Microflora Isolated from Preoperative Conjunctivas, the Prevalence of Methicillin-Resistant Staphylococci and their Antibiotic Profile

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Abstract: The existence of bacterial flora in apparently healthy conjunctiva has been reported in several studies. Methicillin- resistant *Staphylococcus aureus* (MRSA) and Methicillin-resistant coagulase-negative staphylococci (MRCNS) have been isolated from clinically healthy conjunctivas. Chloramphenicol eye drops are useful for the treatment of MRSA ocular surface infection. **The aim** of the study is to determine the prevalence of MRSA and MRCNS isolates. Also to assess the *in vitro* susceptibility of the conjunctival bacterial isolates to the most common used antibiotics in the outpatient eye clinics and the hospital of Research Institute of Ophthalmology (RIO), in Egypt, with evaluation of *in vitro* susceptibility of MRSA and MRCNS to chloramphenicol antibiotic. Our results showed bacterial & fungal growth as 14.3%. CNS dominated the isolated microbial flora, with 76.2% while *S. aureus* was 10.5%. Methicillin-resistant staphylococci represented 24.2% of the isolated staphylococci where MRSA was 40% & MRSE was 22%. Other microbial flora included streptococci 4.2%, *M. catarrhalis* 2.8%, G-ve bacilli 2.8%, G +ve rods 2.1% & *Candida* 1.4%. All the isolated bacteria were highly sensitive to chloramphenicol especially MRSA & MRSE. In the present study chloramphenicol completely inhibited the growth of all (100%) methicillin-resistant staphylococci whether it was *S. aureus* or *S. epidermidis* (MRSA &MRSE), also 100% of methicillin-sensitive *S. aureus* (MSSA) and 96.5% of methicillin-sensitive *S. epidermidis* (MSSE) as well as 100% of *Moraxella catarrhalis* and Gram-negative bacilli also 83% of streptococci and 66.7% of diphtheroids were sensitive to it. Also ofloxacin, gentamicin, rifampicin & fusidic acid were effective against all the isolated bacteria with different percentages. **In conclusion:** MRSA and MRSE are isolated from clinically healthy conjunctivas. Chloramphenicol is very effective antibiotic against MRSA & MRSE as well as all the isolated bacteria.

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1. Introduction

Staphylococcus aureus and coagulase-negative *Staphylococcus* (CNS), including *Staphylococcus epidermidis,* are important bacteria on the ocular surface because the former is the most frequent pathogen that leads to ocular surface infections and the latter, *S. epidermidis* is the most frequent bacterium in normal conjunctival flora *(Fukuda et al., 2002).* Coagulase-negative staphylococci (CNS) are the most normal ocular surface bacterial flora isolated from patients undergoing anterior segment intraocular surgeries *(Ta et al., 2009).* The existence of bacterial flora in apparently healthy conjunctiva has been reported in several studies. Methicillin- resistant *Staphylococcus aureus* (MRSA) and Methicillin-resistant coagulase-negative staphylococci (MRCNS) have been isolated from clinically healthy conjunctivas *(Watanabe et al., 2001).* As a result of its multiple drug resistance and its increasing prevalence, MRSA is a serious cause of morbidity and mortality worldwide *(Shanmuganathan et al., 2005).* Topical antibiotics are commonly prescribed in the preoperative period to possibly reduce the risk of infections following intraocular surgery. Ophthalmologists must carefully choose the antibiotics that are most effective in minimizing ocular colonization with resistant organisms. *In vitro* antibiotic susceptibility testing is the most commonly cited standard and will continue to guide the clinician in antibiotic selection *(Ta et al., 2003 & 2009).* Chloramphenicol was the first broad-spectrum antibiotic discovered. Its isolation in 1974 from the waste products of *Streptomyces venezuelae* was hailed as a milestone in microbiology because the drug was capable of inhibiting a wide variety of Gram-positive and Gram-negative bacteria, as well as several species of rickettsiae and fungi *(Alcamo, 2001).* Chloramphenicol was clinically effective against MRSA conjunctivitis and this may gain favour as a first-line choice of antibiotics outside the USA *(Fukuda et al., 2002).* The success of chloramphenicol as an effective agent against MRSA

in the UK may be due to its limited use systemically for non ocular MRSA infections. As a result, there has not been an overexposure of MRSA to chloramphenicol and thus resistance has not yet developed. *(Shanmuganathan et al., 2005)*.

The aim of this work: is to detect different bacteria isolated from preoperative conjunctival swabs and to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-ve staphylococci (MRCNS) isolates. Also to assess the *in vitro* susceptibility of the conjunctival bacterial isolates to the most common used antibiotics in the outpatient eye clinics and the hospital of Research Institute of Ophthalmology (RIO), in Egypt, with evaluation of *in vitro* susceptibility of MRSA and MRCNS to chloramphenicol antibiotic.

2. Methods

Specimens

A total of 1000 preoperative conjunctival swabs were obtained from eyes of patients who underwent cataract or glaucoma surgery, excluded were those with signs of ocular infection such as blepharitis, conjunctivitis, keratitis and dacryocystitis at the hospital of Research Institute of Ophthalmology in Giza, Egypt.

I.Sampling: Sterile disposable cotton dry swabs were used to swab the entire surface of the conjunctival cul-de-sac from the outer to the inner canthus with special care to avoid lid margins, angels of eye and eye lashes. Any topical antibiotic was stopped for at least 48 hours prior to sampling. These samples were used to inoculate the different culture media. All the plates were incubated at 37ºC for 24 hours for bacterial growth except Sabouraud's Dextrose agar plates were incubated at room temperature (25ºC - 30ºC) for two weeks after sealing the plates with a tape to prevent dryness of the media, they were examined every 48 hours for evidence of fungal growth. Most ocular fungi grow on the media within three days of incubation, however about 25% of cultures may show no growth for as long as 14 days ***(Negm, 2003)***. Therefore cultures were kept incubated for 3 weeks before declaring the culture negative for fungi and discarded.

II. Identification of the isolated strains:

The growth obtained was identified systematically ***(Cruickshank et al., 1975).*** The organisms were identified by the direct smear films using Gram-stain, by their cultural characters and by using specific biochemical tests for each organism using the standard manual technique by ***(Cheesbrough, 2000)***

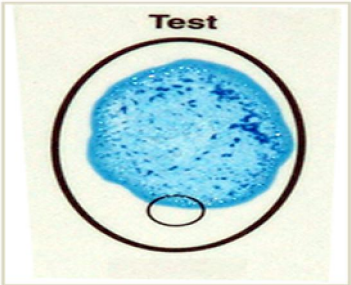

Fig. (1): coagulase +ve test of *S. aureus.*

III. Antibiotic sensitivity tests of the isolated bacteria: The discs of eight different antibiotics were applied from "Oxoid"; methicillin, chloroamphenicol, ofloxacin, vancomycin, gentamycin, rifampicin, polymyxin B, and fucidic acid, were used for disc diffusion Modified Kirby-Bauer- method.

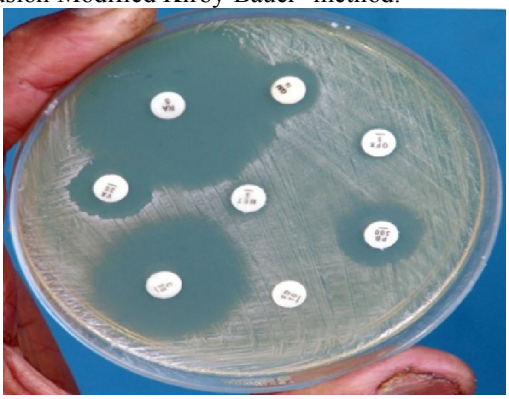

Fig. (2): Methicillin - resistant *S. aureus* (MRSA) strain.

3. Results

The results of culture of one thousand preoperative conjunctival swabs, on different media for bacterial & fungal growth showed microbial growth of 143 samples as shown in table (1).

Table (1): Results of culture of 1000 preoperative conjunctival swabs.

Type of culture	Number	Percentage
Pre-operative conjunctival swabs	1000	100 %
Sterile culture (no bacterial or fungal growth)	857	85.7 %
Microbial growth	143	14.3 %

Then identification of the isolates of 143 microbial growths was done and the results are shown in tables (2 & 3) .

Table (2): Identification of conjunctival isolates from healthy eyes.

Organism	No. of strains	Percentage of total
Coagulase – ve staphylococci (CNS)	109	76.2 %
Staphylococcus aureus	15	10.5 %
Streptococci	6	4.2 %
Neisseria	4	2.8 %
G – ve bacilli	4	2.8 %
G + ve rods	3	2.1 %
Fungi	2	1.4 %

Table (3): Identification of the isolated strains.

Isolated species	No. of strains	% of total
Staphylococcus epidermidis	109	76.2%
Staphylococcus aureus	15	10.5%
Viridians streptococci	5	3.5%
Streptococcus pneumoniae	1	0.7%
Moraxella catarrhalis	4	2.8%
Escherichia coli	1	0.7%
Klebsiella	2	1.4%
Pseudomonas aeruginosa	1	0.7%
Diphtheroids	3	2.1%
Candida albicans	2	1.4%

Table (4): Prevalence of methicillin-resistant staphylococci.

The isolates	No. of strains	Methicillin-sensitive	Methicillin-resistant	% of Methicillin-R
Staphylococci	124	94	30	24.2%
S. aureus	15	9	6	40 %
S.epidermidis	109	85	24	22 %

Table (5): Antibiotic sensitivity tests for staphylococci.

Antibiotic name	*Staphylococcus aureus*				*Staphylococcus epidermidis*			
	No. MS (9)	%	No. MR (6)	%	No. MS (85)	%	No. MR (24)	%
Chloramphenicol	9	100%	6	100%	82	96.5%	24	100%
Ofloxacin	9	100%	1	16.7%	83	97.6%	24	100%
Vancomycin	9	100%	6	100%	85	100%	24	100%
Gentamycin	9	100%	2	33%	81	95%	18	75%
Rifampicin	9	100%	5	83%	79	92.9%	23	95.8%
Polymyxin B	6	66.7%	3	50%	0	0%	0	0%
Fusidic acid	9	100%	5	83%	84	98.8%	23	95.8%

MS = methicillin-sensitive MR = methicillin-resistant

Table (6): Antibiotic sensitivity tests for isolated bacteria other than staphylococci.

Antibiotic name	Streptococci		*Moraxella catarrhalis*		G – ve *bacilli*		G + ve rods	
	No. (6)	%	No. (4)	%	No. (4)	%	No. (3)	%
Chloramphenicol	5	83%	4	100%	4	100%	2	66.7%
Ofloxacin	2	33%	4	100%	4	100%	3	100%
Vancomycin	6	100%	0	0%	0	0%	0	0%
Gentamycin	4	66.7%	1	25%	4	100%	2	66.7%
Rifampicin	3	50%	3	75%	1	25%	1	33%
Polymyxin B	0	0%	0	0%	2	50%	0	0%
Fusidic acid	2	33%	2	50%	1	25%	1	33%

Staphylococci were 124 isolates from 143 growth cultures, representing 86.7% of all isolates. Methicillin-resistant staphylococci were 30 strains representing 24.2% of the isolated staphylococci.

The results of antibiotic sensitivity tests for all the isolated strains are shown in tables (5 & 6).

4. Discussion

The body surfaces support the growth of a variety of bacteria and fungi which collectively are called the normal flora. The viruses and parasites are not considered as members of the normal flora, although they are present in asymptomatic individuals *(Jawetz, 2001).* In the present study the results of culture of 1000 conjunctival swabs showed no microbial growth in 857 cultures representing 85.7%. Our results are almost agree with those of *Javed et al. (2008)* where no growth was found in 412 cultures out of 500 preoperative conjunctival swabs representing 82.4%. *Capriotti et al. (2009)* reported 14.5%. as no growth This lower percentage may be explained by being the study was done in a rural population in Sierra Leone in Africa. In the present study coagulase-negative staphylococci (CNS) (*Staphylococcus epidermidis*) was identified as the most isolated bacteria among the conjunctival microbial flora; 109 strains out of 143 growth representing 76.2%. This result agrees with many studies that proved CNS are the most frequent organism identified by culture of eye flora with different percentages. The same percentage (76%) was reported by *De-Kaspar et al. (2005)* of coagulase-negative staphylococci as the most frequent preoperative normal conjunctival bacteria. *Ta et al. (2009)* made a study to provide an update on antibiotic susceptibility of normal ocular surface bacterial flora isolated from patients undergoing anterior segment intraocular surgeries and among the 116 bacteria isolated 95 (82%) were CNS. Also *Fukuda et al. (2002)* demonstrated that CNS was the most isolated conjunctival bacterial flora of elderly patients 62%. In the present study *Staphylococcus aureus* was 10.5% among the isolated conjunctival microbial flora and came next to *S. epidermidis* which was 76.2%, streptocooci (Viridans streptocooci & *Streptococcus pneumoniae*) were 4.2%, *Moraxella catarrhalis* was 2.8% also Gram-negative bacilli (*Escherichia coli, Klebsiella & Pseudomonas aeruginosa)* were 2.8%, Gram-positive bacilli (Diphtheroids) was 2.1% while *Candida* was the least isolated organism 1.4%. Our results are almost in accordance with the study made by *De-Kaspar et al. (2005)* which involved preoperative normal conjunctival bacteria *Staphylococcus aureus* was 15.3%, and also came next to coagulase-negative staphylococci 76%, streptococci were 6.5% but Gram-negative rods were 8.9% which was higher than our result. *Gündüz et al. (2008)* recorded that *S. aureus* and *Moraxella* species each was 4% and both came next to CNS which were 48%, *Streptococcus* species was 2% and *Candida* also was 2%. It is worthy to mention that *Ta et al. (2009)* reported that *Streptococcus* species was 11.2% representing the second isolated bacteria after CNS 82%, *Staphylococcus aureus* was 5.2%, *Propionibacterium acnes* (Gram-positive anaerobic bacilli) was 3.4% and Gram-negative rods were 2.6% (non were *Pseudomonas*). Fungi do not remain or grow in the conjunctival sac under normal conditions but the surrounding circumstances may have a role in modifying these fungi *(Ando & Takatori, 1982).* It is worthy to mention that isolation of fungi from healthy eyes varies from 2.9% *(Williamson et al., 1968)* to 52% *(Vasquez de Parga & Pereiro, 1965).* In the present study fungi were isolated from two cultures only out of 143 representing 1.4%. In a study done by *Amand et al. (2001)* for definitive and rapid diagnosis of suspected fungal endophthalmitis they had 20 subjects who had clinically healthy eyes with non-infections as control group and the result of the study reported that none of the controls was positive by microscopy, culture or PCR for fungal detection. *El-Mowafy et al. (1983)* reported that *Candida* species were predominant or the only species isolated from clinically healthy eyes and this agrees with our results where *Candida* was the only fungus isolated. Surprisingly *Dalfre' et al. (2007)* in their study to evaluate the incidence of fungi in the ocular conjunctiva of sugar-cane cutting workers it was 67%. They concluded that the environmental condition, the socioeconomic status and the general and personal poor hygienic conditions, together with lack of information about prophylactic standards, surely led to the high incidence of ocular conjunctiva fungus isolation. There is marked variation in the prevalence of MRSA ocular infections geographically and at different time points *(Shanmuganathan et al., 2005).* In the present study the prevalence of methicillin-resistant staphylococci among microbial conjunctival flora of preoperative patients, *Staphylococcus aureus* was 10.5% and MRSA was 40% while *S.*

epidermidis was 76.2% and MRSE was 22%. Regarding MRSA and MRCNS, our results are higher than two studies made in Japan; Which carried by *Kato & Hayasaka (1998)* to evaluate the incidence and characteristics of carriers of conjunctival MRSA and MRCNS among preoperative patients at an eye clinic they reported *S. aureus* as 8% among them MRSA growth was 28.6% while coagulase-negative staphylococci represented by 42% and MRCNS 1.3%. And the other study by *Watanabe et al. (2001)* who reported the incidence of methicillin-resistant staphylococci, among clinically healthy conjunctivas, as *S. aureus* was 5% and MRSA was 12.5% while coagulase-negative staphylococci were 75.5% and MRCNS was 0.8%. But our results agree more or less with a third study made in Japan by *Fukuda et al. (2002)* who evaluate the incidence of methicillin-resistant staphylococci in conjunctival bacterial flora. They reported that *S. aureus* was 20%, of them 57% were MRSA and CNS were 62%, of them 25% were MRCNS. In the present study chloramphenicol completely inhibited the growth of all (100%) methicillin-resistant staphylococci whether it was *S. aureus* or *S. epidermidis* (MRSA & MRSE), also 100% of methicillin-sensitive *S. aureus* (MSSA) and 96.5% of methicillin-sensitive *S. epidermidis* (MSSE) as well as 100% of *Moraxella catarrhalis* and Gram-negative bacilli also 83% of streptococci and 66.7% of diphtheroids were sensitive to it. So in our study all types of the isolated bacteria were sensitive to chloramphenicol. *Shanmuganathan et al. (2005)* reported in their study that all MRSA isolates (100%) were sensitive to chloramphenicol. *Fukuda et al. (2002)* in their study proved that 98% of the isolated MRSA strains were sensitive to chloramphenicol. *Watanabe et al. (2001)* reported all the isolated MRCNS were sensitive to chloramphenicol but all were ofloxacin-resistant. Our results showed 100% sensitivity to ofloxacin by MRSE but only 16.7% by MRSA which are in accordance to the results reported by *Fukuda et al. (2002)* where less than 10% of MRSA strains were sensitive to ofloxacin. *Kato & Hayasaka (1998*) reported that 100% of the MRCNS they isolated were sensitive to ofloxacin and 50% of MRSA were sensitive to it. While *Shanmuganathan et al. (2005)* in their study reported that ofloxacin resistance was observed in all MRSA isolates from patients over 50 years but in younger age group sensitivity were 11.8%. In the present study vancomycin inhibited the growth of all the isolated strains of staphylococci MRSA, MRSE, MSSA and MSSE. Also *Fukuda et al. (2002)* reported sensitivity of MRSA strains to vancomycin as 100%. Vancomycin-resistant MRSA has not yet been reported in MRSA ocular infection *(Shanmuganathan et al., 2005).* But *Kato & Hayasaka (1998)* had completely different result where they reported vancomycin sensitivity by MRSA as 25% only. In the present study gentamycin could inhibit 33% of MRSA and75% of MRSE. Regarding MRSA sensitivity to gentamycin, our results are lower than that reported by *Kato and Hayasaka (1998)* which was 37.5%. Meanwhile our results are higher than that reported by *Fukuda et al., (2002)* which was 27%. On the other hand, *Shanmuganathan et al. (2005)* had completely different result as they reported all MRSA isolates were sensitive to gentamycin. Regarding MRSE our results agree with that of *Watanabe et al. (2001)* who reported sensitivity of MRSE to gentamycin as 75%. While *Kato & Hayasaka (1998)* had lower results as MRSE sensitivity to gentamycin was 50%.

Conclusion:

Chloramphenicol is very effective antibiotic against all the isolated bacteria which included *S. epidermidis* the most frequent isolated bacteria among microbial flora of the eye, *S. aureus*, streptococci, *Moraxella catarrhalis*, Gram-negative bacilli and diphtheroids. Chloramphenicol is very effective against MRSA & MRSE.

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References

1. ***Alcamo, I.E. (2001):*** Fundamentals of Microbiology. 6th edt. pp. 731-736. Jnes and Bartlett publishers. Sudbury,Massachusetts.
2. ***Amand. A.; Madhavan. H.& Neelam, V. (2001):*** Use of polymerase chain reaction in the diagnosis of fungal endophthalmitis. Ophthalmology; 108 (2): 326.
3. ***Ando, N. and Takatori, K. (1982):*** Fungal flora of the conjunctival sac. *Am. J. Ophthalmol.*; 94: 67-74.
4. ***Capriotti, J.A; Pelletier, J.S.; Shah, M.; Caivano, D.M. and Ritterband, D.C. (2009):*** Normal ocular flora in healthy eyes from arural population in Sierra leone. Int. Ophthalmol.; 29(2): 81-4.

5. ***Cheesbrough, M. (2000):*** Medical Laboratory Manual for Tropical Countries. Vol.II Microbiology. Butterworth Heinemann.Oxford.
6. ***Cruickshank. R.; Duguid, J.P.; Mormion, B.P. and Swain, R.H.A. (1975):*** Medical Microbiology. 12th Ed. Vol. 11. Churchil Livingstone, Edinburgh, Lonburgh. London and New York.
7. ***Dalfre', J.T.; Rodrigues, J.P.; Donato, B.G.; Giancoli Neto, A.; de Andrade Oliveira, D.I.; Pereira, M.A. and Fiorini, J.E.N (2007):*** Fungic microbiota of normal conjunctiva, sugar cane and anemophilaus fungi of the region of Monte Belo - Minas Gerais. Arq. Bras. Oftalmol.; 70(3): 445-9
8. ***De Kaspar, H.M.; Koss, M.J.; He, L.; Blumenkranz, M.S. & Ta, C.N. (2005):*** Antibiotic susceptibility of preoperative normal conjunctival bacteria. *Am. J. Ophthalmol.*; 139 (4): 730-3.
9. ***El-Mowafy, N.; Abdella, M.I. and Refai, M. (1983):*** Fungal flora of the conjunctival sac in healthy and diseased eyes. Bull. Ophthalmol. Soc.; 76:19.
10. ***Fukuda, M. ;Ohashi, H.; Matsumoto, C.; Mishima, S. and Shimomura, Y. (2002):*** Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus ocular* Surface infection & efficacy of chloramphenicol eye drops. Cornea; 21 (Suppl.7):S 68-9.
11. ***Gündüz, A., Cumurcu, T. and Seyrek, A. (2008).*** Conjunctival Flora in Behçets patients. Can. J. Ophthalmol.; 43(4): 476-9.
12. ***Javed, E.A.; Sultan, M. and Mehmood, R.Q. (2008):*** Normal conjunctival Flora, A.P.M.C.; 2(1): 17-20.
13. ***Jawetz, E.; Melnick, J. L.; Adelberg, E. A.; Brooks, G. F.; Butel, J.S. &Ornston, L.N. (2001):*** Medical Microbiology. pp. 179-194. 22nd edition Appleton & Lange. Mc Graw-Hill Companies. New York.
14. ***Kato. T. and Hayasaka, S. (1998):*** Methicillin-resistant *Staphylococcus aureus* and methicillin -resistant coagulase- negative staphylococci from conjunctivas of preoperative patients, Jpn. J. Ophthalmol.; 42(6): 461-5.
15. ***Negm, S. (2003):*** Spectrum of ocular mycosis in Egypt. M.D. thesis, *Faculty of Medicine, Cairo University.*
16. ***Shanmuganathan, V.A; Armstrong, M. ; Buller, A. and Tullo, A.B. (2005):*** External ocular infections due to methicillin- resistant *Staphylococcus aureus* (MRSA). Eye; 19 (3): 284-291.
17. ***Ta, C.N.; Chang, R.T.; Singh, K.; Egbert, P.R.; Shriver, E.M.; Blumenkraz, M.S. and De Kaspar, M.M. (2003):*** Antibiotic resistance patterns of ocular flora. Ophthalmology; 110: 1946- 1951.
18. ***Ta, C.N.; He , L.& Mino de Kaspar, H. (2009):*** *In vitro* antibiotic susceptibility of preoperative normal conjunctival bacteria. Eye; 23:559-560..
19. ***Vasquez de Paraga, A.S. & Pereiro, M. (1965):*** Flora micotica de la conjunctiva. *Arch. Soc. Oftal. Hisp. Amer.*; 25: 168.
20. ***Watanabe, K.; Numata-Watanabe & Hayasaka, S. (2001):*** Methicillin-resistant staphylococci and ofloxacin-resistance bacteria from clinically healthy coniunctivas. Ophthalmic Res.; 33: 136-139.
21. ***Wiliamson, J.; Goordon, A.M.; Wood, R.; Mck. Dyer, A.; Yahya, O.A. (1968):*** Fungal flora of the conjunctival sac in health and disease. Influence of topical and systemic steroid .Br. J. Ophthalmol.; 52: 127-137.

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