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Original Article

SCREENING AND ANTIBIOGRAM PATTERN OF BACTERIAL OPHTHALMIC INFECTIONS

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ABSTRACT

Objective: Microbial infections are causing life threatening diseases of which ocular infection is of primary importance, as it can even led to blindness. In order to understand the etiological agents causing bacterial ocular infection, this present study was aimed in screening and understanding the anti biogram pattern of bacterial ophthalmic infections.

Methods: Pus and corneal scrapings were collected from 50 ocular infected patients and were screened for bacterial pathogens for a three months period.

Results: Out of them 88% (44/50) were culture positive. The ocular pathogenic isolates include *Staphylococcus* spp (70.45%), *Streptococcus* spp (22.72%), *Bacillus* spp, *Enterobacter* spp and *Serratia* spp (2.27%). Sex and age wise perspective of ocular infections indicated that females and adults between 21-40 years were highly infected. Antibiotic susceptibility pattern of the isolates revealed that the modern antibiotics namely Moxifloxacin, Ofloxacin, Levofloxacin, Ceftazidime, Cefepime, Vancomycin, Rifampicin exhibited high sensitivity than the conventional antibiotics like methicillin, bacitracin, and oxytetracycline. Isolates exhibited multidrug resistance viz., *Staphylococcus aureus* (52.63%), *Staphylococcus epidermidis* (75%), *Streptococcus* spp (80%), *Enterobacter* spp (100%). Isolation of drug resistant plasmid DNA showed that multidrug resistant isolates harbor high molecular weight plasmid.

Conclusion: All the isolates exhibited the antibiotic resistance to conventional antibiotics, and were sensitive to the current antibiotics.

Keywords: Ophthalmic infection, Antibiotics, Multidrug resistance, Plasmid DNA.

INTRODUCTION

Ophthalmic infections can cause damage to structures of the eye, which can lead to vision loss and even blindness if it is untreated. In tropical countries, the second commonest blindness is caused by corneal infections after operated the cataract. The eye infections are caused by bacteria, virus, fungi and also parasites. The common eye infections caused by bacteria include conjunctivitis, microbial scleritis, canaciculitis, keratitis, cellulitis, endophthalmitis [1]. The bacterial conjunctivitis is the most common ocular infection seen by primary care physicians in world wide.

The epidemiological pattern of corneal infection is significantly varied from country to country even region to region. Globally, purulent bacterial conjunctivitis is mainly caused by gram-positive organisms. The most common causative agents are *Staphylococcus epidermidis* (39% of cases), *Staphylococcus aureus* (22% of cases), and *Streptococcus pneumonia* (6% of cases). The most common gram-negative microorganism found in acute conjunctivitis is *Haemophilus influenzae* (9% of cases). In contact lens wearers, the trend is reversed and more Gram-negative strains are found [2]. Compared to other international surveys, the Hyderabad and Chennai studies showed a reduced percentage of gram positive bacteria (42-47%) and increased percentage of gram negative bacteria (26-42%), fungi (17-22%) and polymicrobial infections (13-17%) [3]. In the 3 month period, 434 patients with central corneal ulceration were evaluated in Madurai. From that, 47.1% had pure bacterial infections, 46.8% had pure fungal infections, 5.1% had mixed bacteria and fungi and 1% was pure culture of *Acanthamoeba.* The most common bacterial pathogen isolate was *Streptococcus pneumoniae* (44.3%) followed by *Pseudommonas spp.* (14.4%) [4].

Several therapeutic classes of antibiotic agents are available for ophthalmic infections. The penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, macrolides, chloramphenicol, and sulfonamides are generally used to treat ocular infections [5]. Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a dreaded organism for its wide range of resistance to several groups of antibiotics. Its prevalence in conjunctivitis is highly variable. A study has shown an increase in MRSA in bacterial conjunctivitis from 4.4% (1994-1995) to 42.9% (2002-2003). There are some reports of Vancomycin resistant *S. aureus* (VRSA) in ocular infections [6].

In developing countries like India, the prevalence of ophthalmic infections is increasing every year. The trends of causative agents are also variable, but predominantly by *S. aureus.* Moreover, the trend in antibiogram pattern of these pathogens is also variable. Hence, there is a need for studying the rate of prevalence, monitoring pathogens and their antibiotic sensitivity. Considering these aspects the present study is focused on "Screening of ophthalmic infection causing bacteria and their antibiogram pattern".

MATERIALS AND METHODS

In the present study, 50 pus and corneal scrapping samples were collected from ocular infected patients in various hospitals located in Erode city, Tamil Nadu, India. Data related to patient's sex, age and socioeconomic status were collected in a pre designed proforma. Collected samples were transported to the laboratory within half an hour and processed without delay.

The specimens were cultured on Nutrient Agar and further subculture was made on Macconkey Agar, Blood Agar, Mannitol salt agar, Todd Hewitt agar etc. The isolated bacteria were identified by standard morphological, physiological and biochemical tests [7].

The antibiotic sensitivity of the isolated bacteria was screened by Bauer et al. [8] disc diffusion method using commercially available antibiotics such as Oxytetracycline, Chloramphenicol, Vancomycin, Amikacin, Ampicillin, Methicillin, Bacitrcin, Rifampicin, Oxacillin, quinalones such as Moxifloxacin, Levofloxacin, Ofloxacin, Ciprofloxacin, and Cephalosporin Derivatives such as Cefepime, Cefixime, Ceftazidime (Hi Media, India). The plasmid DNA was isolated from multi drug resistant bacteria and their molecular weight was determined by Agarose gel electrophoresis [9].

RESULTS

The experimental results showed that 80% samples were culture positive. The bacteria causing ocular infections were identified and their percentage is tabulated (Table 1). The results indicated that *S.*

aureus exhibited high rate of occurrence (43.18%). Followed by *S. epidermidis and Streptococcus* spp showed the prevalence rate of 27.27% & 22.72% respectively. Least occurrence (2.27%) was found with *Bacillus* spp, *Enterobacter* spp and *Serraia* spp.

The sex wise perspectives representation (fig. 1) emphasized that the females are commonly affected by ophthalmic infection than males almost in 2:1 ratio. The reason behind the increase exposure in females may be due to their poor immune power and the working habit in the study area where many textile and dye industries are located. The depiction of the age wise occurrences of the ocular infections is tabulated (Table 2). The result showed that the incidence of ocular infection is higher in adults between 20-40 years and the low rate was found in 1-20 years.

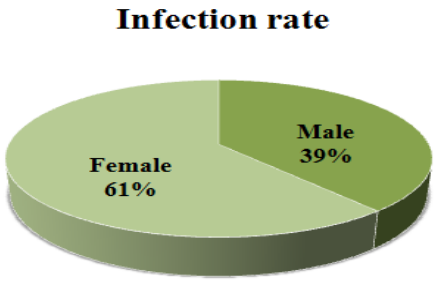

Fig. 1: Sex wise distribution of ocular infection among clinical patients (n=44)

Table 1: Prevalence of ocular bacterial pathogens

S. No.	Name of the isolate	Prevalence (%)
1.	*Staphylococcus aureus*	43.18
2.	*Staphylococcus epidermidis*	27.27
3.	*Streptococcus spp*	22.72
4.	*Bacillus spp.*	2.27
5.	*Enterobacter spp.*	2.27
6.	*Serratia spp.*	2.27

Table 2: Age wise distribution of ocular infection among clinical patients

S. No.	Age group (in years)	Frequency (N=44)
1.	1-10	5
2.	11-20	2
3.	21-40	15
4.	40-60	10
5.	60 and above	12

Table 3: Antibiogram resistogram patterns of bacterial isolates

Antibiotics	Isolates											
	S. aureus (N=19)		*S. epidermidis* (N=12)		*Streptococcus sp* (N=10)		*Bacillus* spp. (N=3)		*Enterobacter spp.* (N=3)		*Serratia* spp. (N=3)	
	S	R	S	R	S	R	S	R	S	R	S	R
Moxifloxacin (MO)	9 (47.37 %)	10 (52.63 %)	7 (58.33 %)	5 (41.67 %)	7 (70%)	3 (30%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)	3 (100%)	0 (0%)
Ofloxacin (OF)	13 (68.42 %)	6 (31.58 %)	9 (75%)	3 (25%)	10 (100 %)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)
Levofloxacin (LE)	10 (52.63 %)	9 (47.37 %)	8 (66.67 %)	4 (33.33 %)	10 (100 %)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)
Oxytetracycline (O)	14 (73.68 %)	5 (26.32 %)	6 (50%)	6 (50%)	8 (80%)	2 (20%)	2 (66.66 %)	1 (33.33 %)	0 (0%)	3 (100%)	1 (33.33 %)	2 (66.66 %)
Vancomycin (VA)	6 (31.58 %)	13 (68.42 %)	5 (41.67)	7 (58.33 %)	8 (80%)	2 (20%)	3 (100%)	0 (0%)	1 (33.33 %)	2 (66.66 %)	3 (100%)	0 (0%)
Rifampicin (R)	10 (52.63 %)	9 (47.37 %)	10 (83.33 %)	2 (16.67 %)	6 (60%)	4 (40%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	3 (100%)	0 (0%)
Methicillin (M)	4 (21%)	15 (79%)	2 (16.67 %)	10 (83.33 %)	3 (30%)	7 (70%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)
Ampicillin (AMP)	2 (10.53 %)	17 (89.47 %)	2 (16.67 %)	10 (83.33 %)	7 (70%)	3 (30%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)
Bacitracin (B)	3 (15.79 %)	16 (84.21 %)	0 (0%)	12 (100%)	0 (0%)	10 (100 %)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	3 (100%)	0 (0%)
Ceftazidime (CAZ)	2 (10.53 %)	17 (89.47 %)	0 (0%)	8 (100%)	2 (20%)	8 (80%)	3 (100%)	0 (0%)	2 (66.66 %)	1 (33.33 %)	0 (0%)	3 (100%)
Cefepime (CFM)	5 (26.32 %)	14 (73.68 %)	2 (16.67 %)	10 (83.33 %)	2 (20%)	8 (80%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)	3 (100%)	0 (0%)
Amikacin (AK)	5 (26.32%)	14 (73.68%)	0 (0%)	12 (100%)	3 (30%)	(70%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)

Table 4: Multi-drug resistance among the bacterial isolates

S. No.	Name of the isolates	No. of mdr	Mdr in %
1.	*Staphylococcus aureus*	**7**	53.8%
2.	*Staphylococcus epidermidis*	6	75%
3.	*Streptococcus spp*	5	83.3%
4.	*Enterobacter spp.*	1	100%

The antibiotic sensitivity of the isolated bacterial strains is shown in table 3. *Staphylococcus aureus* (n=19) demonstrated high degree of antibiotic sensitivity towards ofloxacin (68.42%), oxytetracycline (73.68%) and resistance towards vancomycin (68.42%), ampicillin (89.47%), bacitracin (84.21%), cefazidime (89.47%), cefipime and amikacin (73.68%). *Staphylococcus epidermidis* exhibited high sensitivity to moxifloxacin, rifampicin and resistance to methicillin, ampicillin and cefipime (83.33%) and 100% resistance to bacitracin, ceftazidime and amikacin. *Streptococcus* spp documented 100% sensitivity to ofloxacin, levofloxacin and 100% resistant to methicillin, bacitracin and ceftazidime. The isolates also exhibited resistance to multiple antibiotics (Table 4). *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp and *Enterobacter spp* exhibited resistance to multiple antibiotics as 52.63%, 75%, 80% and 100% respectively. The molecular weight of the isolated plasmid DNA was found to be high (fig. 2).

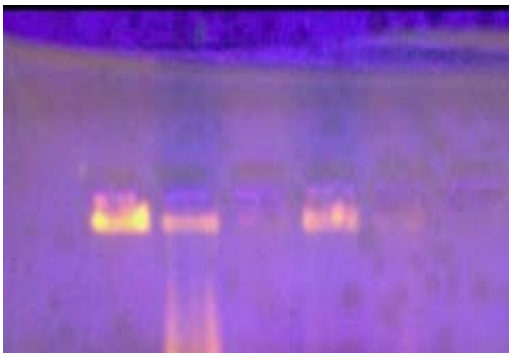

Fig. 2: Plasmid DNA isolated from MDR Staphylococci

DISCUSSION

In the present study, 44 bacterial strains have been isolated. *Staphylococcus aureus* (43.18%) exhibited the high rate of prevalence. 27.27% of *Staphylococcus epidermidis* and 22.72% of *Streptococcus* spp were obtained. Least prevalence was noticed among Bacillus spp, *Enterobacter* spp and *Serratia* spp at the rate of 2.27% each.

In a study of the normal conjunctiva from Rajasthan, India, 86% of eyes were culture positive for bacteria and 12% positive for fungi. The most common bacterial isolates were *S. albus* (32%) followed by *S. aureus* (28%) [10]. In another study from Masungbo, Sierra Leone, where analysis of conjunctival swabs obtained from healthy eyes of 276 residents showed the presence of coagulase-negative staphylococci (28.6%), fungus (26.0%) and *S. aureus* (19.9%) [11]. Many studies have not speciated the staphylococci from normal lids and conjunctiva. However, *S. epidermidis* is reported to be the most common species [12]. Sex wise incidence indicated that out of 31 infected cases, 19 were males and 11 were females. Occurrence in different age group revealed that cases more than 21 years are highly infected.

Results of antibiotic sensitivity envisaged that the isolates have shown sensitivity to Ofloxacin, Levofloxacin, Vancomycin and Rifampicin and resistance to Ampicillin, Methicillin, Amikacin, Cefipime and Ceftazidine. This antibiogram pattern indicated that ocular pathogens are sensitive to quinalones and resistant to penicillin and cephalosporin derivatives. Savithri Sharma, [13] has documented a broad range of three generations of fluoroquinolones are available such as ciprofloxacin (0.3%), ofloxacin (0.3%), levofloxacin (0.5% and 1.5%), gatifloxacin (0.3%) and moxifloxacin (0.5%, preservative free) as eye drops also. Gatifloxacin and moxifloxacin, the newer fourth generation fluoroquinolones that target both DNA gyrase and topoisomerase IV are highly effective against gram positive bacteria including staphylococci in human and animal corneal ulcer model. Iihara et al. [14] has been reported increasing fluoroquinolone resistance in *S. aureus* isolated from ocular as well as non-ocular infections. The development of fluoroquinolone-resistant, methicillin-resistant *S. aureus* (MRSA) has been widespread. The frequent use of OFLX and LVFX since the 1990s may lead to increasing resistance as well as cross-resistance to other fluoroquinolones.

The rate of multidrug resistant isolates found to be increased at the rate of 27/44 (61.36%). *Staphylococcus aureus*(52.63%), *Staphylococcus epidermidis* (75%), *Streptococcus* spp (80%) and *Enterobacter spp* (100%) exhibited multidrug resistance. The prevalence of MRSA in ocular infections varies in different studies. While it is reported to be as low as 3% in England, it is high (25-64%) in Japan. However, Indian workers have also reported increasing prevalence of MRSA over the years [15].

In the present investigation, plasmid DNA was isolated from multidrug resistant ocular pathogens. The molecular weight of plasmid DNA was found to be high with variation in antibiotic resistance.

CONCLUSION

The incidence of bacterial ophthalmic infections was found to be 88%. Staphylococcus spp exhibited the high rate of occurrence. Females (61.29%) were highly infected than males. The ocular infection was highly prevailed in adults with age group of 21-40years. These findings revealed that the quinalone group of drugs namely Ofloxacin, Levofloxacin, Moxifloxacin and others like Vancomycin and Rifampicin are the drug of choices for treating ocular infections in the study area. Development of drug resistance is a threatening problem in the present therapeutic scenario. From our findings, various ocular pathogenic isolates exhibited various degree of sensitivity against the selected antibiotics. Moreover, change in antibiogram pattern was also observed. Many isolates was found to exhibit resistance to multiple antibiotics.

The rate of multidrug resistance was found to be 61.36%. Such resistance may be due to frequent use of antibiotics and poor hygienic practices. The present work has confirmed the presence of the single plasmid in all multidrug resistant isolates with high molecular weight. Hence it is concluded that there is a need for surveillance of multidrug resistance among ocular pathogens and development of remedial measures to control them. Proper treatment and hygienic practices could reduce the incidence of multidrug resistance.

CONFLICT OF INTERESTS

Declared None

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