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Conjunctival Bacterial Flora and Their Antibiotic Sensitivity in Undergoing Ophthalmic Surgery

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Abstract: To evaluate the normal conjunctival bacterial flora in the local region and to ascertain their sensitivity to antibiotics, prospective study was undertaken at Research Institute of Ophthalmology, Giza, Egypt from January, 2014 to December, 2015.Conjunctival swabs were taken from the operated eye of patients scheduled for ophthalmic surgeries before instilling any antibiotic drops or local anesthetics and were used for bacterial isolation by culturing on blood agar plates and antimicrobial susceptibility tests were done on Mueller-Hinton agar using the Kirby-Bauer disk-diffusion technique. Results showed that 1036 out of 11468 conjunctival swabs (9.0%) had bacterial growth preoperatively. The most frequently isolated bacteria were Gram-positive. The predominant bacterial species isolated was Coagulase-negative staphylococci (57%) followed by *S. aureus* (18.1%), *Streptococcus* sp.(11.1%) and Micrococci(7.8%). Other bacterial isolates were Corynebacterium sp. (2.5%), Gram negative rods (1.9%) and Moraxella sp.(1.3%). Most of the bacterial isolates were resistant to Trimethoprim-Sulphamethoxazole (67.2%), Fucidic acid (57.2%), Tetracycline(42.6%) and Erythromycin (33.5%), while Sulbactam-ampicillin showed maximum susceptibility (93.8%) followed by Chloramphenicol (88.5%), Vancomycin (87.7%), Neomycin (86.6%), Tobramycin (85.6%), Cefotaxime (83.9%) and Gentamycin (82.4%). About one third (28.2%) of all bacterial isolates and 30.4% of CNS isolates were multi-drug resistant MDR (resistant to > or = three antibiotics of different groups). In conclusion Coagulase-negative staphylococci are the most common bacteria isolated from the conjunctival sac. Sulbactam-ampicillin, Chloramphenicol, Vancomycin and Neomycin, followed by, Tobramycin, Cefotaxime and Gentamycin are most effective against normal conjunctival flora. Trimethoprim-Sulfamethoxazole and fucidic acid are the most ineffective of all the antibiotics tested. Our *in vitro* results, along with those of other investigators, should prompt further dialogue regarding antibiotic of choice for perioperative surgical prophylaxis in ophthalmic surgery.

Key words: Coagulase negative staphylococci, chloramphenicol, aminoglycosides, fluoroquinolones, antibiotic prophylaxis

INTRODUCTION

Endophthalmitis is one of the most dreaded complications of ophthalmic surgery. It may result in permanent loss of vision if not recognized and treated properly. Eye surgeons use several pre-and postoperative methods to prevent the occurrence of endophthalmitis, the most common of which is the administration of topical antibiotic drops at regular intervals [1]. Use of antibiotic prophylaxis pre-and postoperatively in reducing the risk of endophthalmitis remains controversial [1]. There are also no standard guidelines on the type of antibiotic to be used in this setting. Bacteria are the most common cause of endophthalmitis and Gram-positive pathogens are responsible for 60 to 80% of acute infections [1].Knowing the organisms found most frequently in the ocular flora and their antibiotic sensitivity may provide a better guide in choosing an antibiotic for prophylaxis of postoperative endophthalmitis. The most common site of the organism that is known to cause endophthalmitis is the conjunctiva itself [2].Several studies have shown coagulase-negative staphylococcus to be the most common organism causing endophthalmitis and also the most common organism isolated among the conjunctival flora [3, 4].

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Although there are many published articles [1-3] from Western countries pertaining to normal flora of the eye and their sensitivity pattern to antibiotics, these data cannot be applied directly to our local patients, as several factors affect the type and sensitivity and resistance patterns of bacteria. Moreover, this kind of a study must be done regularly due to the dynamic nature of bacterial resistance to antibiotics [3].

In Egypt with a wide prevalence of trachoma in the past, resulting in alteration of ocular surface subtly in many individuals and markedly in some, the conjunctival flora may be different. And as is well-known going by the various studies [1-6] every region may have a different sensitivity pattern.

This study was undertaken to ascertain the normal conjunctival flora in the region and its sensitivity pattern.

MATERIALS AND METHODS

A prospective study was conducted with patients who underwent ophthalmic surgeries between January 2014and December 2015. 11468 eyes of 11468 patients admitted for intraocular surgeries in Research Institute of Ophthalmology RIO were enrolled in this study as a routine work among preoperative investigations. Patients with ocular surface disease such as meibomitis, dry eye and chronic dacryocystitis were excluded from the study. Conjunctival swab was taken in the microbiology laboratory in RIO within 1 weak before intraocular surgery from operated eye before applying any antibiotic or anesthetic drops. Specimen was taken from the inferior conjunctival fornix with sterile cotton swap without touching the eyelids. The sample was inoculated immediately aseptically by streaking onto blood (5% sheep blood) agar medium and incubated at 37°C for 48 hours. Isolated colonies were identified by morphology, microscopic examination of Gram stained film and biochemical reactions. Antibiotic susceptibility tests to isolated strains were done using the classic agar diffusion (Kirby-Bauer) method [7] performed on Muller Hinton agar (MHA) according to CLSI guidelines [8], to; chloramphenicol, neomycin, gentamycin, tobramycin, ofloxacin, ciprofloxacin, tetracycline, sulfamethoxazole/ trimethoprim, ampicillin/sulbactam, erythromycin, vancomycin, fucidene, cefotaxime and polymixin B

RESULTS

A total of 11468conjunctival swabs were cultured, Yielding 1036bacterial isolates. The rest of samples showed no bacterial growth (Table 1). Coagulase-negative staphylococci (CNS) were the most commonly isolated organisms 591 (57%), 188(18.1%) were *Staphylococcus aureus,* 116 (11.1%) *Streptococcus species (pneumococci*, *Viridans Streptococci* and *Enterococci*), 81(7.8%) grew *Micrococcus species*, 26 (2.5%) *Corynebacterium species,* 20 (1.9%) Gram-negative bacilli and 14 (1.3%) were Moraxella species (Table 2) & (Figure 1).

Overall susceptibility was highest for Sulbactam-ampicillin (94%), Chloramphenicol (89%), Vancomycin (88%), Neomycin (87%), Tobramycin (86%), Cefotaxime (84%) and Gentamycin(82%). Less susceptibility was found with earlier- generation fluoroquinolones; Ofloxacin (74%) and ciprofloxacin (72%), (Table 3) (Fig. 2). This was also true for the CNS isolates, (Table 4), (Fig. 3). A total of 66% of CNS isolates were resistant to Sulphamethoxazole-trimethoprim, 56% resistant to fucidic acid, 44% resistant to Tetracycline and 25% resistant to Ofloxacin and ciprofloxacin. About one third (28.2%) of all bacterial isolates and 30.4% of CNS isolates were multi-drug resistant (resistant to =3 classes of antibiotics).

Table 1: The number and percent of eyes giving bacterial growth and no growth

	Examined eyes(2014) N=6175		Examined eyes (2015) N=5293		Examined eyes in two years N=11468	
Growth status	Number	%	Number	%	Number	%
No bacterial growth	5563	90.0	4869	91.9	10432	90.9
Bacterial growth	612	9.9	424	8.0	1036	9.0%

Table 2: Ocular flora in patients undergoing ophthalmic surgery in 2014 &2015

Organism	Frequency 2014 (n=612)	Frequency 2015 (n=424)	Total 2 year frequency (n=1036)	%
Coagulase-negative Staphylococci	381	210	591	57
Staphylococcus aureus	86	102	188	18.1
Streptococcal sp. (pneumococci, Viridans streptococci& Enterococci)	82	34	116	11.1
Micrococci	31	50	81	7.8
Corynebacterium species.	11	15	26	2.5
Gram negative Bacilli	12	8	20	1.9
Moraxella sp.	9	5	14	1.3

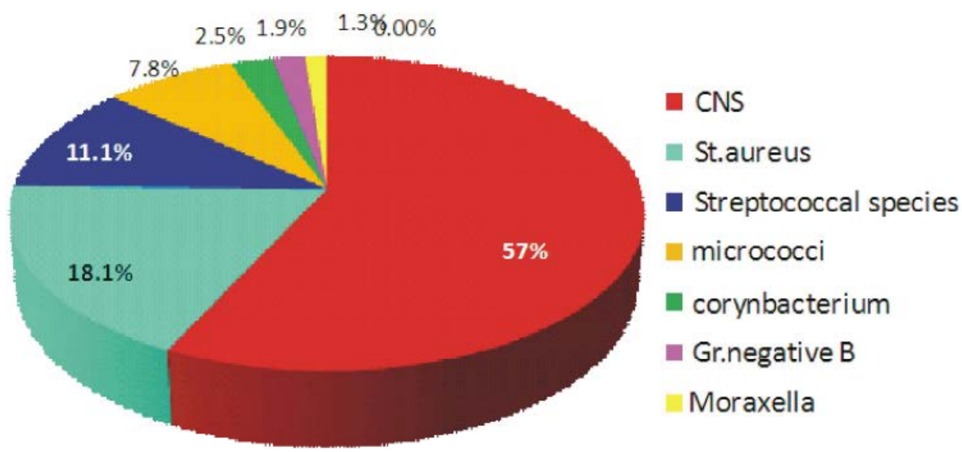

Fig. 1: Types and frequency of isolated organisms %

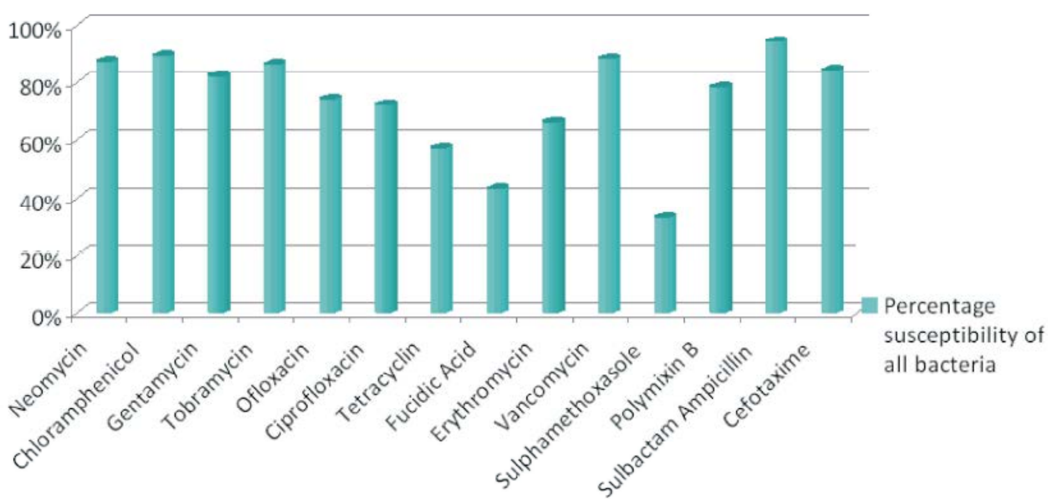

Fig. 2: Percentage susceptibility of all isolates

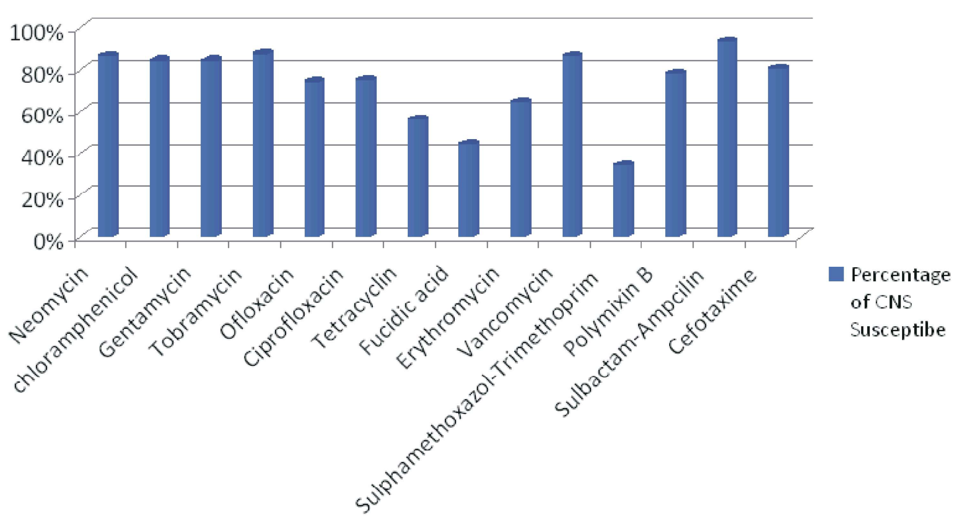

Fig. 3: Percentage susceptibility of CNS

Table 3: Antibiotic susceptibility pattern of all the isolates

	Antibiotic	Sensitive (%)	Resistant (%)
1	Neomycin	423(86.6)	65(13.3)
2	Chloramphenicol	293(88.5)	38(11.4)
3	Gentamycin	504(82.4)	107(17, 5)
4	Tobramycin	514(85.6)	86(14.3)
5	Ofloxacin	489(74.3)	169(25.6)
6	Ciprofloxacin	322(71.8)	126(28.1)
7	Tetracycline	203(57.3)	151(42.6)
8	Fucidene	281 (42.7)	367 (57.2)
9	Erythromycin	174 (66.4)	88 (33.5)
10	Vancomycin	243 (87.7)	34 (12.2)
11	Sulphamethoxasole/Trimethoprim	175 (32.7)	360 (67.2)
12	Polymixin B	546 (77.8)	155 (22.1)
13	Sulbactam/Ampicillin	168 (93.8)	11 (6.14)
14	Cefotaxime	73 (83.9)	14 (16)

Table 4: Antibiotic sensitivity pattern of coagulase-negative staphylococcus

	Antibiotic	Sensitive (%)	Resistant (%)
1	Neomycin	220(85.6)	37(14.3)
2	Chloramphenicol	145(84.3)	27(15.6)
3	Gentamycin	261(84.1)	49(15.8)
4	Tobramycin	269(87)	40(12.9)
5	Ofloxacin	248(74.2)	86(25.7)
6	Ciprofloxacin	174(75)	58(25)
7	Tetracycline	102(56.3)	79(43.6)
8	Fucidene	157 (44.2)	157 (44.2)
9	Erythromycin	92 (64.3)	51 (35.6)
10	Vancomycin	117 (86)	19(13.9)
11	Sulphamethoxasole/Trimethoprim	93 (33.9)	181 (66)
12	Polymixin B	277 (78.2)	77 (21.7)
13	Sulbactam/Ampicillin	81 (93.1)	6 (6.8)
14	Cefotaxime	41(80.3)	10 (19.5)

DISCUSSION

Postoperative endophthalmitis is the most dreaded complication of intra ocular surgery and conjunctival flora has been blamed to be the primary and most frequent source of infection [2, 7, 8]. So evaluation of the conjunctival bacterial flora and their sensitivity pattern is of utmost importance.

In our study, we were able to isolate bacteria in 1036 eyes (9.0%). In a study by Belur R *et al*. [9], the percentage isolation was found to be 48%. Another study by Mashangila B *et al* [10] also showed the ratio of conjunctival isolation was 45%, while lid margin isolates showed higher positivity 59%.

The most common organism isolated in our study was coagulase-negative staphylococcus (57%) which is in confirmation with most of the studies, [9-14] proving that this bacteria is the most common isolate in conjunctival bacterial flora worldwide. This organism is related to approximately 70% of the cases of postsurgical endophthalmitis [15].

With regards to sensitivity patterns particularly in case of CNS, Sulbactam-ampicillin, tobramycin, vancomycin, neomycin, Chloramphenicol, gentamycin and cefotaxime were found to be most effective (>82%) susceptibility pattern. Other studies [9, 10] demonstrated similar sensitivity patterns. Reduction in the susceptibility rates to earlier-generation fluoroquinolones (ofloxacin and ciprofloxacin) was found among CNS isolates (26%) as shown in our study. This was also demonstrated by Chung *et al*. [16]. It may be a therapeutic option to use newer fluoroquinolones in patients undergoing eye surgery to reduce possible infections as methicillin-resistant CNS. In our study CNS showed maximum resistance to Sulphamethoxazole-trimethoprim and fucidic acid followed by tetracycline and erythromycin. Belur *et al*. [9] also found that fucidic acid have the highest resistance pattern. In a study by Muluye *et al.* [14] most of the bacterial isolates were also resistant to erythromycin (43.5%), trimethoprim-Sulphamethoxazole (58.1%) and tetracycline (64.6%). In another study by Mshangila *et al*.[10], CNS showed the highest resistance to tetracycline (58.2%) and erythromycin(38.5%). Similar results were found by Mino *et al.* [12].

In our study, about one third (28.2%) of all bacterial isolates and (30.4%) of CNS isolates were multi-drug resistant (resistant to ≥ 3 classes of antibiotics).This was determined according to CDC and ECDC guidelines; Magiorakos, A. P., *et al.* [17]. This is similar to the study of Miño *et al*. [13], however they defined multi- drug resistant bacteria as being (resistant to > or = five antibiotics). On the contrary Muluye *et al*. [14] considered 87.1% of bacterial isolates as multi drug resistant (MDR) being resistant to two or more drugs.

Tiago *et al.* [18] demonstrated the number of CNS isolates resistant to 4 or more antibiotics (7.4%) was lower than the 39% described by Ta *et al*. [19] and similar to the one found by Pinna *et al*. [20] (9.0%).

Additional studies are necessary to determine how the gender, the age of the patient and seasonal variation affect the ocular microbial populations.

CONCLUSIONS

This study showed that the bacterium most frequently found in the conjunctival flora of the patients undergoing intraocular surgery was the CNS, which was most sensitive to sulbactam-ampicillin, tobramycin, vancomycin and neomycin followed by gentamycin and chloramphenicol. Isolates of this bacterium had low susceptibility rates to fucidic acid and Sulphamethoxazole-trimethoprim, followed by tetracycline and erythromycin. Nowadays, with the considerable increase of bacterial resistance to antibiotics, the understanding of the sensitivity of the conjunctival bacterial flora to antibiotics is of fundamental importance. This study may act as a guide to choosing antibiotics for preoperative prophylaxis or postoperative treatment.

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