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Original Article

Trends of Bacterial Pathogens and their Antimicrobial Susceptibility in Ocular Cultures

Mehwish Sajjad[1], Maryam Shahid[2], Zainullah[3], Bashir Ahmad[4], Hussain Ahmad[5] and Rimal Rashid[6]

[1]Department of Pathology, Dow University of Health Sciences,

[2, 3,4,5,6]Dow University Hospital (Ojha Campus)

ABSTRACT

Background: Eye infections caused by bacteria are a serious public health problem. These diseases, if not properly treated, can cause blindness and impaired vision. The study is designed to evaluate the bacterial etiologies and antimicrobial resistance profiles of the main pathogens involved in eye infections.

Objectives: To isolate different pathogens from ocular culture and evaluate the antibiotic susceptibility pattern of these organisms

Materials and Method: This was a retrospective study in which we retrieved the data of ocular cultures in Dow Diagnostic Reference and Research Laboratory, Dow University of Health Sciences from the period of 1stJanuary 2019 to 31stDecember 2021. All details including bacterial etiologies and antimicrobial susceptibility pattern were noted from the retrospective data.

Results: During the study period, 44 patients were recruited who were requested by a physician for ocular cultures from the period of 1stJanuary 2019 to 31stDecember 2021. Most common bacterial isolate found is Streptococcus pneumonia followed by Staphylococcus aureus and Pseudomonas aeruginosa. In this study all bacterial isolate exhibited 100% susceptibility against chloramphenicol except Streptococcus pneumonia which is 94% susceptible. Streptococcus pneumonia had 100% resistance against Trimethoprim- sulfamethoxazole whereas 100% susceptibility against clarithromycin, linezolid and levofloxacin.

Conclusion: Inference of study is that Coagulase Negative Staphylococcus was resistant to penicillin. This has a significant effect on the options of empirical management of ocular infections.

Key words: Ocular infection, antibiotic resistance, streptococcus pneumonia, staphylococcus aureus.

Introduction

Ocular infections and its complications are among the major causes of blindness. Eye is exposed to an extensive range of pathogens but their natural defense mechanisms like layer of artificial tears, microbial flora protect them from various infections and resist colonization and infection of eye.[1] Certain conditions like improper and prolonged use of contact lens, trauma, surgery, prior eye infection, dry eyes, obstruction of lacrimal duct are the predisposing factors for the ocular infection.[2]

Eye is vulnerable to bacterial, viral, fungal and many parasitic infections.[3] Ocular infections presented as keratitis, conjunctivitis, blephritis and canilicultis.[4]

CORRESPONDENCE AUTHOR

Mehwish Sajjad

Department of Pathology,

Dow University of Health Sciences

Email: mehwish.sajjad@duhs.edu.pk

Contact No: 03332381212

Bacterial infections have high prevalence in ocular infections. Staphylococcus aureus, Coagulase negative staphylococcus Streptococcus pneumonia, Pseudomonas aeruginosa and Hemophilus influenza are the most common cause of bacterial infections.[5] Diagnosis of bacterial ocular infections depends mainly on the clinical symptoms and laboratory assessments including culture analysis and antibiotic susceptibility tests.[2] Empirical treatments with broad spectrum antibiotics for ocular infections were used that results in the emergence of resistance for various ocular pathogens like MRSA.[6] Other factors that also contribute to antibiotic resistance are the parallel use of antibiotics for systemic infections, prophylactic use of antibiotics or repeated exposure to same drug.[7]

Infection with resistant organisms can complicate antibiotic selection, increasing the risk of treatment failure with potentially sight-threatening consequences. Effective management of such infections demands knowledge of the specific etiology. An understanding of resistance patterns among ocular pathogens can help clinicians to select appropriate treatment strategies, improve pre- and postoperative

managements, and positively impact patient outcomes. There is a need of studies that demonstrate the local pattern of antibiotic susceptibility and causative agents of ocular infections. Hence, the study is designed to identify the bacteriological profile and antibiotic resistance pattern in ocular cultures.

Materials and Methods

This is retrospective study will be done in Dow Diagnostic Reference and Research Laboratory, Dow University of Health Sciences after Institutional review board approval. All records of ocular culture and antibiotic susceptibility results will be analyzed from the period of 1st January 2019 to 31st December 2021. Culture and antibiotic susceptibility reports of patient will be retrieved from record files of department. Exact nature of bacterial etiologies and antibiotic susceptibility pattern will be analyzed from the records. Data will be analyzed on SPSS to determine the different causative agents and their resistance pattern. All cases of ocular cultures will be evaluated and included in our study whether it is conjunctival, corneal, aqueous and vitreous fluid. Cultures other than ocular were excluded from our study. All cases with complete demographic details and reports of ocular cultures will be evaluated. Incomplete reports are excluded.

The conjunctival and corneal surfaces should be thoroughly rinsed with sterile, non-bacteriostatic saline or water to remove the anesthetic agents prior to specimen collection because these chemicals may be inhibitory to microorganisms. Because the eyelid and conjunctival surface are normally colonized by a wide variety of bacteria, physicians should be encouraged to collect samples from both the affected and unaffected eyes in order to compare microbial growth to better determine the primary pathogen causing superficial eye infection. We obtain the specimen with a sterile, premoistened cotton or calcium alginate swab which rolls the cotton swab over the conjunctiva and culture of both eyes with separate swabs was taken.

Inoculate sample first on chocolate agar plate than Sheep blood agar (SBA) agar and MacConkey agar plates. Then inoculate in Brain Heart Infusion (BHI) broth for sub culture. Incubate the Chocolate and SBA agar plates in CO2 incubator at 350C and MacConkey agar and BHI in ambient air Incubator 370C. Examine culture plates for growth after 24 hours and 48 hours of incubation. If culture is positive for growth record the type of colony. Identify the isolates and perform the susceptibility test on each isolate, see identification and sensitivities section. Interpret results of antimicrobial susceptibility test and record result. All media, reagents and antibiotics Quality Control was checked and recorded. Quality Control can be checked by the following strains, ATCC Staphylococcus aures 25923, ATCC Escherichia Coli 25922 and ATCC Pseudomonas aeruginosa 27853. Mueller-Hinton agar was used for routine susceptibility testing of non fastdious bacteria by disk diffusion method. To standardize the inoculum density for a susceptibility test, equivalent to a 0.5 McFarland standard was used. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two or more times, rotating the plate approximately 60-degree angles each time to ensure an even distribution of inoculum. Disks were placed and distributed evenly almost 20-24 mm apart from each other so that they were no closer be placed, 6 disks on a 100-mm plate. For catalase positive isolates Penicillin (P) 10 Units, Erythromycin (E) 15 µg, Clindamycin (DA) 2 µg, Co-trimoxazole (SXT) 1.25/23.75 µg, Linezolid (LZD) 30 µg, Gentamicin (CN) 10 µg, Ciprofloxacin (CIP) 5 µg, Chloramphenicol (C) 30 µg and Neomycin (N) 10 µg were tested. For gram positive catalase negative Ampicillin (AMP) 10 µg, Vancomycin (VA) 30 µg, Linezolid (LZD) 30 µg, Erythromycin (E) 15 µg,Clindamycin (DA) 2 µg, Chloramphenicol (C) 30 µg, Oxacillin (OX) 1 µg, Co-trimoxazole (SXT) 1.25/23.75 µg, Levofloxacin (LEV) 5 µg, Optochin (OP) 5 µg and Bacitracin (BC) 0.04 Units were tested. For gram negative rods, Ampicillin (AMP) 10 µg, Gentamicin (CN) 10 µg, Tobramycin(TOB) 10ug, Cefuroxime(CXM) 30ug, Ceftazidime (CAZ) 30 µg, Co-amoxiclav (AMC) 20/10 µg, Pip-tazo (TZP) 100/10 µg, Ceftriaxone (CRO) 30 µg, Meropenem (MEM) 10 µg,Amikacin (AK) 30 µg, Ciprofloxacin (CIP) 5 µg, Co-trimoxazole (SXT) 1.25/23.75 µg and Cefixime (CFM) 5 µg were tested. Diameters of the inhibitory zones were computed through vernier caliper or ruler and noted. The susceptibility breakpoints were interpreted according to CLSI guidelines 2020.[8]

Results

During the study period, 44 patients were recruited who were requested for ocular cultures from the period of 1st January 2019 to 31st December 2021.

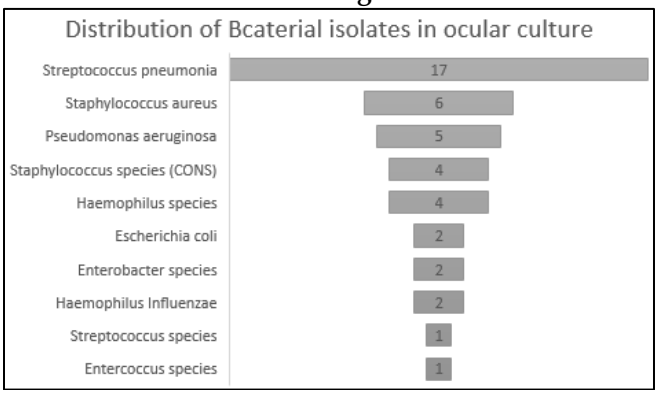
Majority of patients were male about 59% as compare to females which were about 41%. Most of the patients (67%) were between the age group of 1 month to 10 years followed by 11 to 20 years about (7%) as shown in Table I.

Table 1: Gender and Age Distribution of study population

Gender	Number of patients	Percentage
Male	26	59%
Female	18	41%
Age	Number of patients	Percentage
1m-10yr	30	67%
11-20yr	3	7%
21-30yr	0	0%
31-40yr	1	2%
41-50yr	0	0%
51-60yr	2	5%
61-70yr	4	9%
71-80yr	2	5%
81-90yr	2	5%

Mixed bacterial isolates were not found in any of the sample. Most common bacterial isolate found is Streptococcus pneumonia followed by Staphylococcus aureus and Pseudomonas aeruginosa. Other bacteria isolated from ocular culture are given in fig 1.

Figure 1: Distribution of bacterial isolates in ocular culture. X-axis shows organisms where as Y-axis show number of isolates. Length of grey bar shows numbers of organisms.



Antimicrobial susceptibility pattern of bacterial isolates was evaluated on the panel of antibiotics. Substantial numbers of bacteria isolated from culture were resistant to more than one antibiotic. Antibiotic susceptibility patterns of bacteria are shown in Table-2.

Table 2: Antibiotic susceptibility pattern of isolated bacteria in ocular culture.

Antibiotics	Streptococcus pneumonia N=17	Staphylococcus aures N=6	Pseudomonas aerugi nosa	Staphylococcus species (CONS) N=4	Hemophilus species N=4
AK	NT	NT	100%	NT	NT
AMC	88%	50%	NT	75%	100%
AMP	88%	NT	NT	NT	100%
CRO	87%	NT	NT	NT	75%
CFM	NT	NT	NT	NT	100%
C	94%	100%	NT	100%	100%
CLARI THRO	100%	NT	NT	NT	NT
DA	81%	60%	NT	100%	NT
OX	NT	50%	NT	75%	NT
SXT	0%	75%	NT	67%	67%
E	75%	40%	NT	50%	NT
LEV	100%	NT	NT	NT	NT
LZD	100%	NT	NT	NT	NT
TE	81%	50%	NT	67%	NT
VA	82%	50%	NT	100%	NT
OX	NT	50%	NT	100%	NT
FA	NT	100%	NT	NT	NT
CN	NT	50%	100%	100%	NT
NEOM YCIN	NT	40%	NT	100%	NT
PENE CILLI N	NT	NT	NT	0%	NT
CAZ	NT	NT	100%	NT	NT
CIP	NT	NT	100%	NT	NT
COLIS TIN	NT	NT	100%	NT	NT
PB	NT	NT	100%	NT	NT
MEM	NT	NT	100%	NT	100%
TZP	NT	NT	100%	NT	NT
TOB	NT	NT	100%	NT	NT

Sensitivity is shown in percentages (%)NT stands for not tested. Antibiotics AK (Amikacin), AMC (Amoxicillin -clavulanate), AMP (Ampicillin), CRO (Ceftriaxone), CFM (Cefotaxime), C (Chloramphenicol), CLARITHRO (Clarithromycin) , DA (Clindamycin), OX (oxacillin), SXT (Co-trimoxazole), E (Erythromycin), LEV (Levofloxacin), LZD (Linezolid), TE (Tetracycline), VA (Vancomycin), OX (Oxacillin), FA (Fusidic Acid), CN (Gentamicin), CAZ (Ceftazidime), CIP (Ciprofloxacin), PB (Polymyxin B), MEM (Meropenem), TOB (Tobramycin), TZP (Pipra-tazobactam)

In this study all bacterial isolate exhibited 100% susceptibility against chloramphenicol except Streptococcus pneumonia which was 94% susceptible.

Streptococcus pneumonia had 100% resistance against Trimethoprim- sulfamethoxazole whereas 100% susceptibility against clarithromycin, linezolid and levofloxacin. Staphylococcus aureus had 50% resistance against Gentamicin, tetracycline and vancomycin, 60% against neomycin and erythromycin, 40% to clindamycin and 25% to Trimethoprim-sulfamethoxazole. Pseudomonas aeruginosa is the most susceptible and least resistant isolate showed 100 percent sensitivity against ceftazidime, amikacin, ciprofloxacin, colistin, gentamicin, meropenem, tazobactam piperacillin and tobramycin.

Discussion

Ocular infections affected people of all genders and ages. Successful management of Ocular infection is dependent on appropriate and precise diagnosis of along the proper administration of antibiotic after antibiotic susceptibility report. In our study patients from male gender has high rate of ocular infection as compare to female which is also reported by Fahimeh Asadi-Amoli et al.[9] Gender variations might be due to life style and social factors. High incidence of ocular infection was seen in newborn, infants, toddlers and children of 10 years which is in consistent with the study which reported high rates of ocular infection in the age group of 70years.[9, 10]

In our study gram positive were the most isolated bacteria in which Streptococcus pneumonia, the most common organism isolated from ocular culture followed by Staphylococcus aureus and Staphylococcus species (CONS) which was also reported by Khurana et al and Anand et al[1,2,11,13] but in consistent with Panos et al.[12] In our analysis, S. aureus showed 50% resistance against Gentamicin, tetracycline and vancomycin, 60% against neomycin and erythromycin, 40% to clindamycin and 25% to Trimethoprim- sulfamethoxazole whereas Manente R et al showed that S.aureus has 84.2% resistance against penicillin G, and resistance to azithromycin, clarithromycin, clindamycin and erythromycin exceeding 40%. Additionally, significant resistance to oxacillin was found which was also showed by Manente R et al.[10]Oxacillin resistance was related with major progression of the illness and terrible consequence because of the limited options of available antibiotics for the management of these infections .[10]

Majority of bacteria in our study especially staphylococcus species (CONS) were resistant to penicillin which is also reported by other studies.[13,14,15]This resistance might be due to the previous acquaintance of these antibiotics due to low cost and easy accessibility. All of streptococci pnemonea is mostly susceptible to ampicillin, amoxiclav, ceftriaxzone, vancomycin, tetracycline, linezolid, levofloxacin, erthromycin, Trimethoprim-sulfamethoxazole, clindamycin, clarithromycin, which is in line with the study of Mohammad et al but contrary to the Haile Z et al.[2, 15Low] numbers gram negative isolates were reported. In our study Pseudomonas aeruginosa, Haemophilus species and E. coli were isolated. Mohammed AA et al found that E.coli is more prevalent in his study.[13] In our study Pseudomonas aeruginosa was 100 % sensitive to ceftazidime, amikacin, ciprofloxacin, colistin, gentamicin, meropenem, tazobactam piperacillin and tobramycin which is also similar to previous studies.[13,16]

Conclusion

Inference of study is that CONS was resistant to penicillin. This has a significant effect on the options of empirical management of ocular infections

Limitations

Our study has following limitation like it is a single center study, retrospective study design; the exact collection sites were not recorded. Despite of having this limitation our study provides a glance to the problem.

Recommendation

Further studies are required to raise the acquaintance of the pathogen of ocular infections and antimicrobial susceptibility for enhancing the therapeutic approach for the treatment of methicillin resistant staphylococcus and other resistant bacteria that causes ocular infections.

Conflict of Interest: Authors declare no conflict of interest.

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

CONTRIBUTION OF AUTHORS	
AUTHOR	**CONTRIBUTION**
Mehwish Sajjad	A,B,C
Maryam Shahid	A
Zainullah	B,C
Bashir Ahmad	B,C
Hussain Ahmad	B
Rimal Rashid	C