



Prevalence of Aerobic bacterial flora in normal and inflamed conjunctiva

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Abstract

The aerobic bacterial flora in normal and inflamed conjunctiva has been widely studied, although much less work has been done on anaerobic bacteria.

Results: *This study highlights the aerobic bacterial flora in normal and inflamed human conjunctiva. This study comprised of hundred eyes with normal conjunctiva (control group) and an equal number of eyes with inflamed conjunctiva (study group). Staphylococcus albus was the predominant aerobic bacteria isolated from the normal conjunctiva. In the inflamed conjunctiva, 87% of the eyes were positive for bacterial culture and, no growth was seen in 13%. Aerobic bacteria were isolated in 83% (pure and mixed cultures). Staphylococcus aureus (31.69%) was the commonest aerobic bacteria recovered from cases of acute conjunctivitis in (2.81%). The difference between bacterial cultures isolated from normal and inflamed conjunctiva is statistically highly significant at p value of (0.01).*

Conclusion: *It is of utmost importance for the Ophthalmologist to consider the bacterial flora while treating patients with conjunctivitis and prophylaxis prior to surgery.*

Keywords: *Human conjunctiva, Aerobic Bacteria, Aerobic Culture.*

Introduction

Infections of the conjunctiva and cornea are among the most prevalent problems faced by the ophthalmologist (Watson *et al.*, 2018). Bacterial conjunctivitis can be incapacitating and the source of disastrous ocular infections. The normal flora develops a lifelong relationship with the host in a

stable balance between bacterial proliferation and control of their numbers by indigenous antibacterial factors (Epling, 2012). For the host, the normal flora provides protection against colonization by organisms of great virulence and pathogenic potential, (Ribet and Cossart, 2015). This symbiotic relationship exist as long as the

normal flora does not proliferate beyond its usual numbers. The response to this insult is an inflammatory reaction. i.e Conjunctivitis. In a study it was reported that the organisms isolated from the vitreous were genetically indistinguishable from an isolate recovered from the patient's eyelid, conjunctiva, or nose in 82% cases of endophthalmitis (Sharma *et al.*, 2013). Therefore, aerobic bacterial cultures of normal conjunctiva and identification of ocular microflora are generally ordered preoperatively to avoid postoperative endophthalmitis by prophylactic administration of appropriate antibiotics. In the inflammation of the conjunctiva – the mucous membrane covering the white of the eyes and the inner side of the eyelids. Conjunctivitis is a common eye condition, which is not serious, but can be uncomfortable and irritating, usually affects both eyes at the same time – although it may start in one eye and spread to the other after a day or two. It can be asymmetrical, affecting one eye more than the other (Alash, 2015). Bacterial conjunctivitis is an infection caused by bacteria, such as Staphylococci, Streptococci or Haemophilus. These organisms may come from the patient's own skin, upper respiratory tract or caught from another person with conjunctivitis (Okesalo and Salako, 2010). Typically, transmission of the infection involves exogenous routes of infection via airborne fomites, hand-to-eye contact, contact with genital secretions and contact with upper respiratory tract infection secretions. Less frequently, infection may spread to the conjunctiva from the eyelids, lacrimal drainage apparatus, face, or paranasal sinuses. Hematogenous route of infection is however rare. The most common presentation is a red eye and the main tasks are to exclude potentially serious causes of a red eye and then decide whether the conjunctivitis is infective, allergic, or due to other causes, so that a suitable management could be instituted. Clinical presentations are not diagnostic of the causative agent, therefore microbiological analysis, with cytology, cultures, and microbial susceptibilities are mandatory. The specific

antimicrobial therapy should then be based on the laboratory findings (Kowalski *et al.*, 2003). This study was therefore designed to determine the prevalence and pattern of aerobic microflora among bacterial pathogens of conjunctivitis and study their antibiotic susceptibility.

Material and Methods

The study was conducted on one hundred clinically diagnosed patients of untreated acute conjunctivitis in the Outpatient Department of Ophthalmology, Govt. Medical College, Jammu. Fifty patients attending the outpatient Department for refractive errors without any other non infectious problems served as control. The cases of conjunctivitis were examined for ocular symptoms and signs

Specimen Collection:

Conjunctival cultures were obtained from both the eyes of fifty patients enrolled in control group. In patients with conjunctivitis, excess exudates was removed with sterile saline, and the surface of the lower conjunctival sac was wiped with a sterile swab. Two swabs of conjunctival discharge were taken from each eye, one for Gram staining and other for aerobic culture. The Tubes of nutrient broth were used for transportation of swabs and were processed in the Department of Microbiology, Govt. Medical College, Jammu for isolated and identification of micro organisms within one hour. Sheep blood Agar, Macconkey Agar and Chocolate Agar was used for the isolation of Aerobic cultures. After inoculation the plates were incubated at 37°C for 48 to 72 hrs, maximum for 5 days and examined in detail for morphology and culture characteristics. Bacterial identification was done on the basis of colonial morphology, Gram staining and various biochemical tests.

Statistical analysis

The difference between bacterial cultures isolated from normal and inflamed conjunctiva is statistically significant at p value of 0.01.

Results

In the present study 100 cases of conjunctivitis eyes were examined for the ocular symptoms and signs. (Table 1) In the above study 100 eyes from the Ophthalmology, Department of GMC, Jammu of various age and sex were studied. (Fig. 1 & 2) In the samples from healthy normal eyes, bacteria were isolated in 57% while 43% eyes were sterile. (Fig.3) Among 57% of positive cultures 37% were aerobic while as 20% had mixed presence of microbes.

From the normal conjunctiva *Staphylococcus albus* dominated with 36 isolates (50%), *Staphylococcus aureus* with 6 isolates, Diptheroids 4 isolates while both *Klebsiella* and *E.coli* had 3 isolates each. (Fig.4)

While, in case of inflamed conjunctiva 53 was mixed, 34% were aerobic while only 13% was

sterile. (Fig.5) In case of inflamed conjunctiva *Staphylococcus aureus* was the predominant with 45 isolates (31.69%), followed by *Klebsiella* and *S. albus* 8 isolates each (5.63%). *E.coli* and *H. influenzae* had 5 isolates each (3.52%), Diptheroids (2.81%) had 4 isolates and *Proteus* had 3 isolates (2.11%). *Pseudomonas*, *S. pneumonia*, *Enterobacter* had 2 isolates each (1.40%) along with 2 unidentified. Only one isolate was reported for *S.viridans*, *Neisseria* and *Citrobacter* (0.70%) (Fig.6).

Further, the aerobic flora isolated was subjected to antibiotic susceptibility by disc diffusion method and plates observed after 24h - 48h. it was observed that Ciprofloxacin is the most effective drug for Gram positive and Gram negative organisms (100%). The other potent antibiotics are Gaberomycin (96.25%) and Cloxacillin (87.7%) while Ampicillin was the most resistant.(65%) (Table 2(a) & 2(b))

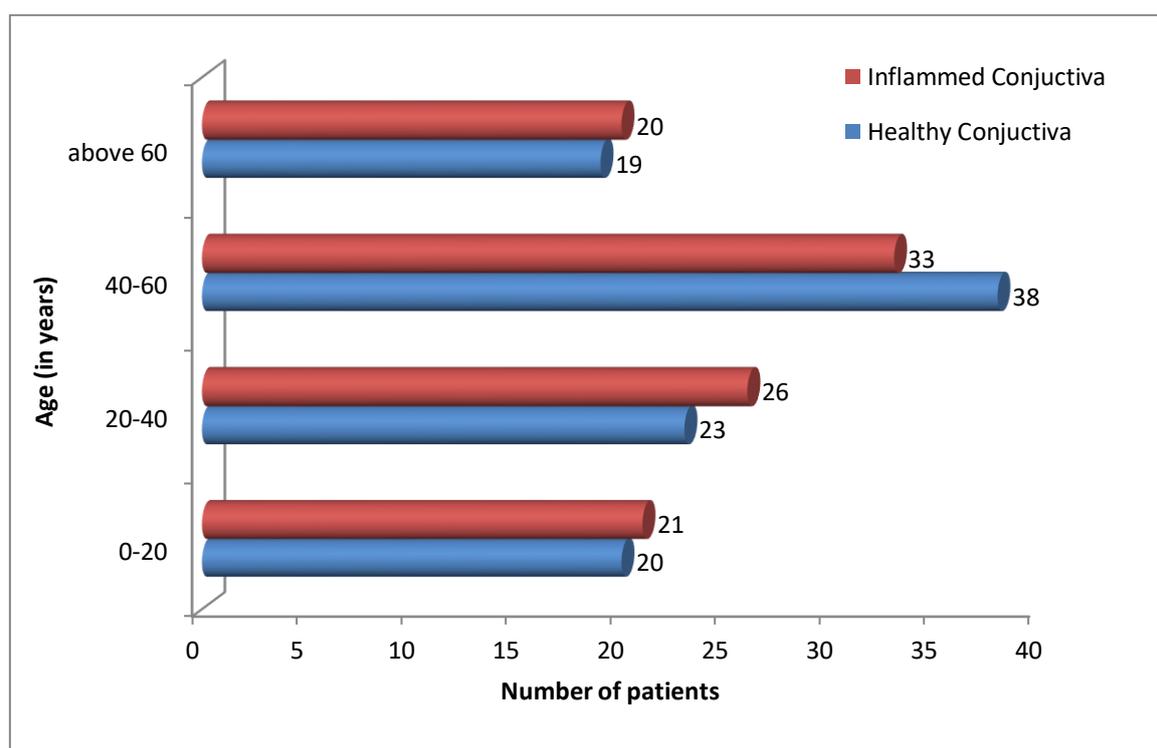


Fig.1 Age incidence in cases of acute conjunctivitis and normal conjunctivitis

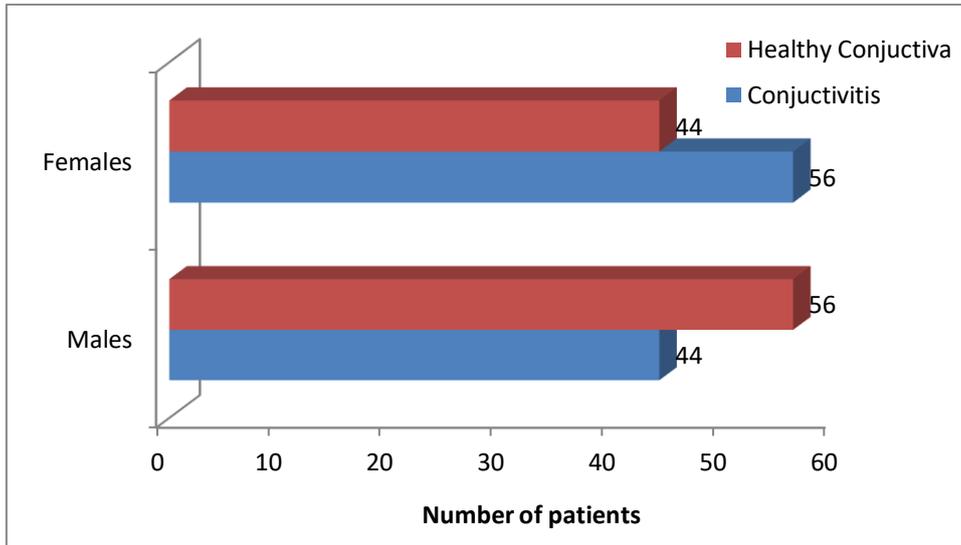


Fig 2: Sex distribution in control and study group

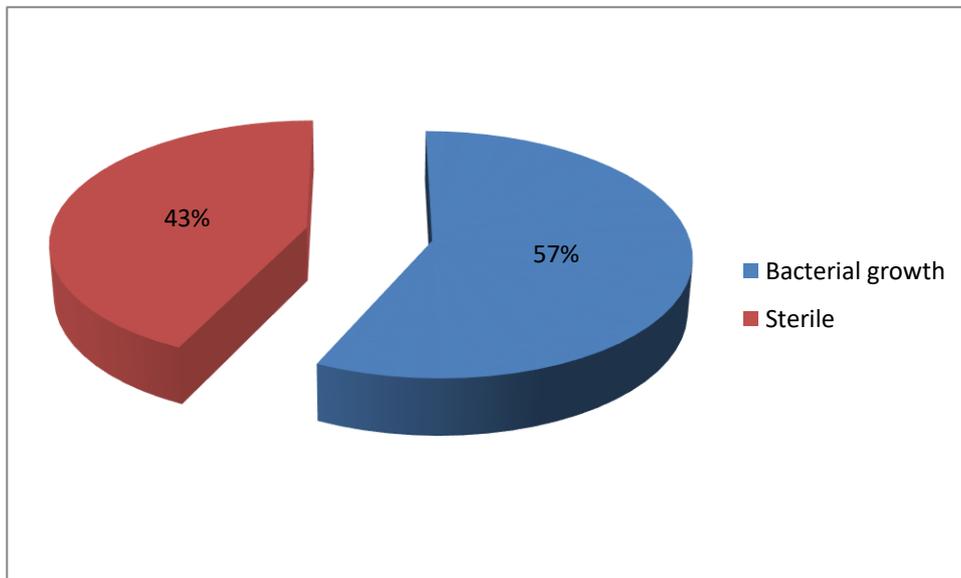


Fig 3: Bacterial flora in Normal Conjunctiva

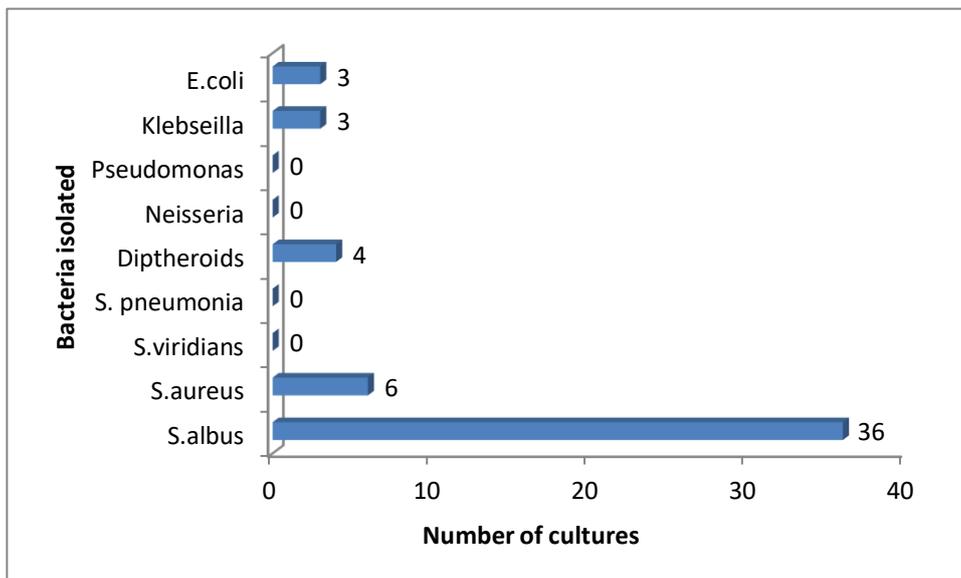


Fig 4: Bacteria isolated from normal conjunctiva

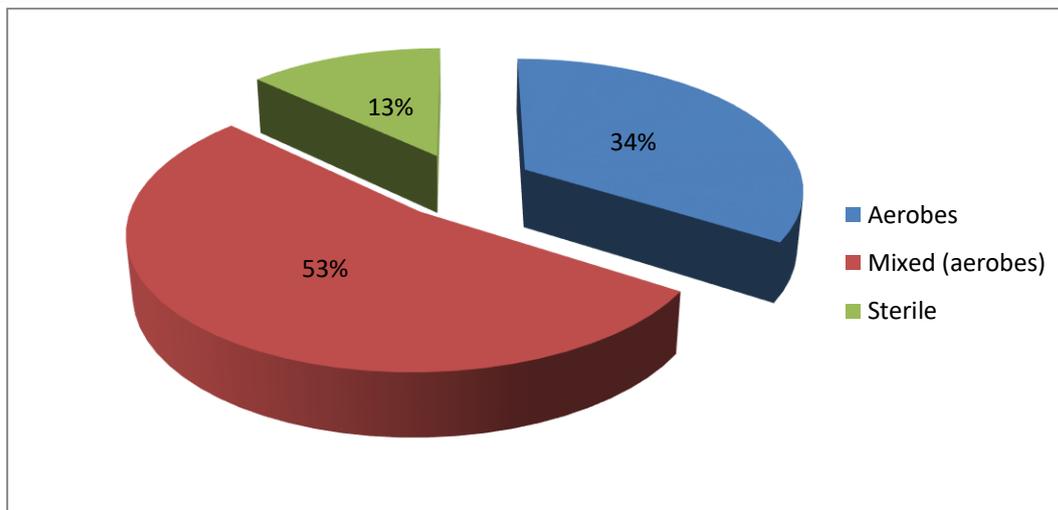


Fig 5: Bacterial flora in inflamed conjunctiva

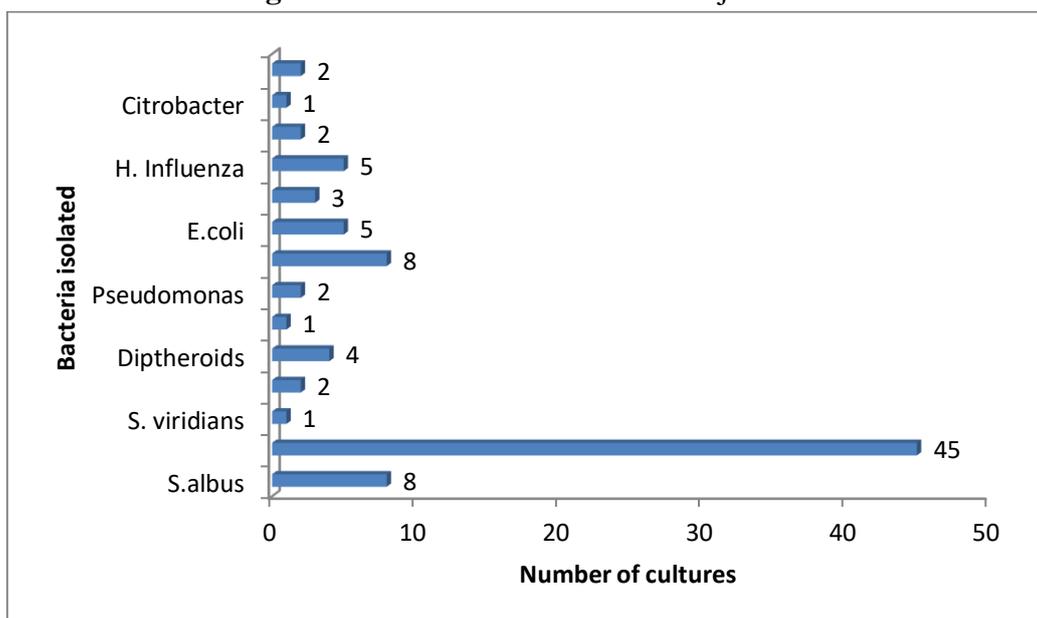


Fig 6: Bacteria isolated from inflamed conjunctiva

Table 1: Clinical presentation of the patients

Ocular symptoms	No. of cases
Discomfort	90
Pain	50
Tearing	84
Photophobia	-
Itching	65
Ocular signs	No. of cases
Erythema & swelling of the eyelids	6
Discharge	
Serous	12
Mucoid	25
Mucopurulent	36
Purulent	7
Redness of the conjunctiva	98
Chemosis of bulbar conjunctiva	16
Subconjunctival haemorrhages	5
Corneal involvement	-
Palpable lymphnodes	-
Disturbances of the vision	-

Table 2(a): Antibiotic sensitivity patterns of aerobic bacteria isolated from healthy conjunctiva

Organisms	No. of cases	Am	Ch	Tet	Clo	Ery	Gen	Sep	Str	Neo	Gab	Cip
<i>S.albus</i>	36	21	-	16	25	12	-	10	-	15	31	35
<i>S.aureus</i>	6	3	-	3	5	3	-	4	-	4	6	6
<i>Klebsiella</i>	3	2	2	-	3	-	2	-	2	-	3	3
<i>E.coli</i>	3	2	2	-	3	-	3	-	2	-	3	3

Table 2(b): Antibiotic sensitivity patterns of Aerobic bacteria isolated from Inflamed Conjunctiva

Organisms	No. of cases	Am	Ch	Tet	Clo	Ery	Gen	Sep	Str	Neo	Gab	Cip
Gram positive												
<i>S.albus</i>	8	5	-	4	7	5	-	4	-	3	8	8
<i>S.aureus</i>	45	30	-	39	40	37	-	34	-	36	43	45
<i>S.viridans</i>	1	1	-	1	1	R	-	R	-	1	1	1
<i>S.pneumonia</i>	1	1	-	1	1	R	-	R	-	1	1	1
Gram negative												
<i>Neisseria</i>	1	R	1	-	1	-	R	-	1	-	1	1
<i>Klebsiella</i>	8	6	6	-	7	-	6	-	7	-	8	8
<i>E.coli</i>	5	3	3	-	4	-	4	-	4	-	5	5
<i>Pseudomonas</i>	1	R	R	-	R	-	1	-	-	-	1	1
<i>Proteus</i>	3	2	2	-	3	-	2	-	2	-	3	3
<i>H.influenzae</i>	4	2	3	-	3	-	2	-	3	-	3	4
<i>Enterobacter</i>	5	2	2	-	3	-	2	-	2	-	3	3

Discussion

The conjunctiva is exposed to the outer world but is unprotected by keratinized layer. It is liable to a host of exogenous infections. Several studies have been performed to isolate aerobic commensals and pathogenic bacteria from normal and inflamed conjunctival sac but limited work has been done on presence of anaerobic bacteria. The present study was done on one hundred eyes with normal conjunctiva (control group) and an equal number of eyes with inflamed conjunctiva for aerobic bacterial flora.

In this study out of 100 patients, 56 were females (56%) while 44 were male (44%). In a similar study on cataract patients females predominated. In another study by Karthika *et al.*, (2014) 54% were females while 46% were males. In the study conducted by Belur and Keshav 2012) 23 (33.9%) were males and 37 (66.1%) were females. In our study, we were able to isolate bacteria in 57% cases of healthy eyes and 48% of inflamed eyes. In another study by Karthika *et al.*, (2014) isolation of bacteria was done from 58% of eyes while Reza *et al.*, (2008) and Belur and Basu (2012) reported 52.4% and 48.3% of isolation.

Studies on the frequency of normal conjunctival sacs which are sterile have shown marked disparity. At the low end of the spectrum, 9.4%, 9%, 2-5%, and 0% of conjunctivae to be sterile are reported in various studies. (Karthika *et al.*,2014). In our study, the conjunctiva was sterile in 43% of cases in healthy cases and 13% sterile in inflamed cases. The aerobic bacterial flora of the normal adult human conjunctiva has been studied by numerous authors during the past 50 years. In this study the most common isolate was *S. aureus* (31.69%) followed by *S.albus* and *Klebsiella* (5.63%), in the patients with inflamed conjunctiva. On the contrary, in cases of normal conjunctiva *S. albus* (50%) was most abundant. The isolates were subjected to antibiotic sensitivity testing to commonly used antibiotics like Ampicillin, Chloramphenicol, Tetracycline, Cloxacillin, Erythromycin, Gentamycin, Neomycin and Ciprofloxacin and the isolates were found to be sensitive to above, antibiotics, which is similar to study conducted by Kudva *et al.*,(2012). It is concluded that the difference between bacterial cultures isolated from normal and inflamed conjunctiva is statistically highly significant at p value of (0.01).

Abbreviations

Am-Ampicillin, Ch-chloramphenicol, Tet-Tetracycline, Clo- Cloxacillin, Ery-Erythromycin, Gen- Gentamycin, Sep-Septran, str- Streptomycin, Neo-Neomycin, Gab- Gabromycin, Cip- Ciprofloxacin, R-resistant.

References

1. Watson S, Cabrera-Aguas M, Khoo P. Common eye infections. *Aust Prescr* 2018;41:67–72
2. Epling J. Bacterial conjunctivitis. *Clinical Evidence* 2012;02:704
3. Ribet D and Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. *Microbes and Infection* 17 (2015) 173e183
4. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW and Saravanan M. Bacterial profile of ocular infections: a systematic review. *BMC Ophthalmology* (2017) 17:212
5. Van Gelder RN. Ocular Pathogens for the Twenty-First Century. *American Journal of Ophthalmology*. 2010, Volume 150, Issue 5, Pages 595–597
6. Johnson SA, Goddard PA, Iliffe C, Timmins B, Rickard AH, Robson G and Handley PS. Comparative susceptibility of resident and transient hand bacteria to para-chloro-meta-xyleneol and triclosan. *Journal of Applied Microbiology* 2002, 93, 336–344.
7. Nigam D. Microbial Interactions with Humans and Animals. *International Journal of Microbiology and Allied Sciences*. Nov 2015, 2(2):1-17.
8. Sharma PD, Sharma N, Gupta RN, Singh P. Aerobic bacterial flora of the normal conjunctiva at high altitude area of Shimla Hills in India: a hospital based study. *Int J ophthalmology* 6(5) 2013.
9. Alash SAAAA. Study the Prevalence of Bacterial Conjunctivitis in Iraq. *Iraqi Journal of Science*, 2015, Vol.56, No.4C, pp: 3371-3375
10. Okesola AO and Salako AO. Microbiological profile of bacterial conjunctivitis in Ibadan, Nigeria. *Annals of Ibadan Postgraduate Medicine*. Vol.8 No.1 June, 2010
11. Kowalski R.P., Karenchak L.M. and Romanowski E.G. Infectious disease: changing antibiotic susceptibility. *Ophthalmol Clin North Am* 2003;16(1-9):13.
12. Karthika N, Neelima A, Ramchandran S. A study of normal bacterial flora of the conjunctiva in patients undergoing cataract surgery in a rural teaching hospital in R. R. district. *J. Sci and Inn Res* 2014; 3 (2): 164-167
13. Belur RK, Basu S. Normal conjunctival flora and their antibiotic sensitivity in Omanis undergoing cataract surgery. *Oman j of ophthalmology* 2012;5(1):16-18.
14. Reza M, Madani H, Ghaderi E. Conjunctival bacterial flora and antibiotic resistance pattern in patients undergoing cataract surgery. *Pak j med sci* 2008;24:581-5
15. Kudva AA, Kadari R, Bhandary R, Hegde KS, Achar A, Devika P, Serrao V. A study of normal bacterial flora on the conjunctiva of patients undergoing cataract surgery to select the best pre-operative topical antibiotic. *Ij-Ajims* 2012;1(2):139-142.