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Original Article

Frequency and Antibiotic Susceptibility of Bacteria Isolated in Patients with Blepharitis

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ABSTRACT

Objective: To determine the frequency and antibiotic susceptibility of bacteria isolated in patients with blepharitis.
StudyDesign: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan, from Oct 2019 to Oct 2021.
Methdology: 160 cases of blepharitis, during a period of 2 years, who were not using topical or systemic antibiotics were recruited in this cross-sectional study. After detailed history and slit-lamp examination of eyelids and tears film each patient underwent sample collection under aseptic conditions, from the eyelid margin. The samples were inoculated in a culture of blood agar and chocolate agar (Biomeriux) , Then the susceptibility of the identified bacteria to common systemic and topical antibiotics were tested using the disc diffusion method (Rosco Neo-Sensitab).
Results: In 160 patients,118(73.7%) were males and 42(26.3%) were females. According to type of blepharitis, 98(61.3%) had posterior blepharitis and 62(38.7%) had anterior blepharitis.Culture of eyelid sample of 77(48.1%) patients showed growth of *coagulase-negative staphylococcus Coagulase-negative staphylococcus* (CoNS) show greater resistance towards Penicillin 77(100%), Ciprofloxacin 55(72.5%) and Erythromycin 33(42.8%) however more susceptibility was shown towards Linezolid (CoNS). 71(45%) had *Staphylococcus aureus* isolated from their culture. 77(100%),Vancomycin 77(100%), Gentamycin 66(85.8%), Chloramphenicol 56(74.5%) and Cotrimoxazole 55(72.5%).Staphylococcus aureus show greater resistance towards Penicillin 72(100%),Ciprofloxacin 41(56.9%) and Tetracyclins 30(41.6%) however more susceptibility was found towards Gentamycin 72(100%), Linezolid 72(100%),Vancomycin 72(100%) and Chloramphenicol 61(84.7%).
Conclusion: *Coagulase-negative staphylococcus* (CoNS) and *Staphylococcus aureus* have been the main isolated organisms in blepharitis. These bacteria have been shown to be resistant to Penicillin, Ciprofloxacain Erythromycin and Tetracyclins However both are highly Sensitive to Vancomycin and Linezolid along with Significant Sensitivity towards Chloamphenicol, Gentamycin, Amoxicillin-Clavulanate and Cotrimoxazole.

Keywords: Antibiotics, Blepharitis, Resistance.

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INTRODUCTION

Blepharitis is inflammation of eyelid margins; It is a common ocular disorder encountered in clinical practice by gender, age, or ethnicity.[1] Blepharitis is classified as anterior or posterior according to anatomical location, although the two types overlap and are often present together.[2] In anterior blepharitis outer edge of the eyelid margin that is eyelid skin and eyelash follicles are affected due to Staphylococcal Infection or seborrhoea. In posterior Blepharitis inner edge of the eye lid is affected due to meibomian gland dysfunction and alternations in meibomian gland secretions.[3] Clinically, blepharitis is chronic in nature with remissions and exacerbations. Mostly bilateral and symmetrical. Symptoms are caused by tear film instability and disturbance in normal ocular function. Itching, burning, redness, photophobia, and grittiness are common and usually worse in early morning.[4] In Staphylococcal blepharitis hard scales and crustings are present around the bases of eye lashes and it is often associated with dry eye syndrome. Hyperemia, greasy anterior lid margin, soft scales and overlapping of eyelashes are signs of seborrheic blepharitis.[5] In posterior blepharitis there is hyperemia and telengectasia of posterior lid margin with pouting, recession or plugging of meibomian gland orifices.[6] The tear film is usually oily, foamy and often unstable. Complications due to anterior blepharitis include scarring and notching of the lid margin, madarosis, trichiasis, stye, and marginal Keratitis. Posterior blepharitis is associated with Inferior corneal punctate erosions, corneal scarring, and vascularization.[7]

Pathogens responsible for blepharitis include chronic bacterial infection (especially *Staphylococci*) and inflammation caused by bacterial components.[8] Further more demodex, acne rosacea, air pollution , atopic

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dermatitis, seborrheic dermatitis, and graft versus host disease have also been associated with blepharitis.[9] At present, standard treatment focuses on controlling symptoms and inflammatory signs. Treatment options include lid hygiene, lubricants, topical and systemic antibiotics, warm compresses, and topical steroids. New therapies includes pulsed light application, topical cyclosporine and designed devices to probe and express meibomian glands. The management of blepharitis with antibiotics is mainly empirical; therefore, It can lead to resistance to commonly used antibiotics.[10] Antibiotics are being rendered ineffective by the rapid emergence of resistant bacteria. This cross-sectional study aims to find the bacteria on the eyelids in blepharitis and evaluate its antibiotic susceptibility and resistance pattern in order to avoid misuse of antibiotics, prevent treatment failure and develop effective antibiotic regimes to improve treatment.

METHADOLOGY

The cases affected with blepharitis during a period of 2 years (October 2019 to October 2021) were recruited in this cross-sectional study after taking approval from the Institutional Review Board (IRB), Armed Forces Institute of Ophthalmology (AFIO Rawalpindi dated 29 August 2019) and approved from College of Physician and Surgeon (CPSP Dated 01 October 2019). A sample size was calculated with the help of the WHO sample size calculator software.The sample size calculation was based on 89% prevalence of chronic blepharitis, 80% study power, 95% level of confidence and 10% precision. The minimum required sample size was calculated to be 160.[11]

Inclusion Criteria: Patients aged 8-75 years, of either gender, affected with blepharitis, having no history of antibiotic use in the past six weeks or any ocular intervention. Patients willing to participate and follow up were included in our study.

Exclusion criteria: Patients with evidence of demodex infestation, ectropion, entropion, Using systemic and topical antibiotics with in last 6 weeks, Inability to follow up for the duration of the study and repeated samples from same patient were excluded in our study.

All patients gave written consent for the collection of their specimens by consecutive random sampling technique, which was approved by the Institutional Review Board(266/ERC/AFIO) and the confidentiality of their data was maintained at all times. A detailed history was taken from each patient, followed by a slit-lamp examination (Haag Streit BM900 LED) of eyelids and tear film. The patients who underwent sample collection were not receiving topical or systemic antibiotics since last six weeks. To collect the specimens under aseptic conditions, swabs soaked in sterile physiologic solution were rubbed against the base of eyelashes and inserted into agar transport media (Amies Eurotubo Deltalab S.L.). These samples were shifted to Microbiology department ,Army Medical College at PEMH.The samples were inoculated on the cultures of blood agar and chocolate agar (Biomeriux) followed by incubation at 37°C for 48 hours. Based on the disc diffusion method (Rosco Neo-Sensitab), the identified bacteria were tested for their susceptibility to common systemic and topical antibiotics like *Fusidic acid, Penicillin Clotrimazole, Cloxacillin, Minocycline, Gentamycin, Linezolid, Tetracycline, Chloramphenicol, Erythromycin, Vancomycin, Ciprofloxacin and Amoxicillin-clavulanate*. Descriptive statistics data was analyzed by using Statistical Package for the social sciences (SPSS) version 22.00 software. Mean±SD was calculated for continuous variable. Frequency and percentage was calculated for categorical variables. Paired T-test was used. The *p*-value ≤0.05 was considered significant.

RESULTS

Out of the total 160 participants in our study 118(73.7%) were males and 42(26.3%) were females. Patients were grouped into three age groups, with 87(54.4%) over 45 years of age, 42(26.3%) between 19 and 45 years of age, and only 31(19.4%) from 5-18 years. According to type of blepharitis,62(38.7%) had anterior blepharitis while 98(61.3%) had posterior blepharitis. 77(48.1%) patients showed growth of *coagulase negative staphylococcus* (CoNS). 71(45%) had *Staphylococcus aureus* isolated from their culture. While only 11(6.9%) patient showed growth *of Streptococcus pneumoniae*. *Staphylococcus aureus* was more common isolate among the age group of 5-18 years However in patients of age group of 19-45 years(22 out of 42) and above 45 years (55 out of 87) *coagulase-negative staphylococcus* (CoNS) was the major cause of blepharitis. Anterior blepharitis was more common among patients below 18 years of age as all the patients among the age group of 5-18 years presented with anterior blepharitis 31(100%). In age group of 19-45 years, equal number of patients presented with anterior 10(50%) and posterior belpharitis 10(50%) and among the patients of age >45 years, posterior blepharitis was far more common, where 99(90.8%) patients showed posterior blepharitis. Coagulase-negative staphylococcus (CoNS) show greater resistance towards Penicillin 77(100%), Ciprofloxacin 55(72.5%) and Erythromycin 33(42.8%) however more susceptibility was shown towards Linezolid

77(100%), Vancomycin 77(100%), Gentamycin 66 (85.8%) Chloramphenicol 56(74.5%) and Cotrimoxazole 55 (72.5%). *Staphylococcus aureus* show greater resistance towards Penicillin 72(100%), Ciprofloxacin 41(56.9%) and Tetracyclins 30(41.6%) however more susceptibility was found towards Gentamycin 72(100%), Linezolid 72(100%), Vancomycin 72(100%), Chloramphenicol 61(84.7%) and Amoxicilin Clavulanate 52(72.3%).

Streptococcus pneumoniae showed resistance only towards erythromycin .Details of antibiotic resistance and susceptibility is given in Table below according to type of bacteria isolated from cultures of 160 patients included in the study.

Table: Pattern of Antibiotic resistance and susceptibility of Bacterial Culture Isolates in Blepharitis (n=160)

Antibiotics	Staphylococcus Aureus (72)		Coagulase-Negative Staphylococcus (77)		Streptococcus Pneumoniae (11)		Not Done
	Resistance	Sensitivity	Resistance	Sensitivity	Resistance	Sensitiviy	-
Chloramphenicol	11(15.2%)	61(84.7%)	22(28.5%)	55(71.5%)	-	11(100%)	-
Ciprofloxacin	41(56.9%)	31(43.1%)	55(72.5%)	22(28.5%)	-	11(100%)	-
Cloxacilin	21(29.1%)	51(70.9%)	33(42.8%)	44(57.2%)	-	-	11(100%)
Cotrimoxazole	32(44.4%)	40(55.6%)	22(28.5%)	55(72.5%)	-	-	11(100%)
Erythromycin	20(27.7%)	52(72.3%)	33(42.8%)	44(67.8%)	11(100%)	-	-
Fusidic Acid	30(41.6%)	42(58.4%)	44(57.1%)	33(42.9%)	-	11(100%)	_
Gentamicin	-	72(100%)	11(14.2%)	66(85.8%)	-	11(100%)	-
Linezolid	-	72(100%)	-	77(100%)	-	-	11(100%)
Minocycline	-	72(100%)	-	77(100%)	-	11(100%)	-
Penicillin	72(100%)	-	77(100%)	-	-	11(100%)	-
Tetracycline	30(41.6%)	42(58.4%)	22(28.5%)	55(71.5%)	-	11(100%)	-
Vancomycin	-	72(100%)	-	77(100%)	-	-	11(100%)
Amoxicilin-Clavulanate	20(27.7%)	52(72.3%)	33(42.8%)	44(57.2%)	-	11(100%)	-

DISCUSSION

Blepharitis is one of the common diseases that an ophthalmologist need to manage, being present in 37-47% of patients visiting eye clinics. According to studies staphylococcal blepharitis being the most common has a prevalence of 37–42%.[12] As it is uncommon for an ophthalmologist to do a bacterial culture for treatment of blepharitis, very few studies have been published regarding bacterial isolates.[13] Most common bacteria isolated from ocular infections are CoNS and *Staphylococcus aureus*.[14] In a study carried out by Belyhun *et al*,[15] blepharitis patients were diagnosed with these bacteria in 29% of cases. Similar results were reported by Teweldemedhin *et al*.[16] in his research study.[16] In our study, CoNs was isolated in 48.1% of the cultures and *Staphylococcus aureus* was present in 45% of cultures. Comparing our results about antibiotic resistance and susceptibility of bacteria isolated from blepharitis cases with a study done in the late 1990s by Pinna *et al*.[17] there appears to have been an increase in resistance to penicillin from 67% in that study to 100% in our study, and ciprofloxacin 7-60% in the current study. This can be attributed to the wrong practice of prescribing antibiotics without taking sample for culture and sensitivity. The reason for increased resistance to penicillin may be prior exposure of the isolates to these antibiotics. According to literature, among the drugs that are effective against CoNS and *Staphylococcus aureus* Vancomycin is by far the most popular, it was reflected in our study as well where 100% of the growth of both these organisms showed susceptibility to Vancomycin.[19] The most commonly used antibiotics for treating eye infections like blepharitis are Tetracyclines, Chloramphenicol and Cotrimoxazole to name a few.[20] 55.6% and 72.5% susceptibility was shown towards Cotrimoxazole by *Staphylococcus aureus* and CoNS while susceptibility towards chloramphenicol was 84.7% for *Staphylococcus aureus* and 71.5% for CoNS. These results were similar to the study carried out by Miler *et al*.[21] Treatment of blepharitis with antibiotics have become ineffective as a result of rising resistance to antimicrobials as was evident in our study results. In order to reduce resistance to antimicrobial agents close monitoring of resistance and sensitivity patterns can guide the clinicians to choose the antibiotic treatment.[22] Given the high percentage of eye infections caused by *Staphylococcus aureus* and CoNS and the increasing resistance to various antibacterial agents, our study

aimed to evaluate the antibiotic resistance and sensitivity patterns of these organisms in blepharitis in order to avoid misuse of antibiotics, prevent treatment failure and develop effective antibiotic regimes to improve treatment.

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LIMITATION OF STUDY

Antibiotic susceptibility and resistance of *Streptococcus pneumonia* is unclear in this study. Single Center Study.

CONCLUSION

Coagulase-negative staphylococcus *CoNS* and *Staphylococcus aureus* have been the main isolated organisms in blepharitis. These bacteria have been shown to be resistant to Penicillin, Ciprofloxacain Erythromycin and Tetracyclins However both are highly Sensitive to Vancomycin and Linezolid along with Significant Sensitivity towards Chloamphenicol, Gentamycin, Amoxicillin-Clavulanate and Cotrimoxazole.

Conflict of Interest: None

Author's Contribtiion

Following authors have made substantial contributions to the manuscript as under:

IM & MS: Supervision, Conception, Study design, analysis and Interperitation of data, Critically reviewed manuscript & approval for the final version to be published.

FH & MM: Co-supervision, Data entry, analysis and interpretation, manuscript writing & approval for the final version to be published.

MA & MA: Critically reviewed, Drafted manuscript & approval for the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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