primary inoculation was done at the site of sample collection. The samples were inoculated directly onto the blood agar (Aerobic incubation), chocolate agar (5-10% CO2), nutrient agar, macconkey agar and liquid media such as brain heart infusion broth and for the fungal culture on sabouraud's dextrose agar containing gentamycin. Further processing was done in the microbiology laboratory, and kept in an incubator at 37°C for 18- 24 hr. These media were examined on next day for the presence of growth of any bacteria. For the fungal isolates two sets of sabouraud's dextrose agar plates were inoculated, one incubated at 25°C for isolation of filamentous fungi and the other at 37°C for isolation of yeast form of fungi. Cultures were examined for growth daily during first weeks and twice a week during next three weeks. Collected specimens was subjected to 10% KOH wet mounting, Gram's staining. A standardized protocol was followed [16,17] *In vitro* susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted using Clinical and Laboratory Standards Institute's [18] The antibacterial agents used were amikacin (30 µg/disk), tobramycin (10µg/disk), gentamicin (10 µg/disk), cefazolin (30 µg/disk), cefotaxime (30 µg/disk), ceftazidime (30 µg/disk), ciprofloxacin (5 µg/disk), norfloxacin (10 µg/disk), ofloxacin (5 µg/disk), gatifloxacin (5 µg/disk), chloramphenicol (30 µg/disk) and vancomycin (30 µg/disk).

Results

Ocular infections were commonly seen in younger and adult age group. 53% of patients were in ≥40 years of age group, which was followed by 41% in 18 to 39 year of age, 3% in 0-2 year and 3-11 year each. Ocular infections were more common in male amounting 65% as compared to female 35%. Male to female ratio was 1.8:1. Various predisposing risk factors were studied. In case of keratitis, most common risk factor was trauma 42% followed by foreign body exposure 14%, other ocular disease 11%, systemic diseases 5% and use of contact lens 4%, post operative 1%, use of topical steroids 1% in case of keratitis. 11% of cases were idiopathic. In case of conjucntivitis, most common risk factor was exogenous infection 97%, followed by trauma 3% In case of endophthalmitis, post operative infections 75%, followed by traumatic injury 25%. Exogenous infection was also associated with dacryocystitis and infections of eyelid in all cases.

Of the 130 samples were collected from 116 clinically diagnosed patients with ophthalmic infections attending ophthalmic outpatient department (OPD) and indoor during the study period between March 2010 to Feb 2011. They were submitted for microbiological evaluation as shown in [Table-1]. The culture was positive in 44 (34%) samples, from which 37 (29%) had bacterial growth, 7(5%) had fungal growth. Mixed bacterial growth was obtained in keratitis only. Among the 22 positive samples from keratitis, 21(95%) having pure bacterial growth while 1(5%) sample having mixed bacterial growth. Thus, a total of 38 bacterial isolates were recovered from case with ocular infections. The remaining 86 (66%) ocular specimens were culture negative for microbial growth. From the total 44 isolates (bacterial + fungal), 66% was in keratitis, 30% in conjunctivitis, 2% in dacryocystitis, 2% in endophthalmitis. One patient with eyelid infection was negative for both fungal and bacterial culture.

The predominant bacterial species isolated was *Coagulase negative staphylococci* 37%, followed by *Pseudomonas aeruginosa* 21%, *Acinetobacter spp* and *Staphylococci aureus* were 13%, *Klebsiella spp.* 7%, *Enterococci* spp., *Streptococci spp*. and *E.coli* were 3% each. The predominant bacterial species isolated from eyes with keratitis 60%, conjunctivitis 34%, endophthalmitis 3% and dacryocystitis 3%. Of the total 23 of isolates from patients with keratitis, *Coagulase negative staphylococci* was 8(35%) common isolates followed by *Pseudomonas aeruginos*a 6(26%), *Acinetobacter spp.* 4(18%), *Klebsiella* 2(9%) followed by *Enterococci, Streptococci and Staphylococcus aureus* 1(4%). Of the total 13 of isolates from patients with conjunctivitis, *Coagulase negative staphylococci* was 5 (38%) common isolates followed by *Staphylococcus aureus* 4 (31%), *Pseudomonas aeruginos*a 2(15%), *Acinetobacter spp 1* (8%), *Klebsiella* 1(8%).

After the culture, the samples were processed for direct examination by gram's staining and KOH preparations. Gram stain was positive in 55 samples where as negative in 75 samples. The sensitivity and specificity of gram's stain was calculated by considering culture as gold standard. The sensitivity of gram's stain was 82% (Confidence interval: 68.04-90.49), where as specificity was 78% (Confidence interval: 68.05-85.38). The positive predictive value was 65% (Confidence interval: 52.25-76.64) and the negative predictive value was 89% (Confidence interval: 80.34- 94.5).

The sensitivity and specificity of KOH preparations was calculated by considering culture as gold standard. The sensitivity of KOH preparations was 86% (Confidence interval: 48.69- 97.43), where as specificity was 98% (Confidence interval: 94.26-99.55). The positive predictive value was 75% (Confidence interval: 40.93-92.85) and the negative predictive value was 99% (Confidence interval: 95.5- 99.86).

Results of antibiotic susceptibility pattern of *Staphylococci aureus* shows 100% susceptibility to vancomycin and 80% to the cefazolin, cefotaxime, amikacin, tobramycin, gentamycin and gatifloxacin, chloramphenicol and 60% to ciprofloxacin and ofloxacin. *Coagulase negative staphylococci* were mostly susceptible to vancomycin and gatifloxacin 93%. Susceptibility of *Acinetobacter spp*. to amikacin, ciprofloxacin and ofloxacin were 80%. *Pseudomonas aeruginosa* was mostly susceptible to amikacin, ofloxacin, norfloxacin, ciprofloxacin, gatifloxacin which was 88%. *E.coli* was 100% susceptible to amikacin, ceftazidime, norfloxacin, ofloxacin, gatifloxacin and chloramphenicol. *Klebsiella spp.* was mostly susceptible to gatifloxacin 100% followed by 67% susceptibility to amikacin, gentamycin, ceftazidime, norfloxacin, ciprofloxacin, ofloxacin and chloramphenicol. Fungi were isolated only in case of keratitis. Most common fungal isolate was *Aspergillus flavus* 57%, followed by *Candida spp.* 29%, *Curvularia spp.* 14%.

Table 1- Percentage of Microbial growth pattern from specimens obtained in ocular infections subjected to culture and sensitivity test between march 2010 to feb 2011 at a tertiary care New civil hospital.

Clinical Presentation	Total No. of samples	No. of specimens with Culture positive	No. of specimens bacterial growth alone	No. of specimens fungal growth alone
Keratitis	64	66	59	8
Conjunctivitis	30	30	35	0
Endophthalmitis	3	2	3	0
Dacryocystitis	2	2	3	0
Infections of eye lid	1	0	0	0

Table 2- Susceptibility bacterial isolates to various antibiotics in %

Name of the Bacterial Isolates	Amikacin	Tobramycin	Gentamicin	Cefazolin	Cephotaxime	Ceftazidime	Norfloxacin	Ciprofloxacin	Ofloxacin	Gatifloxacin	Chloramphenicol	Vancomycin
Staphylococcus aureus	80	80	80	80	80	60	60	60	60	80	80	100
Coagulase negative staphylococci	71	36	50	71	57	43	43	50	50	93	71	93
Enterococci spp.	..	..	0	0	0	..	0	0	0	0	100	100
Streptococci spp.	0	0	100	100	100	..	100	100	100	100	100	100
Acinetobacte spp.	80	80	60	0	80	60	80	80	80	80	60	0
Pseudomonas aerusinosa	88	50	63	0	75	75	88	88	88	88	63	0
E.coli	100	0	0	0	0	100	100	0	100	100	100	0
Klebsiella spp.	67	33	67	0	67	67	67	67	67	100	67	0

Discussion

Culture-positivity was 34% among the total samples collected, in which 28% had bacterial growth, 5% had fungal growth. Predominant cause of infection in eye was bacteria and predominant species isolated in keratitis 60%. Fungi were isolated only in case of keratitis. The most common bacteria isolated from all ocular samples were *Coagulase negative staphylococci* 14(37%) followed by *Pseudomonas aeruginosa,* 8(21%). *Coagulase negative staphylococci* were mostly isolated from the keratitis, conjunctivitis and endophthalmitis. Most common fungus isolate was *Aspergillus flavus* are responsible for majority fungal infection in patients with keratitis in our study.

Though *Staphylococci* and *Streptococci* along with other bacteria like *Moraxella, Haemophilus*, and *Neisseria, Corynebacterium* are commensal of the conjunctiva but it may causes infection under certain conditions [24,25]. *Staphylococcus aureus* is commonly involved in primary pyoderma and acts as a secondary invader on diseased skin. It produces Coagulase enzyme, which is capable of clotting the plasma which may play a role in the development of staphylococcal abscess by producing local fibrin thrombi that protect organisms and concentrate toxic factors [26]. *Coagulase negative staphylococci* elaborate slime on their surface that facilitates adherence to the surface and may play a role in the pathogenesis of endophthalmitis. The surface slime protects the organism from phagocytosis and the action of antimicrobial agents. *Coagulase negative staphylococci,* especially *Staphylococcus epidermidis* are the commonest cause for postoperative endophthalmitis. Being a normal inhabitant of the upper respiratory tract, *Streptococci pneumoniae* is frequently found in the lacrimal apparatus and conjunctiva. Any minor corneal epithelial disruption facilitates invasion of the bacteria and corneal ulcer may produced.

Resistance and sensitivity based on *in vitro* testing may not reflect true clinical resistance and response to an antibiotic because of the host factors and penetration of the drug. In this study, vancomycin (100%) and cefazolin (80%) revealed a higher efficacy against gram -positive isolates as compared to other antibacterial agents. Vancomycin is basically a glycopeptides; which inhibits cell wall mucopeptide synthesis in early stages and it exhibited greatest potency against ocular gram-positive isolates. We found greatest coverage of gatifloxacin and amikacin against gram-negative isolates. Ciprofloxacin and ofloxacin were introduced initially and have been widely used in past, whereas gatifloxacin's usage has started in recently. Methoxy side chain at the C-8 position and methyl group on the piperazinyl ring is present in gatifloxacin. There was a decrease in susceptibilities to antibiotics in pathogens' to ciprofloxacin and ofloxacin along with subsequently increase in the effectiveness of gatifloxacin. The relationship between use of antibiotic and its resistance is complex. Improper selection, inadequate dosing and poor compliance to antibiotic therapy may also play important a role in increasing resistance like their overuse.

Conclusion

Majority of ocular infections are associated with bacterial etiology, which was more commonly due to gram-positive organisms than gram negative organism. Most of the gram-positive isolates were susceptible to vancomycin and cefazolin, whereas gram-negative organisms were susceptible to amikacin and gatifloxacin. Gatifloxacin was also effective against both the type of bacterial isolates.

Fungus was also associated with ocular infections, but to the lesser extent. Gram stain also has good sensitivity and specificity so it can help in early diagnosis and treatment as many eye clinics in locality do not have microbiological facility. The information provided in this article would aid the clinician in formulating rationale-based decisions in the empirical antibiotic treatment of bacterial ocular infections that cause major public health problems.

Abbreviation

KOH preparation: Potassium Hydroxide preparation

CONS: Coagulase negative staphylococci

ABST: Antibiotic susceptibility testing

CLSI: Clinical and Laboratory Standard institute

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