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Original Research Article

Analysis of antimicrobial susceptibility pattern of ocular infections at Regional Ophthalmic Institute in India

Qudsia Nuzhat[1], Arkapal Bandyopadhyay[2]*, Rakesh Chandra Chaurasia[3], Satya P. Singh[4], Monica Singh[5]

[1]Department of Pharmacology, Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh, India
[2]Department of Pharmacology, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India
[3]Department of Pharmacology, Government Medical College, Azamgarh, Uttar Pradesh, India
[4]Department of Ophthalmology, [5]Department of Microbiology, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India

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***Correspondence:**
Dr. Arkapal Bandyopadhyay,
Email: drarkapal@gmail.com

ABSTRACT

Background: Ocular infections are a result of alteration in the normal microbial flora of eye. They are not only responsible for increase in morbidity varying from self-limiting trivial infection to sight threatening infection but also blindness.
Methods: Patients with ocular infections were recruited at Regional Institute of Ophthalmology, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh. Bacterial profile in ocular infections and susceptibility pattern to commonly used antibiotics were analyzed amongst these patients. The isolated organism was then identified by colony morphology, gram stain and biochemical test following which in vitro susceptibility test was performed by Kirby-Bauer disc diffusion method and interpreted clinically.
Results: *Staphylococcus aureus* and coagulase negative *Streptococcus* were most common etiological agents of ocular infections in the present study. It was observed that bacterial isolates were highly (in 100% of cases) susceptible to vancomycin and chloramphenicol among gram positive organisms. Gram negative organisms showed higher susceptibility to moxifloxacin, tobramycin and gentamycin. *Pseudomonas* was seen to have sensitivity towards ceftazidime and cefazolin.
Conclusions: The present study gives an insight into use of ocular antimicrobials in northern India. These findings illustrate the need for constant bacterial surveillance before starting empirical treatment.

Keywords: Antibiotics, Bacteria, Infections, Ocular, Resistance

INTRODUCTION

The eye is impermeable to almost all external infectious agents, though the ocular surface invariably is exposed to a wide array of microorganisms.[1] Virulence of the pathogenic microorganisms and host's reduced resistance can cause ocular infections. According to various studies, the most common microorganisms causing ocular infections include *Staphylococcus aureus*, Coagulase negative staphylococci, *Streptococcus*, *Corynebacterium*, *Bacillus*, *Nocardia*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*.[2,3] In developing countries like India, the prevalence of ophthalmic infections is increasing every year. Penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, macrolides, chloramphenicol and sulfonamides are generally used to treat ocular infections. Treatment of *Staphylococcus aureus* infections has become more complicated with emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) strain.[4] Increased resistance to commonly used antimicrobials is also challenging the empirical choice of an effective treatment for ocular infections.[5,6] The changing spectrum of microorganisms involved in ocular infections and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy.[7,8] For specific antibacterial treatment, isolation and identification of bacterial pathogens along with antibiotic susceptibility spectrum is essential.[9]

The present study was done with the aim to isolate, identify the pathogens and analyze the pattern of antibacterial sensitivity in patients of ocular infection.

METHODS

The present prospective observational study was carried out in the Department of Ophthalmology at Regional Institute of Ophthalmology (M.D. Eye Hospital) and MLN Medical College, Allahabad over a period of 6 months from November 2017 to April 2018.

Sample size was calculated based on study by Daniel et al.[10] With prevalence of 20%, precision 5%, 95% confidence interval, Z value of 1.96 the sample size was 245.6. So, a sample size of 250 was taken for the present study. All the patients attending Ophthalmology OPD were examined on the slit-lamp biomicroscope and infective diseases were diagnosed clinically by a group of ophthalmologists. Patients of either sex aged more than 12 years, attending Ophthalmology OPD, who were either newly diagnosed with ocular infection (i.e. patients not on antibiotics- either topical or systemic, for last 4weeks) or recurrent/old cases who were not responding to antibiotics (either topical or systemic for 4 weeks), who were able to comprehend interview questions and follow study related advice were included in the study. Patients not willing to give consent, noninfectious etiology of ocular diseases, immunocompromised patients like AIDS, malignancy, malnutrition, diabetes and patients on steroids were excluded. Diagnosed patients of ocular infection who fulfilled the inclusion criteria were recruited consecutively. Recruitment was done on all working days of OPD (Monday to Saturday) till the completion of sample size. Written informed consent was taken from all patients in the study.

Data related to patient's sex, age and socioeconomic status was collected in a pre-designed questionnaire. Clinical examination, slit lamp examination and investigations were done in all the enrolled cases. Pus and corneal scraping samples were taken from cases of blepharitis, corneal ulcers, suppurative scleritis, suppurative abscess, conjunctivitis, canaliculitis and dacryocystitis.

Collected samples were transported to the laboratory within one hour and processed without delay. The specimens were then cultured on blood agar and Mac-Conkey agar, mannitol salt agar and Todd Hewitt agar etc. The isolated organism was there after identified by colony morphology, gram stain and biochemical tests according to Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing.[11]

In this study, a positive culture was defined as growth of the same organism on more than two solid phase media or confluent growth on one solid medium. A standardized protocol was followed for each ocular specimen for the evaluation of significant microbiological features. In vitro susceptibility testing was performed by Kirby-Bauer disc diffusion method and interpreted using Clinical and Laboratory Standards Institute's serum standards.[11] Antibiotics were classified as sensitive (S), resistant (R) and moderately sensitive (MS). Antimicrobial which is moderately sensitive to organism in high concentration is considered as sensitive. So, total sensitive microorganisms were denoted as MS+S (Figure 1).

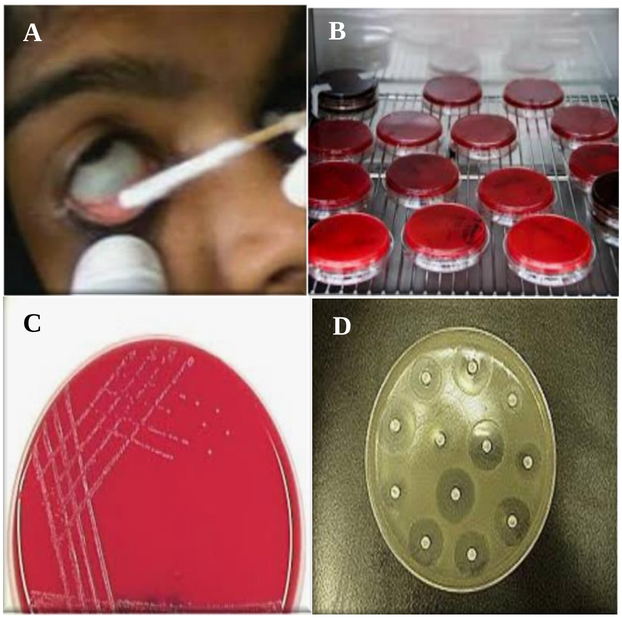

Figure 1: Procedure of sample collection and analysis, (A) taking a swab from the infected eye; (B) incubation of the culture plates; (C) colonies of bacterial growth on culture plate; (D) antibiotic sensitivity test by zone of inhibition method.

The antibacterial agents used were tobramycin (10 µg/disk), gentamicin (10 µg/disk), cefazolin (30 µg/disk), cephotaxime (30 µg/disk), ceftazidime (30 µg/disk), ciprofloxacin (5 µg/disk), norfloxacin (10 µg/disk), ofloxacin (5 µg/disk), gatifloxacin (5 µg/disk), moxifloxacin (5 µg/disk), chloramphenicol (30 µg/disk),and vancomycin (30 µg/disk). They were consistently tested for their efficacy against Standard American Type Culture Collection [ATCC]- bacteria (*Staphylococcus aureus* ATCC 25923, *S. pneumoiae* ATCC 49619, *H. influenzae* ATCC 49241, *P. aeruginosa*

ATCC 27853, *E. coli* ATCC 25922), fungus (*Aspergillus fumigatus* ATCC 1022, *Candida albicans* ATCC10231) as a general quality control laboratory procedure. The primary effectiveness outcome was measured by microbial culture on Mac-Conkey agar and blood agar and further subculture was done based on isolated organism. (Figure 1). Observations were made under supervision of qualified microbiologist in microbiology department.

The study was conducted after approval from the institutional ethical committee. Data was entered using Microsoft Excel 2013. Statistical analysis was done using SPSS version 23 and STATA 18.

RESULTS

Demographic and disease characteristics of patients

In the present study, 54% were males and 46% were females. Age group of patients ranged from 12-70 years. Patients within age group of 21-30 years were most commonly affected followed by 51-60 years.

Among 250 samples collected from patients, 50 were positive to infections. Amongst them, 29 were males and 21 were females. Majority (88%) were bacterial infections followed by fungi (12%). Out of bacterial infections, *Staphylococcus aureus* (40.9%) was the most common followed by Coagulase Negative *Streptococcus* species (CONS) (22.7%), E. coli (13.6%), *Pseudomonas* (6.8%) and *Klebsiella* (4.5%). Remaining (11.3%) were mixed infections. *Aspergillus fumigatus* (66.6%) and *Candida albicans* (33.4%) were the fungal infections found in the study. In this study it was observed that rural population was more sensitive for infection (56%) than urban (44%).

Swabs were collected from varied ocular infections. Most common infection prevalent was conjunctivitis (42%) followed by corneal ulcer (25%). Least common diseases were Endophthalmitis (4%) and recurrent hordeolum externum (2.8%) (Table 1).

Culture and isolation of microbes

Culture of 50 growth positive specimens showed that 56% were Gram positive bacteria, 22% were Gram negative bacteria, 10% were mixed growth and 12% were fungal isolates. Most common bacterial isolate was *Staphylococcus aureus* (36%) followed by CONS (20%). Among gram negative isolates, *E. coli* was most commonly isolated (12%) followed by *Pseudomonas* (6%) (Table 2).

Antimicrobial susceptibility pattern

In *S. aureus* isolates, maximum sensitivity was seen for Vancomycin (100%) followed by Chloramphenicol (78%) in this study. Methicillin sensitivity was present in 72% of cases. Rifampicin was also observed as sensitive anti-staphylococcal agent. Chloramphenicol, Rifampicin and Cefazolin showed moderate sensitivity. Maximum resistance was seen for Norfloxacin (77%) and Ciprofloxacin (66%) (Table 3).

Table 1: Distribution of patients by type of ocular infection.

Infection	N (%)	Positive cases (%)
Conjunctivitis	105 (42)	18 (36)
Corneal ulcer	62 (25)	10 (20)
Blepharitis	33 (13.2)	7 (14)
Dacrocystitis	19 (7.6)	6 (12)
Keratitis	14 (5.6)	4 (8)
Endophthalmitis	10 (4)	3 (6)
Recurrent stye	7 (2.8)	2 (4)
Total	250 (100)	50 (100)

Table 2: Distribution of cases according to culture.

Groups	Organism cultured	N (%)
Gram positive bacteria	*Staphylococcus aureus*	18 (36)
	CONS	10 (20)
Gram negative bacteria	*E. coli*	6 (12)
	Pseudomonas	3 (6)
	Klebsiella	2 (4)
Mixed infections		5 (10)
Fungi	*A. Fumigatus*	4 (8)
	C. albicans	2 (4)
Total	-	50 (100)

Table 3: Distribution of *Staphylococcus aureus* cases according to sensitivity.

Staph. aureus (n=18)	S	MS	R	Total sensitivity (S+MS) N (%)
Ciprofloxacin	3	3	12	6 (33.3)
Norfloxacin	2	2	14	4 (22.2)
Vancomycin	14	4	-	18 (100)
Chloramphenicol	5	9	4	14 (77.7)
Erythromycin	2	6	10	8 (44.4)
Methicillin	3	10	5	13 (72.2)
Netlimycin	2	7	8	9 (50)
Rifampicin	2	16	-	2 (11.1)
Cefazolin	4	14	-	4 (22.2)
Ceftazdime	4	14	-	4 (22.2)

Among CONS, maximum sensitivity (100%) was seen towards vancomycin and chloramphenicol followed by erythromycin (80%) and methicillin (80%), shown in Table 4.

Among the Gram-negative isolates, *E. coli* was most commonly isolated. It showed maximum sensitivity (100%) to moxifloxacin, gentamycin, tobramycin, ceftazidime and ciprofloxacin and 83.3% sensitivity to cefazolin, erythromycin and netilmycin. With ofloxacin, maximum resistance (66.7%) was seen.

Table 4: Distribution of CONS cases according to sensitivity.

CONS (n=10)	S	MS	R	Total sensitivity (S+MS) N (%)
Ciprofloxacin	-	2	8	2 (20)
Norfloxacin	-	1	9	1 (10)
Vancomycin	10	-	-	10 (100)
Chloramphenicol	7	3	-	10 (100)
Erythromycin	4	4	2	8 (80)
Methicillin	3	5	2	8 (80)
Netilmycin	4	3	3	7 (70)
Rifampicin	3	4	3	7 (70)
Cefazolin	2	4	4	6 (60)
Ceftazdime	3	3	4	6 (60)

Klebsiella showed sensitivity to moxifloxacin, gentamycin and tobramycin in 100% cases. It showed 50% sensitivity in cefazolin, cetazidime, ciprofloxacin, erythromycin and netlimycin. Resistance of *Klebsiella* was seen with ofloxacin in 100% of cases.

Pseudomonas aeruginosa, an important causative agent of a variety of infectious diseases leading to blindness and serious eye consequences was isolated in 3 cases. It showed sensitivity for cefazolin, moxifloxacin and ceftazidime in 100% cases. With gentamycin 100% resistance was seen. Ofloxacin, tobramycin and netilmicin showed resistance in 2 out of 3 cases (66%). Ciprofloxacin and erythromycin showed resistance in 1 out of 3 cases (33.3%).

As far as fungal isolates were concerned, *Aspergillus fumigatus* and *Candida albicans* showed sensitivity for fluconazole and ketoconazole in 100% cases.

DISCUSSION

In the present study, males were predominantly affected. Male predominance may be due to many social factors like involvement in outdoor works, lifestyle habits like smoking increasing sensitivity for infection and exposure to pollution etc. Maximum patients infected were in age group of 21-30 years. A study done at Ethiopia showed similar trends of gender distribution with male predominance for most of the infections but majority of infections occurred in the age group of 40-60 years age.[12] This may be due to the site of the present study where majority of the participants resided in the rural areas, belonged to low socio-economic status and were working outdoors from an early age. Hence, population in the younger age group was more susceptible to ocular infections.

Most common positive culture ocular infection in this study was conjunctivitis (36%) followed by corneal ulcer (20%), blepharitis (14%) and dacrocystitis (12%). A study by Bharathi et al showed infections of lacrimal apparatus (28.05%) as the most common ocular infection followed by infection of eyelids (26.05%) and conjunctiva (22.14%).[13] Dacrocystitis was present in most of the cases.

present study had an overall isolation rate of 20% with 42% in conjunctivitis, 25% in corneal ulcers and 13.2 % in keratitis. A study conducted by Moinuddin et al had an increased isolation rate of 69.6%.[14] The isolation rate was 70.54% in conjunctivitis, 77.05% in keratitis and 54.05% in blepharitis. This can be due to the difference in the disease prevalence of the area and site of the study.

In the present study, 56% were Gram positive bacteria, 22% were Gram negative bacteria, 10% were mixed growth and 12% were fungal isolates. The results were similar to a study done by Bharathi et al (bacterial- 58% followed by fungal infection- 10.3%).[13] A study done by Srinivas et al in Madurai also showed predominant gram-positive isolates (47%) similar to present study but also high fungal culture positivity (46.8%).[15] A study by Sharma et al also showed high bacterial isolates (69.1%) similar to present study.[16]

In present study, the most common bacterial isolate was *Staphylococcus aureus* (36%) followed by CONS (20%). Among gram negative isolates, *E. coli* was most commonly isolated (12%) followed by Pseudomonas (6%). The study conducted by Bharathi et al also showed *Staphylococcus aureus* (26.69%) followed by *Streptococcus pneumonia* (22.14%) as the predominant microbe isolated.[13] A study conducted by Mohammed et al in Jordan had a very similar picture with *Staphylococcus aureus* (56.6%) being the most common microbe isolated followed by *Streptococcus pneumoniae* (14.9%).[17] A study by Maurer et al in Switzerland over 20 years on 7862 patients showed 38.3% positivity for aerobic bacteria.[18] The strains isolated most frequently were *Staphylococcus aureus* (23.9%), Coagulase-negative staphylococci (16.1%), *Pseudomonas aeruginosa* (10.0%), *Escherichia coli* (5.1%), *Streptococcus pneumoniae* (3.9%) mostly similar to present study.

Present study had 27.78% isolates of MRSA. A 10-year retrospective study by Nithya et al showed 21% isolates of MRSA.[19] This may be attributed to the small sample size and shorter duration of the study

In present study, gram positive organism was most sensitive (100%) to vancomycin and chloramphenicol whereas gram negatives to moxifloxacin, tobramycin and gentamycin. Overall, ciprofloxacin was the most effective antimicrobial agents with susceptibility rate of 78.0%. In a study by Mohammad et al most of gram-positive isolates were susceptible to vancomycin (93.6%) and cefotaxime (84.3%) and gram-negative isolates to ciprofloxacin (77.7%) and tobramycin (48.1%).[17] *Pseudomonas* was isolated 3 cases in this study (6%). It was similar to a study done by Mohammad et al wherein

Pseudomonas was isolated in 8 cases (5.2%) over 4 years.[17]

The present study is the first of its kind in the region. The distribution of the ocular infections and their antibiotic susceptibility profile provides a good understanding of the nature of the infection in the state of Uttar Pradesh and northern part of India. Owing to the limited resources, the study was conducted for a short duration of time and hence the sensitivity pattern over a longer period of time could not be evaluated.

CONCLUSION

The present study on the susceptibility pattern shows the need for broad spectrum antibiotics with greater antibacterial efficacy based on current spectrum and trends. These findings illustrate the need for constant bacterial surveillance before starting empirical treatment. Strict hygienic practice, routine ocular check-up and early treatment of the suspected infections are the need of the hour.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee MLN Medical College and Associate Hospitals, Allahabad (Ethics Committee Registration No. ECR/922/inst/UP/2017 issued under Rule 122DD/of the Drugs and Cosmetics Rule 1945)

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