Original article

BACTERIOLOGIC FINDINGS IN PATIENTS WITH OCULAR INFECTION AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF ISOLATED PATHOGENS

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ABSTRACT

Objective: Isolation of common bacterial pathogens involved in ocular infection and their *in vitro* susceptibility to commonly used ocular antibiotics and the trends in antibiotic resistance developed by these pathogens.

Patients & Methods: Corneal scrapings were obtained from 318 hospitalized patients through Central laboratory, Imam Khomeini Hospital, Ahwaz, Iran from October 2005 to March 2006.They were inoculated directly onto blood agar and thioglycollate broth and were incubated. The isolates were identified as per standard procedures. Antibiotic susceptibility was determined for all positive cultures and susceptibility of isolated pathogens to commonly used ocular antibiotics was examined.

Results: Of the isolats (n=70) Gram positive cocci accounted for 47 (67.2%) of all bacterial isolates gram negative bacilli for 23 (32.8%). Coagulase negative staphylococci (33%) and Pseudomonas sp. (24%) were most common isolated. In susceptibility testing, Gentamicin had coverage against 35 (74.5%) of 47 gram positive cocci and 19 (82.6%) of 23 gram negative isolates. The coverage of Tetracycline, Cephalotin and Ceftriaxon against the gram positive cocci isolates were 61.7%, 55% and 53% respectively. All the gram positive cocci isolats showed resistance to Cefotaxime and Penicillin. Ceftriaxon and Tobramycin had coverage against 17 (73.9%) and 14 (60.8%) of 23 gram negative isolates respectively. The coverage of Vancomycin against coagulase negative staphylococci was 100%. However all the isolates of *S. aureus* were resistant to Vancomycin.

Conclusions: Susceptibility analysis revealed that the most effective antibiotics were Gentamicin (77.1% of 54 isolates) and Ceftriaxon (42% of 42 isolates). Both antibiotics had good coverage against gram-positive cocci which constitute the majority (67.1%) of ocular isolates in this study.

KEY WORDS: Corneal pathogens, Antibiotic, Susceptibility test.

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INTRODUCTION

The eye is a unique organ that is virtually impermeable to most environmental agents. Continuous tear flow, aided by the blink reflex, mechanically washes substances from the ocular surface and prevents the accumulation of microorganisms. In addition, lysozyme, lactoferrin, secretory immunoglobulins, and defensins present at high levels in tears can specifically reduce bacterial colonization of the ocular surface.[1,2] However in some circumstances infectious agents gain access to the posterior segment of the eye following one of three routes: (i) as a consequence of intraocular surgery,[3,4] (ii) following a penetrating injury of the globe,[5] or (iii) from hematogenous spread of bacteria to the eye from a distant anatomical site. Although uncommon, endophthalmitis can also result from keratitis, an infection of the cornea with potential complications.[6]

Bacterial keratitis is one of the most threatening ocular infections. *Pseudomonas aeruginosa* and *Staphylococcus aureus* frequently cause severe keratitis that may lead to progressive destruction of the corneal epithelium and stroma.[7,8] Successful treatment of ocular infection including bacterial keratitis requires multiple administrations of antibacterial agents to maintain drug concentration in the corneal tissue high enough and for a sufficient period of time to have a useful antibacterial effect.[9] Besides, in the case that the pathogen is not yet known, the choice of antimicrobial agents is commonly made empirically. In case of access to microbiology facilities, once the organism has been identified, the effective antimicrobial is chosen according to susceptibility testing. In this paper, the commonly isolated bacterial pathogens involved in ocular infection were studied. Furthermore the *in vitro* effectiveness of commonly used ocular antibiotics describing their coverage of bacterial species and the trends in antibiotic resistance developed by common ocular pathogens was investigated.

MATERIALS AND METHODS

The study population was 318 hospitalized patients who mostly underwent operation, in Imam Khomeini Hospital, Ahwaz, Iran between October 2005 and March 2006. The samples obtained from patients which consisted of 138 females and 180 males, age ranging from44-83 years with the mean of 63.6. For sampling, corneal scrapings were obtained in the operation room with a sterile blade using standard technique.[10] The scrapings were inoculated directly onto blood agar and thioglycollate broth and were immediately transferred to the microbiology laboratory of the hospital and were incubated at 37°C for 24 hours. Subculture on blood agar medium, MacConkey agar and Mannitol salt agar (Hi-Media, Mumbai, India) were performed. Blood agar plates were incubated at 5-10% CO2 atmosphere. The cultures were examined after 24 hours of incubation. The necessary biochemical tests were conducted and the organisms identified as per standard procedures.[11] Antibiotic susceptibility was determined for all positive cultures using the Kirby-Bauer disc-diffusion method.[12] Antibiotic discs were purchased from Padtan Teb, Tehran, Iran and susceptibility of isolated pathogens to commonly used ocular antibiotics was examined. They were defined as sensitive (S) resistant (R) or intermediate resistant (I) by the diffusion method. SPSS software was used for data analysis.

RESULTS

Among isolates (n=70) from seventy patients gram positive cocci accounted for 47 (67.2%) and gram negative bacilli for 23 (32.8%) Tables-I. Coagulase negative Staphylococci (33%) and Pseudomonas sp. (24%) were the most common isolated organisms.

Based on results from susceptibility testing (Table-II), Gentamicin covered against 35 (74.5%) of the 47 gram positive cocci isolates and was the most effective antibiotic against

Table-I: Bacterial strains isolated from ocular infection

Organism(s)	*Total no. of isolated Recovered*	*% of isolates*
Coagulase negative Staphylococci	23	32.9
Pseudomonas sp.	17	24.2
Staphylococcus aureus	9	12.9
Streptococcus pneumoniae	6	8.6
Enteric gram negative bacilli	6	8.6
Alpha hemolytic negative bacilli	5	7.1
Micrococcus SP.	4	5.7
Beta hemolytic streptococci	1	1.4

Table-II: Susceptibility results of various bacteria isolated from ocular infection to commonly used ocular antibiotics

Organisms	*# isolated*	*No. sensitive and % sensitive*								
		GM	*AM*	*TE*	*SXT*	*CRO*	*CF*	*CTX*	*TOB*	*K*
Staphylococci	23	16 (69)	4 (17)	14 (61)	11 (48)	12 (52)	12 (52)	---	---	---
Pseudomonas	17	13 (76)	2 (12)	1 (6)	4 (23)	11 (65)	4 (23)	9 (53)	14 (82)	4 (23)
S. aureus	9	9 (100)	1 (11)	9 (100)	8 (89)	8 (89)	5 (55)	---	---	---
S. pneumoniae	6	3 (50)	1 (17)	3 (50)	0	3 (50)	4 (67)	---	---	---
Alpha hemolytic-Streptococci	5	3 (60)	0	2 (40)	0	2 (40)	3 (60)	---	---	---
Micrococcus	3	3 (100)	2 (67)	2 (67)	2 (67)	3 (100)	0	---	---	---
Proteus	3	3 (100)	---	2 (67)	2 (67)	3 (100)	0	---	---	---

GM: Gentamicin, AM: Ampicillin, TE: Tetracycline, SXT: Co-trimoxazole,
CRO: Ceftriaxone, CF: Cephalotin, CTX: Cefotaxime, TOB: Tobramycin, K: Kanamycin.

Staphylococcus aureus. The coverage of Gentamicin against gram negative bacilli was 19 (82.6%) of the total 23 tested isolates. The coverage of this antibiotic for Pseudomonas sp. was 13 of 17 isolates (76.4%).

The coverage of Tetracycline, Cephalotin and Ceftriaxon against gram positive cocci tested isolates were 61.7%, 55% and 53% respectively. All the isolates of gram positive cocci showed resistance to Cloxacillin and Penicillin. Ceftriaxon and Tobramycin had coverage against 17 (73.9%) and 14 (60.8%) of 23 gram negative bacilli tested isolates respectively. Tobramycin was the most effective antibiotic against Pseudomonas sp. in the study and showed good coverage against other isolated enteric, i.e. Klebsiella, *E. coli*, Proteus and Entrobacter as well. Amikacin had excellent coverage against *S. aureus* and coagulase negative Staphylococci. The coverage of Vancomycin against coagulase negative Staphylococci was 100%. However all the isolates of *S. aureus* were resistant to Vancomycin.

DISCUSSION

The organisms which caused ocular infections are generally exogenous and result from entry of organisms into eye during surgery. Sources of contamination include instruments, and infusion fluids. In addition, the conjunctival sac itself normally harbors several commensals. Contamination of the multidose topical preparations used preoperatively could also be a source.[13] In present study 70 out of 318 eye samples were culture positive, so the contamination rate was estimated as 22%. In similar studies the contamination rates of 25.5%, 43% and 37.7% were reported.[3,13,14] Similar to these studies, our study has also documented coagulase negative Staphylococcus as the most common isolated organism.

Based on the results from susceptibility analysis of the ocular pathogens isolated, the antibiotics with great coverage were Gentamicin (77.1% of 54 isolates) and Ceftriaxon (42% of 42 isolates). Both antibiotics had good coverage against gram-positive cocci which constituted the majority (67.1%) of ocular isolates in this study. Besides, the efficacy of these antibiotics against gram negative organisms was also good.

Despite that some of the antibiotics used in present study showed good coverage against pathogens isolated, for instance Amikacin against *S. aureus* and Tobramycin against Pseudomonas, however some of the other antibiotics showed no effectiveness against tested organisms. The highest resistance was seen in gram negative and positive cocci to Penicillin and Cloxacillin (98% of 47 isolates), Oxacillin (91.5%) and Ampicillin (87.2%). Hence, we do not recommend treatment of bacterial keratitis and other ocular infections on an empirical basis, particularly in the centers that ophthalmologists have access to Microbiology facilities, which an attempt should be made to identify the ocular pathogen and perform susceptibility testing.

It should be dept in mind that these are *in vitro* results and do not always mirror the clinical response to antibiotics due to variety of reasons including direct topical delivery, corneal penetration of an antibiotic and host factors.[15] However, these results do provide information that allows a clinician to make rationale- based decision in choosing an initial regimen for treatment of ocular pathogens. Furthermore, the result of present study provides susceptibility information for ocular pathogens resistant to commonly used ocular antibiotics.

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