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Prevalence of Bacteria in the External Ocular Infection and Determination of their Antibiotic Resistance Pattern in a Tertiary Care Unit of India

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Abstract—*External eye infection is an important public health problem as vision is critical to every individual. The knowledge of the etiologic agents causing these infections is crucial in proper management of the cases. There is a scarcity of published data on the spectrum of etiologic agents responsible for regular external ocular infections in India which makes this study even more significant.*
The study was conducted to determine the bacterial spectrum of keratitis and conjunctivitis. The study was conducted between February 2018 to July 2018. Samples from 75 patients with symptoms for bacterial keratitis and conjunctivitis were collected.
Out of 75 ocular samples collected, 50 samples showed culture positivity. 64 bacterial isolates were identified into 8 different types of bacteria. Gram-positives accounted for 76.56 % while Gram-negatives comprised of 23.44%. The most common etiologic agents isolated in case of keratitis were Staphylococcus aureus (72.22%) and Pseudomonas aeruginosa (27.78%). In case of conjunctivitis, Staphylococcus aureus (73.39%), Micrococcus lactis (8.69%), and Klebsiella pneumoniae (13.04%) were isolated. Antibiotic susceptibility test revealed all strains were susceptible to commercial preparations of ciprofloxacin (0.3%), gatifloxacin (0.3%), besifloxacin (0.6%), moxifloxacin (0.5%) and chloramphenicol (0.5%), however resistant to carboxymethyl cellulose (0.5%).
This study provides an insight regarding prompt choice of appropriate antimicrobial agent that would be most effective in the management of external ocular microbial infections.

Keywords: *keratitis, conjunctivitis, antibiotic resistance, bacteria.*

1. INTRODUCTION

The eye is probably the most significant amongst the other sense organs when it comes to interaction with the environment. It is a unique organ that is almost impermeable to almost all external agents [1]. Even then it is vulnerable to infections, made possible through lapse of the immune system, cell damage, old age or breach by pathogenic or mutualistic strains.

Ocular infections, defined as infections in the inner or external eye, are caused by various agents, however, bacterial infections are quite common and occur in several forms, ranging from mild, self-limiting conditions to those that could be extremely serious and visually threatening. The prevalence of these infections and the responsible bacterial organisms varies with the age of the patient and his or her geographic location. Ocular infections are among the leading causes of ocular morbidity and blindness in developing countries like India [2]. According to the World Health Organization, corneal diseases are among the major causes of vision loss and blindness in the world today, after cataract and glaucoma [3]. Ocular infections are classified mainly on the basis of the location of the infection. The conjunctiva, eye lid and cornea are most frequently infected parts of the eye. The most common manifestations are conjunctivitis, blepharitis, keratitis, canaliculitis, dacryocystitis, scleritis, orbital cellulitis, endophthalmitis, panophthalmitis and other infections. Bacteria, viruses, fungi and even parasites can cause these ocular infections [4, 5]. Thus ocular infections can affect different eye structures, and their presentation and treatment vary accordingly. In deciding on appropriate treatment, both the causative pathogen and the structure(s) affected must be considered [6, 7].

Inflammation or infection of the conjunctiva (the membrane that lines the eye lids and covers the exposed surface of the eyeball) is known as conjunctivitis and is characterized by dilatation of the conjunctival vessels, resulting in hyperemia and edema of the conjunctiva, typically with associated discharge which may be watery, mucoid, mucopurulent or purulent [8]. Keratitis is an inflammation of the cornea. It is characterized by corneal edema, cellular infiltration, and ciliary congestion disability [9]. The severity of the corneal infection usually depends on the underlying condition of the cornea and the pathogenicity of the infecting bacteria [10-12]. Superlative Keratitis is the second most common cause of ocular blindness after un-operated cataract in countries such as India and other tropical countries [13- 15].

Popular choices of treatment for bacterial conjunctivitis and keratitis in India included antibiotics in the group of fluoroquinolone, which is a transcription and translation inhibitor [15, 16]. Enquiry revealed that few of the commonly prescribed fluoroqinolones were commercial preparations of ciprofloxacin, gatifloxacin, besifloxacin and moxifloxacin. Often Carboxymethyl cellulose was used in tandem to prevent associated allergic reactions or dry eyes [17].

This study was carried out to isolate and characterize the bacterial population occurring on the ocular surface of patients with keratitis and conjunctivitis infection. Analysis for the development of resistivity against the commonly prescribed antibiotics was also carried out. Statistical analysis and data compilation allowed better understanding of the scenario of conjunctivitis and keratitis in Siliguri and adjoining areas.

2. MATERIALS AND METHODS

2.1 Study design:

A cross sectional study of external ocular infections was conducted between February 2018 to July 2018 with the approval of the Siliguri District Hospital and Siliguri Greater Lions Eye Hospital. All the patients with keratitis and conjunctivitis, confirmed by an ophthalmologist were considered for study population.

2.2 Study population and Data collection:

After describing the intention of the study, the documented consent of the patients, willing to donate the samples, were collected. Demographical data (age, gender, and residence) were collected from patients consenting to provide the sample for the study. Proper medical ethics were maintained for collecting samples.

2.3 Sample Collection:

A conjunctiva swab was obtained from the affected area from each patient with a sterilized cotton swab (placed in 10 ml phosphate saline solution). The swab was dipped directly in phosphate buffer solution placed in a screw cap tube immediately after swabbing.

In patients with symptoms of keratitis, corneal scrapings were collected by a professional ophthalmologist with sterile spatula, following a two-drop single application of topical benoxinate (0.4%) to the cornea. Corneal scrap blade were used as direct inoculum for nutrient agar plates and they were appropriately labelled and the plates were transported. Each sample was subjected to microbial investigation. All protocols were conducted as previously reported [19, 20].

2.4 Isolation of bacteria from the infected patients:

The conjunctiva swabs were vortexed at low intensity and then 100 µl of each of the samples were then spread on nutrient agar plates and incubated at 37^{o}C for 24 hrs.

The keratitis samples of corneal scrapings were directly inoculated on the Nutrient Agar were incubated for 24 hours at 37^{o}C. The isolated colonies in both the cases were picked up and subsequently streaked on N.A plates for pure culture isolation.

2.5 Identification of the isolates:

The morphological and colony characteristics (size, shape, pigmentation, elevation, colour, surface, texture and margin of colony) of the isolates were recorded. To determine the shape of the bacterial cultures, the isolated strains were gram stained and observed under light microscope (100x). Commercial identification kits were used to identify the isolates up to species level Different type of API kits Analytab product, Plainview), and Vitek system, different card for identification of gram-positive bacteria and gram negative bacteria.

2.6 Antibiotic susceptibility test:

The susceptibility of the bacterial isolates to antimicrobial agents was determined using well diffusion method. Commercial preparations of besifloxacin (0.6%), moxifloxacin (0.5%), ciprofloxacin (0.3%), chloramphenicol (0.5%), carboxymethyl cellulose (0.5%), gatifloxacin (0.3%) and a combination of carboxymethyl cellulose and besifloxacin, moxifloxacin, gatifloxacinciplofloxacin were used for each set of isolated strain. The concentrations of the antibiotics was not standardised to allow analysis at the exact concentration at which these are applied to the eye. Overnight grown bacterial isolates were spread evenly on the Mueller Hinton Agar plate. The antibiotic solutions were placed in the wells dug on plates and were incubated for 24 hours at 37˚C. The results were recorded after incubation.

2.7 Statistical analysis:

Data was stored and managed in Microsoft Excel. Statistical analysis was done by performing the X2 (chi-square test) [21]. Probabilities of $p < 0.01$ were considered statistically significant. The α-level was set at 0.05.

3. RESULT

3.1 Demographic characterization:

Samples were collected from a total of 75 patients with ocular infection. Most of the patients were of the age group of 11-40 years (56.01%), followed by 51-60 years (16%), 41-50 years (13.33%) and 61-70 years (6.67%). Also 8 % patient population was of 10 years or less of age (Fig.1).

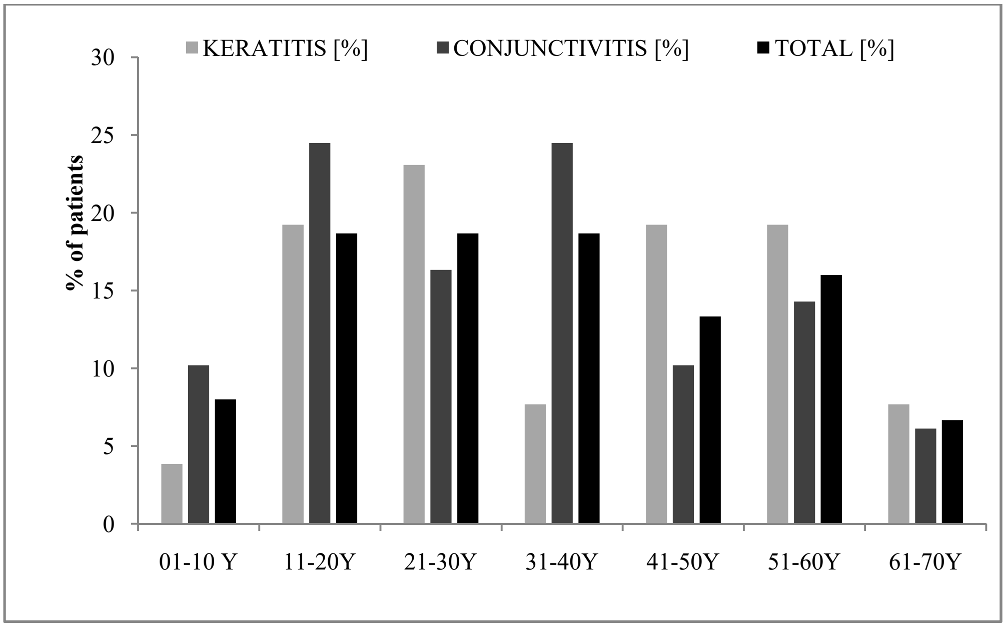

Figure 1: Distribution of the age group of patients with respect to percentage of patients suffering from keratitis and conjunctivitis.

Age group of 21-30 showed maximum percentage of keratitis cases (23.08%) followed by 11-20 years (19.23%), 41-50 years (19.23%), 51-60 years (19.23%) while in conjunctivitis it was 11-20 years (24.49%) and 31-40 years (24.49%). Among the collected samples, 18 males (69.23%) and 8 females (30.76%) resulted positive for keratitis while 27 males (55.10%) and 22 females(44.90%) showed positive for conjunctivitis (Fig. 2).

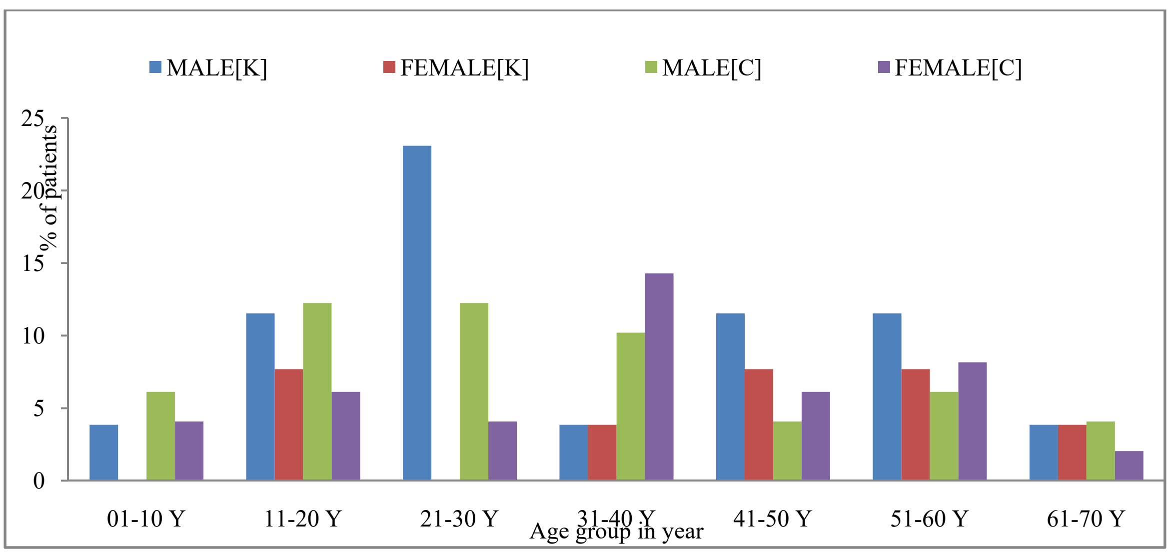

Figure 2: Percentage of male & female patients affected by keratitis(K) and conjunctivitis(C).

Statistical analyses of the residence of the patients revealed 14 cases in urban (18.67%) and 12 in rural (16%) for keratitis In contrast, conjunctivitis patients showed a greater population from rural area (41.33%) compared to 24% in urban areas (Fig. 3).

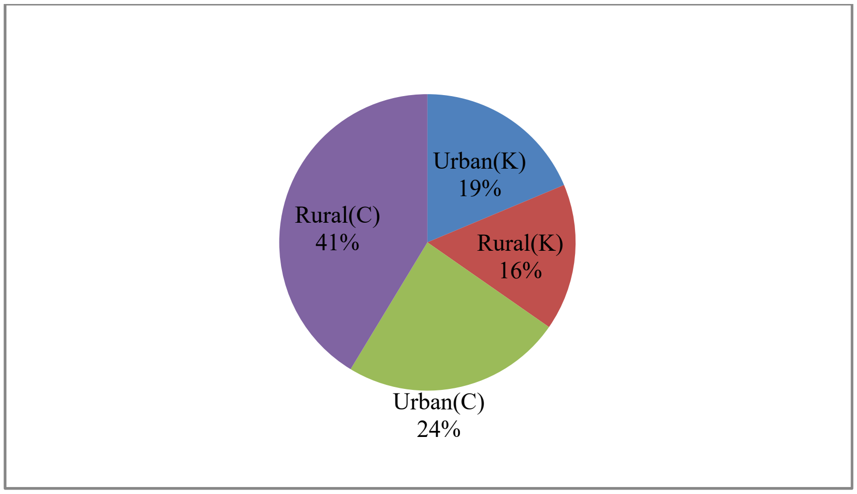

Figure 3: Frequency distribution of residence of patients.(K) stands for keratitis while (C)stands for conjunctivitis.

3.2 Isolation and Identification of the isolates:

A total of 64 bacterial strains were isolated from 75 ocular samples. Bacterial identification confirmed 5 different genera of bacteria among 64 strains.18 strains of keratitis were identified to be either *Staphycococcus aureus* or *Pseudomonas aeruginosa* (Table 1).

Table 1: Identification of the bacterial isolates causing keratitis

Organism	Gram Nature	Shape	Colony morphology	Frequencyof isolate
Staphylococcus aureus	+	Circular, Pinhead	Entire, opaque, convex, smooth, golden yellow	13
Pseudomonas aeruginosa	-	Oval, Medium	Wavy, convex, smooth, opaque, diffusible green	5

In case of conjunctivitis, 46 strains were identified to be *Staphycococcus aureus, Micrococcus lactis* and *Klebshiella pneumonia* (Table 2). The rate of culture positivity was found to be significantly higher ($P \leq 0.01$) amongst conjunctivitis (71.8%), than keratitis (28.1 %).

Table 2: Identification of the bacterial isolates causing conjunctivitis

Organism	Gram Nature	Shape	Colony morphology	Frequencyof isolate
Staphylococcus aureus	+	Circular, Pinhead	Entire, convex, golden yellow, smooth, opaque	34
Micrococcus lactis	+	Circular, Pink Pigment	Entire, slightly convexed, orange, smooth, opaque	6
Klebsiella pneumoniae	-	Rod	Undulate, round, mucoid, convex opaque	6

Among the bacteria isolated from ocular infection, 20.31% (keratitis) and 59.38% (conjunctivitis) were Gram-positive while 7.81% (keratitis) and 9.38% (conjunctivitis) were Gram-negative (Fig 4).

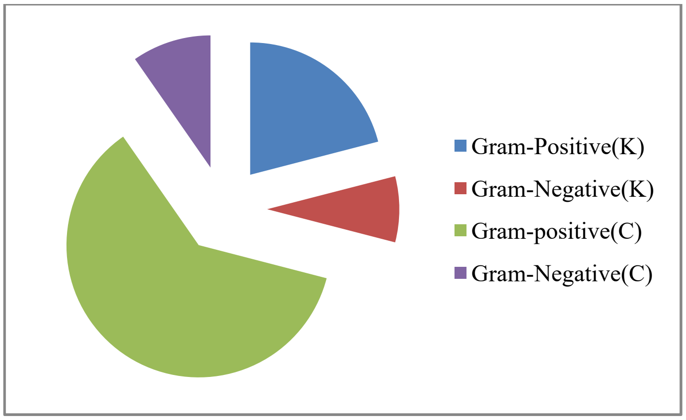

Figure 4: Distribution of isolated bacteria on basis of gram nature

3.3 Bacterial population analysis from isolates:

In the present study, *Staphylococcus aureus* (72.22% in keratitis & 73.92% in conjunctivitis) was the most dominant amongst the other strains in both the ocular infections. *Pseudomonas aeruginosa* (27.78%) was isolated in cases of Keratitis only. (Fig 5) *Micrococcus lactis*(8.69%) and *Klebsiella pneumonia* (13.04%) were isolated only in case of conjunctivitis.

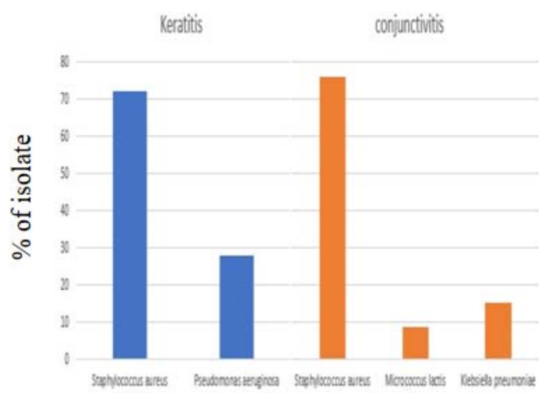

Figure 5: Frequency distribution of isolated bacteria from ocular infection.

3.4 Antibiotic Susceptibility test

On analysis of antibiotic susceptibility of the different commercial eye drops by well diffusion method, it was found that all the strains were sensitive to the 3rd and 2nd generation antibiotics; however *Staphylococcus aureus* was seen to be partially resistant to Chloramphenicol. Carboxymethyl Cellulose did not show any inhibition as it had a mild antimicrobial effect.

Table 3.Antibiogram of the isolated strain

Antibiotics		CIP 0.3%	BESI 0.6%	CHL 0.5%	MOX 0.5%	GAT 0.3%	CMC 0.5%
Generation of antibiotics		2nd	2nd	1st	3rd	3rd	1st
KERATITIS	*Staphylococcus aureus*	S	S	S,R	S	S	R
	Pseudomonas aeruginosa	S	S	S	S	S	R
CONJUNCTIVITIS	*Staphylococcus aureus*	S	S	S,R	S	R	R
	Micrococcus lactis	S	S	S	S	S	R
	Klebsiellapneumoniae	S	S	S,R	S	S	R

S means Sensitive, R means Resistant; S, R means some were sensitive and some were resistant*CIP stands for ciprofloxacin, CHL for chloramphenicol, CMC for carboxy methyl cellulose, MOX for moxifloxacin, GAT for gatifloxacin and BESI for besiflo***xacin**

4. DISCUSSION

In the present study, the highest incidence of ocular infection occurred between the age groups of 21 to 30 years. The predominance of infections amongst age groups above 20 indicated pathogenic factors or probable immune response compromise due to selective medical conditions. In general, males (60%) were more susceptible to ocular infection compared to females(40%) probably because more males are involved in outdoor activities thus they have a higher chance of interactions with pathogens. A study conducted by Srinivasan et al. [22] at Madurai observed patients of the lower socio-economic group like farmers were more affected by external ocular infections. The current study too reciprocated the observation as there were more cases of infection from rural areas as compared to urban areas.

A total of 75 samples were taken into consideration, swab samples of 49 patients, diagnosed with conjunctivitis, and corneal scrapings of 26 patients, diagnosed with keratitis were collected for the microbiological study. On culturing the 75 samples, 64 samples showed bacterial growth. The probable reasons for no bacterial growth in some of the samples could be due to various factors such as low amount of inoculums [22], the empirical treatment received before collection of the samples[23] and also that keratitis and conjunctivitis are caused not only by a variety of microorganisms (fungi and virus) but also many eye allergens [4].

In this study, the culture-positivity varied from centre to centre and patient to patient. The probable reasons were factors like age, environmental conditions, and personal hygiene. In this study conducted, the culture positivity is 66.67% which is similar to the study conducted in Arvind Eye Hospital Tamil Nadu [24]. Chances of entry of eye irritants were higher since this study has been carried out immediately after the festival of Holi [25, 26]. Studies by Idu et al., [27] stated bacterial conjunctivitis was the most commonly seen external ocular infection which was similar in the present study also. The rate of positive culture result per sample taken was found to be significantly higher ($P \leq 1\%$) among eyes with conjunctivitis (65.33%), than keratitis (34.67 %) because of a greater variety of bacterial causative agents.

Out of the 64 isolates obtained, Gram-positives accounted for 79.69 % while Gram-negatives comprised of 20.31%. This result is similar to the 69.1% and 75.65% Gram-positives reported previously by some Indian investigators [22].

In the study, *S. aureus* was the predominant organism isolated in both cases followed by *P. aeruginosa* and *K. pneumonia*. This may be due to the dominance of *Staphylococcal* strains in causing upper respiratory infections. Other associated microorganisms isolated were previously reported to have caused keratitis and conjunctivitis. However *M. lactis* findings indicate a new developing causative agent. The analysis of the different antibiotics on the isolated bacterial cultures showed effect in most cases. However *Staphylococcal* strains and *Klebsiella sp*. were seen to be developing resistance against 1st gen antibiotics (Chloramphenicol). The zones of inhibition were maximum in case of 3rd gen antibiotics followed by 2nd gen antibiotics. The probable reason behind this could be bacterial modification and resistance development. Ocular surfaces are sensitive thus lower concentrations of antibiotics are recommended which can also be a reason for bacterial growth.

5. CONCLUSION

This study would spread awareness about the possible methods that can potentially reduce chances of external ocular infections. Our study on the effect of the commercially developed eye drops on the bacterial isolates determines the prevalence of the resistant strains and identifies the possible drugs to overcome the bacterial resistance. Thus this study could potentially trigger the development of the better concept of the pathogenic strains and help in providing prompt medicine selection and reduce the span of application and relief.

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