Original Article

# Comparison of Indigenous Microbial Flora of The Eye to That Found in Conjunctival and Corneal Infections in A Hospital Based Study

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*Pak J Ophthalmol 2006, Vol. 22 No.2*

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Received for publication June 2005

**Purpose:** To determine the normal indigenous microbial flora and to compare it with pathogens, which cause conjunctivitis and keratitis.

**Material and Methods:** A case-control prospective hospital based study was done. The control group (350 persons) included patients waiting for cataract surgery, hospital personnel, visitors and accompanying persons, while the cases group (150 persons) comprised of individuals with bacterial conjunctivitis, fungal and bacterial keratitis. Gram and Giemsa staining was performed on all specimens from conjunctival swabs and corneal scrapings. Blood, chocolate and Sabouraud's agars were used to grow the bacteria and fungi. Sensitivity of yielded bacteria was checked against antibiotics using standard sensitivity discs.

**Results:** The study demonsrtated a male preponderance in both groups. Common organisms found in the control group were Staph. epidermidis, Staph. aureus, Strep. pneumoniae and Diptheroides spp, while those found as pathogens were Staph. aureus, Strep. pneumoniae, Staph. epidermidis and Strep. pyogenes in the conjunctivitis group and Strep. pneumoniae, Staph. aureus, Hemophilus spp. and Pseudomonas in the keratitis group. In the cases group, over 80% of Staph. aureus showed sensitivity against gentamicin, chloramphenicol and cephradin. About 80% of pneumococci were sensitive to chloramphenicol, cephradin and erythromycin. The presence of Staph. epidermidis in the control group was highly significant ($p<0.000$). Staph. aureus did not show any significant difference in the either groups. Strep. pneumoniae, Staph. pyogenes, Pseudomonas and fungi had a significantly higher prevalence in the keratitis and conjunctivitis groups.

**Conclusions:** Gentamicin and chloramphenicol are still an effective and economical first line treatment for most cases of conjunctivitis and keratitis.

**Key Words:** Ocular commensals, Ocular opportunistic pathogens, Microbial flora of the eye, Conjunctivitis, Keratitis, Eye infections.

Bacterial and fungal infections are an important but complex group of ocular diseases. A variety of infective processes may involve the eye. The source of bacteria or fungus can be local i.e. from the lids or conjunctival sac, or it may be from a remote site like sinuses or nasopharynx. Bacterial or fungal keratitis can result in severe visual impairment or even blindness. Thus, it is vital to understand the pathogenic mechanisms of infective disease, role of commensals, bacterial resistance and newer antibiotics that are more specific in their coverage. Data from different geographical areas differ with regard to the leading cause of bacterial and fungal infections.

This study was performed to determine the normal indigenous microbial flora and to compare it with pathogens, which cause conjunctivitis and keratitis.

## MATERIAL AND METHODS

A prospective case control study was conducted at Al-Shifa Trust Eye Hospital, Rawalpindi over one year period. This is a tertiary care and referral eye hospital and WHO Collaborating Centre for Prevention of Blindness with modern facilities for examination and diagnosis. All laboratory work was done at the Al-Shifa Reference Laboratory for Blindness caused by Infections (EYE LAB). EYE LAB is well equipped and has all the facilities for diagnosis of ocular infections.

Patients for the study were drawn from the outpatient clinics of the hospital. All newly diagnosed and consecutive patients attending the outpatient clinics with conjunctivitis or keratitis were considered for the study. The controls were selected randomly from accompanying persons, hospital personnel and patients with no corneal and conjunctival disease. 150 patients with corneal and conjunctival infection and 350 healthy controls were studied

All patients and controls recruited for the study had a complete external eye examination on slit-lamp and where indicated, posterior segment examination with indirect ophthalmoscope.

The sample size for subjects and controls were calculated using the EPI-INFO version 6 program to give results with 95% confidence interval.

### Specimen collection protocol

**a. Controls**

Conjunctival swabs were taken from both inferior fornices and the lid margins of both eyes. Cultiplast cotton swabs were used for specimen collection.

**b. Conjunctivitis**

In case of patients with conjunctivitis, swabs were taken from both eyes. The lower eyelid of the infected eye was pulled down gently and the swab rolled across the inferior tarsal conjunctiva and fornix. The same procedure was repeated in the other eye. Cultiplast cotton swabs were used for specimen collection.

**c. Keratitis**

In case of keratitis, material was collected with a sterilized Kimura spatula. The Kimura spatula was sterilized in an autoclave. The infected eye was anaesthetized with 0.5% Proparacaine eye drops. The necrotic material was first debrided and infected material collected from the base and the advancing edge of the ulcer.

Gram and Giemsa staining was performed on all the specimens. Blood agar, chocolate agar and Sabouraud's agar were used to grow the bacteria and fungi. Sensitivity of yielded bacteria was checked against antibiotics using standard sensitivity discs.

An informed consent was taken from all subjects and controls. The data obtained was recorded on a questionnaire and all information kept confidential only to be used for academic purposes. The study was approved by the ethics and research board of Al- Shifa Trust Eye Hospital, Rawalpindi.

## RESULTS

The study was conducted in two groups. One group (cases) included 150 patients with microbial conjunctivitis and keratitis. The other group (control) comprised of 350 individuals with healthy conjunctiva and cornea.

In the control group (350 individuals), there were 182 males and 168 females. The male to female ratio was 1.1:1. The distribution of healthy individuals in different age groups is shown in Table-1.

In the cases group, out of 150 patients, there were 90 males and 60 females, giving a ratio of 3:2. The distribution of patients in different age groups is given in (Table 1).

In 150 affected patients (300 eyes), the right eye was involved in 58 patients while the left eye was involved in 63 patients and both eyes in 29 patients.

The common presenting complaints included redness in 85 patients (56.7%), watering in 70 patients (46.7%), pain in 68 patients (45.3%), reduced vision in 44 patients (29.3%), discharge in 33 patients (22%) and other complaints like itching and blepharospasm in 5 patients (3.3%). 27 patients (18%) had a history of trauma causing a corneal ulcer.

In the cases group, the common clinical findings included mild conjunctivitis in 58 patients (38.7%), purulent conjunctivitis in 22 patients (14.7%), corneal ulcer in 36 patients (24%), corneal abscess in 17 patients (11.3), hypopyon in 14 patients (9.3), descemetocele in 5 patients (3.3%) and corneal perforation in 4 patients (2.7%) (Table 2).

Visual acuity of all the individuals was checked at the time of examination. Visual acuity of patients with keratitis are shown in Table 3. 52 (74.3%) out of 70 affected eyes with keratitis were suffering from severe visual impaired or blindness (<6/60 to no perception of light).

In the control group, 700 eyes of 350 individuals were tested for normal conjunctival flora. In 120 patients (34.3%), the micro organisms detected included Staphylococcus epidermidis in 69 individuals (57.5%), Staphylococcus aureus in 27 individuals (22.5%), Streptococcus pneumoniae in 10 individuals (8.3%), Diptheroides spp. in 4 individuals (3.3%), Streptococcus viridans in 3 individuals (2.5%), Haemophilus spp. in 2 individuals (1.7%), E. coli in 2 individuals (1.7%) and non-filamentous fungi (Candida albicans) in 3 individuals (2.5%) (Table 4)

80 out of 150 patients (cases group) presented with conjunctivitis. In 52 individuals (65%), a positive growth was obtained while 28 persons (35%) had false negative results. In the conjunctivitis patients that had a positive yield, the common organisms were Staphylococcus aureus in 15 patients (28.8%), Streptococcus pneumoniae in 13 patients (25.1%), Staphylococcus epidermidis in 11 patients (21.2%) Streptococcus pyogenes in 5 patients (9.6%), Diptheroides spp. in 3 patients (5.8%), Pseudomonas spp. in 2 patients (3.8%) and Neisserria gonorrhoeae in one patient (1.9%), filamentous and non-filamentous fungi (Candida albicans) in one patient each (1.9%) (Table 4).

70 patients (cases group) presented with keratitis. Causative agents were detected in 40 patients (57.1%). 30 patients showed false negative results. In these 30 patients (42.9%) keratitis was present clinically but no micro organism was detected. The causative agents detected in 40 (57.1%) patients were as follows - Streptococcus pneumoniae in 10 patients (25%), Staphylococcus aureus in 7 patients (17.5%), Pseudomonas spp. in 4 patients (10%), Staphylococcus epidermidis in 3 patients (7.5%), Streptococcus pyogenes in 3 patients (7.5%), Haemophilus spp. in 5 patients (12.5%), filamentous fungi in 5 patients (12.5%) and non-filamentous fungi (Candida albicans) in 3 patients (7.5%) (Table 4).

700 swabs were taken from 700 eyes of the control group. A positive growth was detected in 147 swabs (21%) of 120 persons (240 eyes). Staphylococcus epidermidis was detected in 86 swabs (58.5%), Staphylococcus aureus in 34 swabs (23.1%), Streptococcus pneumoniae in 13 swabs (8.8%), Streptococcus viridans in 4 swabs (2.7%), Diptheroides spp. in 4 swabs (2.7%), Haemophilus spp. in 2 swabs (1.4%), E. coli in 2 swabs (1.4%) and Candida albicans in 3 swabs (2.4).

Conjunctival swabs were taken from both eyes of 80 individuals who presented with conjunctivitis. 120 swabs were taken from affected eyes and 40 swabs were taken from healthy eyes. Out of 120 swabs from affected eyes, 70 swabs (58.3%) showed positive growth. Out of 40 swabs from healthy eyes, 12 swabs (30%) showed positive bacterial growth.

In the 120 swabs taken from affected eyes, Staphylococcus aureus was detected in 25 swabs (20.8%), Staphylococcus epidermidis in 17 swabs (14.2), Streptococcus pnuemoniae in 12 swabs (10%), Streptococcus pyogenes in 6 swabs (5%), Pseudomonas spp. in 2 swabs (1.7%), Diptheroides in 2 swabs (1.7%), and Neisseria gonorrhoae in 1 swab (0.8% each) (Table 10).

In 6 patients (12 eyes) with conjunctivitis, multiple organisms were detected. In 3 patients, Staphylococcus aureus was found in one eye and Staphylococcus epidermidis in the other eye. The 4th patient had Streptococcus pyogenes in one eye and Staphylococcus aureus in the other. The fifth patient revealed Streptococcus pneumoniae in one eye and Diptheroides spp. in the opposite eye. The sixth patient had a growth of Escherichia coli in one eye and Staphylococcus aureus in the fellow eye. Polymicrobial growth was detected in one eye of one patient. The culture from the conjunctival swab grew Staphylococcus aureus, Staphylococcus epidermidis and Diptheroides spp.

Conjunctival swabs were taken from diseased as well as healthy eyes of the patients presenting with keratitis. A positive growth was obtained from 8 conjunctival swabs from diseased eyes and 4 conjunctival swabs from healthy eyes. The growth pattern was similar to that detected from corneal scrapings in case of diseased eyes.

Out of 53 corneal scrapings, 40 (75.4%) gave a positive yield. Streptococcus pneumoniae was detected in 10 (18.8%) scrapings, Staphylococcus aureus in 7 (13.2%) scrapings, Haemophilus spp. in 5 (9.4%) scrapings, Pseudomonas spp. in 4 (7.5%) scrapings, Staphylococcus epidermidis in 3 (5.7%) scrapings, Streptococcus pyogenes in 3 (5.7%) scrapings, filamentous fungi in 5 (9.4%) scrapings and non-filamentous fungi (Candida albicans) in 3 (5.7%) scrapings.

Conjunctival swabs taken from 70 eyes with keratitis revealed Streptococcus pneumoniae in 4 swabs (5.71%), Haemophilus species in 2 swabs (2.85%) and Staphylococcus aureus and Pseudomonas spp. in 1 swab each (1.42%). The growths obtained through the conjucntival swabs in these 8 eyes corresponded to the growth obtained from corneal scrapings.

Conjunctival swabs were also taken from healthy eyes of patients presenting with keratitis. Staphylococcus aureus and Staphylococcus epidermidis were

**Table 1:** Age distribution by sex

| **Age in years** | **Control group** | | | **Cases group** | | |
|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total |
| 0 - 4 | 9 | 4 | 13 | 10 | 3 | 13 |
| 5 - 9 | 7 | 4 | 11 | 8 | 4 | 12 |
| 10 - 19 | 9 | 6 | 15 | 6 | 4 | 10 |
| 20 - 29 | 12 | 6 | 18 | 14 | 5 | 19 |
| 30 - 39 | 14 | 5 | 19 | 11 | 2 | 13 |
| 40 - 49 | 12 | 17 | 29 | 12 | 11 | 23 |
| 50 - 59 | 33 | 33 | 66 | 8 | 11 | 19 |
| 60 - 69 | 47 | 64 | 111 | 13 | 11 | 24 |
| 70 – 79 | 30 | 16 | 46 | 5 | 5 | 10 |
| 80 – 89 | 6 | 7 | 13 | 2 | 3 | 5 |
| 90 - 100 | 4 | 5 | 9 | 1 | 1 | 2 |
| Total | 183 | 167 | 350 | 90 | 60 | 150 |

**Table 2:** Type and frequency of clinical findings

| **Clinical findings** | **Male** | **Female** | **Total n (%)** |
|---|---|---|---|
| Corneal Ulcer | 22 | 14 | 36 (23.1) |
| Corneal abcess | 7 | 10 | 17 (11) |
| Hypopyon | 8 | 6 | 14 (9) |
| Corneal perforation | 3 | 1 | 4 (2.6) |
| Descemetocele | 3 | 2 | 5 (3.2) |
| Mild conjunctivitis | 32 | 26 | 58 (37.2) |
| Purulent conjunctivitis | 15 | 7 | 22 (14.1) |
| Total | 90 | 66 | 156 |

**Table 3:** Visual acuity of effected eyes with keratitis

| **Visual acuity** | **No. of effected eyes n (%)** |
|---|---|
| 6/6 - 6/ 18 | 3 (4.3) |
| < 6/18 - 6/60 | 15 (21.4) |
| < 6/60 - 3/ 60 | 20 (28.6) |
| < 3/60 - PL + | 29 (41.4) |
| NPL | 3 (4.3) |
| Total | 70 |

obtained from 2 swabs each (2.85% each). In one patient with keratitis, Haemophilus spp. was detected in the corneal scraping of one eye while Staphylococcus aureus was found in the conjunctival swab taken from the fellow eye. Polymicrobial growth was detected in one eye of one patient with Keratitis. Candida albicans and Staphylococcus aureus were isolated from the corneal scrapings from the affected eye.

The type and frequency of microorganisms detected, was reviewed in different age groups. In the control group, Staphylococcus aureus and Staphylococcus epidermidis were common among the 50-79 years age group In the case group, Staphylococcus aureus was found in almost all age groups and Streptococcus pneumoniae was common in children (0-4 years).

Antibiotic sensitivity was checked against all the organisms detected, using standard sensitivity discs. In the control group, about 70% of Staphylococcus epidermidis were sensitive to gentamicin, chloramphenicol, cephradin and erythromicin. More than 70% of Staphylococcus aureus were sensitive to gentamicin, chloramphenicol and cephradin. In contrast, 60% of pneumococci showed resistance against gentamicin (Table 5).

In the cases group, more than 80% of Staphylococcus aureus showed sensitivity against gentamicin, chloramphenicol and cephradin. About 80% of Pneumococci were sensitive to chloramphenicol, cephradin and erythromicin. Sensitivity of Staphylococcus epidermidis against cephradin was more than 70% (Table 5).

The prevalence of differe nt organisms was subjected to statistical analysis using the chi square test ($X^2$). Compared to the control group, the prevalence of any microbial growth in patient with conjunctivitis (52/80, 65%) was highly significant at $\alpha = 0.01$ with $p<0.000$. Furthermore, compared to the Control Group, the prevalence of any microbial growth in patients with Keratitis (40/70, 57%) was also highly significant at $\alpha = 0.01$ with $p<0.005$ (Table 6).The detection of Staphylococcus epidermidis in the Control Group was highly significant ($p<0.000$), as compared to the Keratitis and Conjuctivitis Groups table 6. Staphylococcus aureus did not show any significant difference in its prevalence in the Control, Keratitis or Conjuctivitis Groups (Table 6).

Streptococcus pneumoniae had a significantly higher prevalence in the Keratitis Group ($p<0.012$) and the Conjunctivitis Group ($p<0.006$) as compared to the Control Group (Table 6).

Fungi, Strep pyogenes and Pseudomonas spp. all demonstrated a significantly higher prevalence in the Keratitis and Conjunctivitis Groups compared to the Control Group (Table 6).

## DISCUSSION

Ocular infections are one of the leading causes of blindness throughout the world. In Pakistan, blinding infections caused by bacteria, fungi, viruses and parasites are a major public health problem. Ocular infections are responsible for blindness in both eyes in 260,000 people and in one eye in about 390,000 people [1].

There are 2.4 million people blind in both eyes, 2.5 million people blind in one eye and 5 million have visual impairment. Therefore, almost 10 million people are visually handicapped in Pakistan. Among these corneal opacities cause blindness in 13% of 10 million people. Over 300,000 people are blind from corneal opacities due to infection[2].

A population based ocular survey was conducted in NWFP (North Western Province of Pakistan) in 1994. It revealed that 1.99% of the population of NWFP was blind in both eyes and 1.90% was blind in one eye by WHO standards. Corneal opacities were a cause of blindness in 11% of the affected population in the Northern zone and 12% of the affected population in the Southern zone of NWFP[3].

Other studies done elsewhere have also emphasized the importance of corneal infections as an important cause of visual loss. Daghfous and colleagues found that in the rural areas of southern Tunisia, corneal blindness was the second commonest cause for sight loss and responsible for almost 25% of all cases of blindness. One of most frequent etiologies was corneal ulcer[4]. A population-based survey was conducted in Tanzania to determine the prevalence of major blinding disorders. Corneal opacities were responsible for 44% of bilateral and 39% of monocular blindness. Corneal infection was one of the major causes[5]. Our study showed a preponderance of male patients with anterior segment infections (Male: Female = 3:2). A possible cause for this could be that since men are the main bread-earners of the family, they cannot afford for their disease to be prolonged, thus causing financial problems. On the other hand, women tend to remain with the families to perform their duties and oftentimes do not report in the hospital for mild to moderate infections. Another contributory factor could be their dependence on the males for their treatment. There is also a clear predominance of males (72%) in the paediatric age group (0-9 years). This is consistent with similar work done by Cruz and associates in Florida, in which they found a male preponderance of 68% [6]. More than halves of the males were between 20-70 years of age and about 50% of the females were between 40-70 years, indicating that these age groups are prone to corneal and conjunctival infections.

Out of 150 patients in the cases group, 25 patients (18 males, 7 females) were in the paedriatic age group (0-9 years). About two-thirds of the population lives in the rural areas and children are often exposed to a dry and dusty environment. Insufficient supply of clean water may also be a contributing factor. Furthermore, parents are more conscious about the health of their children and tend to report early to the hospital for their treatment.

Most of the patients with corneal infections presented as ulcerative keratitis (36 cases). The second most common presentation was corneal abscess (17 cases). On a review of recent literature from western countries, similar presentations have been found by other workers[7-9]. Mehmood A in his study on corneal infections found that almost equal number of cases presented with ulcer and abscess [10]. This difference may partly be explained by the heightened community awareness campaigns in the last few years with patients now seeking medical advice early.

**Table 4**: Type and Frequency of microorganisms

| Organisms | No of individuals | | | |
|---|---|---|---|---|
| | **Control n (%)** | **Conjunctivitis n (%)** | **Keratitis n (%)** | **Total n (%)** |
| Staph. epidermidis | 69 (57.5) | 11 (21.2) | 3 (7.5) | 83 (39.2) |
| Staph. aureus | 27 (22.5) | 15 (28.8) | 7 (17.5) | 49 (23.1) |
| Strep. pneumoniae | 10 ( 8.3) | 13 (25) | 10 (25) | 33 (15.6) |
| Diptheroides spp. | 4 ( 3.3) | 3 (5.8) | 0 (0) | 7 (3.3) |
| Strep. viridans | 3 ( 2.5) | 0 (0) | 0 (0) | 3 (1.4) |
| Hemophilus spp. | 2 ( 1.7) | 0 (0) | 5 (12.5) | 7 (3.3) |
| E. coli | 2 ( 1.7) | 0 (0) | 0 (0) | 2 (0.9) |
| Neisseria Gonorrhoeae | 0 (0) | 1 (1.9) | 0 (0) | 1 (0.5) |
| Strep. Pyogenes | 0 (0) | 5 (9.6) | 3 (7.5) | 8 (3.8) |
| Pseudomonas | 0 (0) | 2 (3.8) | 4 (10) | 6 (2.8) |
| Fungi (Candida spp.) | 3 ( 2.5) | 1 (1.9) | 3 (7.5) | 7 (3.3) |
| Filamentous fungi | 0 (0) | 1 (1.9) | 5 (12.5) | 6 (2.8) |
| Total | 120 (100) | 52 (100) | 40 (100) | 212 (100) |

**Table 5:** Antibiotic sensitivity of bacteria

<table>
<tr><th rowspan="3">Antibiotics</th><th colspan="4">Staph Epidermidis</th><th colspan="4">Staph Aureus</th><th colspan="4">Pneumococci</th></tr>
<tr><th colspan="2">Control Group</th><th colspan="2">Cases Group</th><th colspan="2">Control Group</th><th colspan="2">Cases group</th><th colspan="2">Control Group</th><th colspan="2">Cases group</th></tr>
<tr><th>S n (%)</th><th>R n(%)</th><th>S n (%)</th><th>R n(%)</th><th>S n (%)</th><th>R n(%)</th><th>S n (%)</th><th>R n(%)</th><th>S n (%)</th><th>R n(%)</th><th>S n (%)</th><th>R n(%)</th></tr>
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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gentamicin | 67 (78) | 19 (22) | 9 (64) | 5 (36) | 27 (79) | 7(21) | 19(86) | 3(14) | 5(38) | 8(62) | 13(57) | 10(43 ) |
| Chloramphenicol | 60 (70) | 26 (30) | 77 (50) | 77 (50) | 257 (73) | 97 (27) | 187 (82) | 47 (18) | 117 (85) | 27 (15) | 217 (91) | 27 (9) |
| Cephradin (velosef) | 707 (81) | 167 (19) | 107 (71) | 47 (29) | 267 (76) | 87 (24) | 207 (91) | 27 (9) | 107 (77) | 37 (23) | 207 (84) | 37 (13) |
| Erythromycin | 627 (72) | 247 (28) | 97 (64) | 57 (36) | 197 (56) | 157 (44) | 167 (73) | 67 (27) | 97 (69) | 47 (31) | 217 (91) | 27 (9) |
| Tobramycin | 607 (70) | 267 (30) | 87 (57) | 67 (43) | 247 (70) | 107 (30) | 177 (77) | 57 (23) | 107 (77 | 37 (23) | 177 (74) | 67 (26) |
| Cloxacillin | 65 (76) | 21 (24) | 9 (64) | 5 (36) | 21 (62) | 13 (38) | 15 (68) | 7 (32) | 9 (69) | 4 (31) | 18 (78) | 5 (22) |
| Penicillin | 62 (72 | 24 (28) | 8 (57) | 6 (43) | 22 (65) | 12 (35) | 12 (55) | 10 (45) | 10 (77) | 3 (23) | 19 (83) | 4 (17) |

**S**= Sensitive **R**= Resistant

**Table 6**: Level of significance of microbial detection in control versus keratitis and conjunctivitis groups

| **Types of Microbes** | **Control n (%)** | **Keratitis n (%)** | **Conjunctivitis n (%)** | **Test of significance=X2** | |
|---|---|---|---|---|---|
| | | | | **Keratitis VS control** | **Conjunctivitis VS control** |
| Staph. Epidermidis | 69 (57.5) | 3 (7.5) | 11 (21.2) | P <.000 | P <.000 |
| Staph. Aureus | 27 (22.5) | 7 (17.5) | 15 (28.8) | N.S | N.S |
| Strep. Pneumoniae | 10 (8.3) | 10 (25) | 13 (25.1) | P <.012 | P <.006 |
| Diptheroides SPP | 4 (3.3) | 0 (0) | 3 (5.8) | N.S | P <.028 |
| Strep. Viridans | 3 (2.5) | 0 (0) | 0 (0) | N.S | N.S |
| Haemophilus SPP | 2 (1.7) | 5 (12.5) | 0 (0) | P <.011 | N.S |
| E. Coli | 2 (1.7) | 0 (0) | 0 (0) | N.S | N.S |
| Fungi | 3 (2.5) | 8 (20) | 2 (3.8) | P <.000 | P <.03 |
| Strept. Pyogenes | 0 (0) | 3 (7.5) | 5 (9.6) | P <.012 | P <.012 |
| Pseudomonas SPP | 0 (0) | 4 (10) | 2 (3.8) | P <.000 | P <.003 |
| N. Gonorrhoea | 0 (0) | 0 (0) | 1 (1.9) | N.A | N.S |
| | 120 (100) | 40 (100) | 52 (100) | | |

X2 -Chi Square
N.S -Not significant
NA -Statistical analysis not advised due to very small numbers

The common organisms detected in the control group were Staph. epidermidis (58.5%), Staph. aureus (22.5%), Strep. pneumoniae (8.3%) and Diptheroides spp. (2.7%). This suggests that these microorganisms are commonly found in the conjunctival sac of the Pakistani population in this region of the country. This compares well with other studies on the normal flora of the conjunctival cul-de-sac. Locatcher-Khorazo and Seegal studied the normal flora of 10,000 healthy eyes and isolated Staph. epidermidis (37%), Staph. aureus (17%), Diptheroides (1%), combination of these three organisms (35%) and miscellaneous growth in (9%)[11].

Gritz et al studied the conjunctival flora of 42 persons. They isolated Staph. Epidermidis in 54.8% and Diptheroides in 9.5% subjects. But they did not detect any Staph. aureus. This may be due to the small sample size of their study[12].

Larkin and Leeming studied the normal ocular flora of 34 individuals and compared it with that of contact lens users. Staph. epidermidis was the most prevalent species among healthy individuals. They also found Corynebacterium spp. in 6 individuals. This however, was not detected in our series[13].

The types of bacteria were almost similar in all age groups. Staph. epidermidis was the most common organism followed by Staph. aureus and Strep. pneumoniae. Thiel and Schumacher studied ocular flora of 135 persons of various age groups (3-90 years). They found characteristic changes in the flora at different stages of life, which suggested that with increasing age, aerobic cocci were found less frequently and the proportion of anaerobic cocci increased[14].

Diptheroides spp. was detected in 3.3% of normal individuals. Soudakoff in 1954 cultured Diptheroides from 2.8% of eyes in his Los Angeles based series[15]. The frequency of Diptheroides detection was found to increase with increasing age. This pattern has also been found in other studies[16,17].

Weis et al studied normal flora of 91 children and found Staphylococci, Corynebacteria and alpha hemolytic Streptococci as predominant organisms[18]. Corynebacteria were not encountered in our series.

Other species like Strep. viridans, Haemophilus and E.coli were detected less frequently. Perkin and coworkers in their study of the flora of 90 healthy eyes, found Propionibacterium acnes and anaerobic Diptheroides predominantly, and Lactobacillus spp., Eubacterium spp. and Peptostreptococcus spp. less frequently[19]. Thiel and Schumacher detected Megasphaera elsdenii, Bacteroides urolyticus, Bacteroides pneumosintes, Stomatococcus mucilaginosos and group ANF Corynebacterium for the first time in the eye[14].

No fungi were detected in the paedriatic and younger age groups in our study. Candida albicans was detected in 3 individuals above 40 years. Filamentous fungi were not detected as normal commensals of the cul- de- sac. This is in contrast to the study conducted by Rao and Rao who detected Aspergillus spp. from normal conjunctiva[20].

80 of 150 patients presented with conjunctivitis. 65% yielded a positive growth. Studies done previously have found that 32% - 88% of patients with external ocular bacterial infections have positive cultures[21,22]. In our study, Staph. aureus was the most common organism detected in 28.8%. Strep. pneumoniae and Staph. epidermidis were found in 25.1% and 21.8% respectively. Our results differ from those of Spitzy et al, who studied 120 patients with conjunctivitis and found Staph. epidermidis as the most common cause of bacterial conjunctivitis followed by Staph. aureus[23]. In a series by Grabsons et al, Staph. epidermidis was again the most prevalent (74%) followed by Staph. aureus (12%)[24].

Weiss et al studied 95 children with acute conjunctivitis. The major pathogens cultured from conjunctival specimens were Haemophilus influenzae, Strep. pneumoniae and Moraxella. The common organisms in the paediatric age group (0 - 9 years) in this study were Strep. pneumoniae, Staph. epidermidis and Staph aureus. This indicates that Strep pneumoniae is one of the common organisms that causes conjunctival infection in children[18]. Work done by Mahajan et al also supports Strep. pneumoniae as a cause of bacterial conjunctivitis in children[25].

Diptheroides, Pseudomonas spp. and N.gonorrhoeae were detected in a small percentage of patients. It is possible that these are opportunistic organisms that are able to cause ocular infection. The lowered resistance of these eyes against infection may have played a role in predisposing them to microbial keratitis.

Fungi (filamentous and Candida spp.) were also found in a small number of cases with conjunctivitis (1.9%). This finding is significant because oftentimes conjunctivitis is thought to be bacterial and a possible fungal cause is neglected. It also emphasizes the need for a conjunctival culture before instituting treatment.

70 out of 150 patients presented with keratitis. 52 (74.3%) out of 70 affected eyes with keratitis were either severely visually impaired or blind by World Health Organization standards (<6/60 to No Perception of Light). A significant number of eyes with corneal infection end up blind. Out of 927 keratoplasties done at a Toronto Hospital in Canada, 4.3 % were due to bacterial infections[26].

We used a sterilized Kimura spatula for specimen collection in case of corneal ulcers. Cotton swabs were used for conjunctival specimens. Microorganisms were detected more frequently when corneal ulcers were scraped with a spatula. Banson and Lanier have shown that calcium alginate swabs give a better yield than spatulas for detecting microorganisms in corneal ulcers[27]. Jacob et al found that a Bard Parker blade no.15 was as efficient as a calcium alginate swab for detection of bacteria. However, the swabs were more efficient in case of mycotic ulcers[28].

Conjunctival swabs were also taken along with corneal scrapings in keratitis patients. A positive growth was obtained from 8 conjunctival swabs from diseased eyes. The isolates detected from both specimens were similar. Wahl et al also found an association between corneal and ipsilateral conjunctival isolates[29].

Strep. pneumoniae was the most common organism causing keratitis (25%). It was followed by Staph. aureus (17.5%), Haemophilus spp. (12.5%) and Pseudomonas spp. (10%). This compares well to work done by Tassaduq[1] a few years earlier who found Strep. pneumoniae (27.4%), Staph. epidermidis (19%) and Staph. aureus (9.5%). Ammous and Noorsunba in Kuwait, on the other hand, found Staph. epidermidis to be predominant followed by Pseudomonas and Strep. pneumoniae[30]. Another study showed Staph. epidermidis as the most common isolate followed by Staph. aureus and Pseudomonas[29]. Strep. pneumoniae appears to be more prevalent as a cause of ulcerative keratitis in our population.

Pseudomonas spp. was not a common cause of keratitis in our study. In a series from South Florida, P. aeruginosa was found to be the single most common organism responsible for corneal ulceration[31]. We found Pseudomonas mostly in older patients (above 50 years) with keratitis. Ormerod also found Pseudomonas along with Strep. pneumoniae as a common cause of microbial keratitis in older patients[32].

In the paediatric age group, Strep. pneumoniae was the most common organism causing infections of the conjunctiva and cornea. It was followed by Staph. aureus and Staph. epidermidis. Pseudomonas was not detected in children in this study. In a large series at Bascom Palmer Eye Institute in Florida, the most common organisms were Pseudomonas (34%). Staph. aureus (20%) and fungi (18%)[6].

8 out of 70 patients (11.4%) with corneal ulcer presented with fungal keratitis. In 5 patients, filamentous fungi were detected and in 3 patients non-filamentous fungi (Candida albicans) were isolated. Filamentous fungi like Aspergilus spp. are the most common cause of fungal keratitis in India[33]. Aspergilus has been found to be the most prevalent fungus in other studies as well[34,35]. The high prevalence of Aspergilus spp. may be due to the fact that spores of Aspergilus can survive the hot and dry weather of these countries. No fungus was detected in children in our study. A probable cause for this could be the small sample size, since the overall prevalence of childhood mycotic keratitis is about 10.8% according to Panda et al. Panda et al in India studied 211 cases of childhood mycotic keratitis and found in decreasing frequency Aspergilus spp., followed by Fusarium, Alternaria, Curvularia and Penicillium[36].

The sensitivity of all the isolates was checked against Gentamicin, Chloramphenicol, Erythromycin, Tobramycin, Cloxacillin and Penicillin. In the control group, more than 70% of Staph. epidermidis showed sensitivity against all these drugs. About 70% of Staph. aureus showed sensitivity against these drugs except Penicillin. This indicates effectivity of these drugs for Staphylococcal infections. More than 45% of Staph. aureus were resistance against Penicillin. In case of Staph. Epidermidis, the sensitivity was about 60% against these drugs except for Chloramphenicol (50%). A significant number of Staphylococci showed resistance against Gentamicin, 14% in case of Staph. aureus and 36% in case of Staph. epidermidis. With changing sensitivity patterns, ophthalmologists should be aware of the strains that are resistant to Gentamicin[37]. Similarly Erythromycin resistant Staphylococci causing conjunctivitis have also been reported[38].

73%-90% of Pneumococci in the control group were sensitive against Chloramphenicol, Erythromycin, Tobramycin, Cloxacillin, Penicillin and Cephradin. The resistance to Erythromycin and Chloramphenicol was 8.6%. Mahajan et al in India found pneumococcal resistance to Erythromycin and Chloramphenicol to be 17.6% and 31% respectively[25]. Significant number of Pneumococci 43% showed resistance against Gentamicin in our study.

About 66% of Pseudomonas were sensitive to Gentamicin and Cloxacillin. However, more than 50% showed resistance to Chloramphenicol, Cephradin, Erythromycin, Tobramycin and Penicillin. Gentamicin is highly effective against Pseudomonas keratitis and it has been the mainstay in the treatment of Pseudomonas corneal ulcer[39]. The resistance of Pseudomonas to Tobramycin in our series was 50%. A resistance to Tobramycin has been found in many Gentamicin-resistant strains of Pseudomonas in other studies[40].

Staph epidermidis was the most common organism detected from normal individuals. It was also found in patients with external ocular infection. The role of Staph. epidermidis as a commensal of the cul-de-sac is highly suggestive and that it can cause infection of the conjunctiva and cornea. The second most common organism in healthy individuals and in patients with keratitis was Staph. aureus. It was the most common organism causing conjunctivitis. They are probably not pathogens since they are found with equal or greater frequency in normal eyes[41,42].

Strep. pneumoniae was not detected in the control group, but was found in patients with conjunctivitis (25%) and keratitis (25%). This indicates that Staph. pneumoniae is a pathogen and not a commensal.

Similarly, Pseudomonas was not detected in conjunctival swabs of healthy persons but was present in cases of conjunctivitis and keratitis, indicating the pathogenicity of Pseudomonas. Other microbes like Haemophilus spp., Diptheroides spp., Strep. pyogenes and N.gonorrhoeae were detected in a very small number of cases.

## CONCLUSION

The key findings of this study may be summarized as below:

The common organisms found harboring the normal conjunctival sac as commensals were Staph. epidermidis (57.5%), Staph. aureus (22.5%), Strep. pneumoniae (8.3%) and Diptheroides spp (3.3%).

The common organisms found as pathogens in the conjunctivitis group were Staph. aureus (28.8%), Strep. pneumoniae (25.1%), Staph. epidermidis (21.2%), and Strep. pyogenes (9.6%).

The common organisms found as pathogens in the keratitis group were Strep. pneumoniae (25%), Staph. aureus (17.5%), Hemophilus spp. (12.5%), and Pseudomonas spp. (10%).

In the control group, about 70% of Staph. epidermidis were sensitive to gentamicin, chloramphenicol, cephradin and erythromicin.

In the control group, over 70% of Staph. aureus were sensitive to gentamicin, chloramphenicol, and cephradin, while 60% of pneumococci showed resistance to gentamicin.

In the cases group, over 80% of Staph. aureus showed sensitivity against gentamicin, chloramphenicol, and cephradin. About 80% of pneumococci were sensitive to chloramphenicol, cephradin and erythromicin.

Staph. epidermidis the most common organism detected in all age groups of healthy individuals.

Conjunctival swabs are helpful in detection of microorganisms in case of conjunctivitis and keratitis. Scraping with Kimura spatula is a better way of specimen collection than cotton swabs in case of keratitis.

Fungi are an important cause of keratitis and conjunctivitis.

The detection of Staphylococcus epidermidis in the Control Group was highly significant ($p<0.000$), as compared to the Keratitis and Conjuctivitis Groups suggesting its presence as a normal commensal.

Staphylococcus aureus did not show any significant difference in its prevalence in the Control, Keratitis or Conjuctivitis Groups suggesting its role as an opportunistic pathogen.

Streptococcus pneumoniae had a significantly higher prevalence in the Keratitis Group ($p<0.012$) and the Conjunctivitis Group ($p<0.006$) as: compared to the Control Group indicating it to be a pathogen.

Fungi, Strep. pyogenes and Pseudomonas spp. all demonstrated a significantly higher prevalence in the Keratitis and Conjunctivitis Groups compared to the Control Group, suggesting their roles as pathogens.

There is an overriding need to establish specialised ocular microbiology laboratories in every eye hospital or eye unit. These ocular microbiology services can be provided by existing general microbiology personnel provided they have adequate training. Ophthalmologists and General Practitioners need to be aware of the conjunctival commensals and pathogens causing conjunctivitis and keratitis.

There is also a need to disseminate information to ophthalmologists at teriary and secondary levels about changing microbiological sensitivity patterns. It is reassuring to confirm that Gentamicin and chloramphenicol are still an effective and economical first line treatment for most cases of conjunctivitis and keratitis.

A thorough understanding of the differences in the conjunctival flora of healthy and diseased eyes is essential for ophthalmologists. This knowledge can play an important role in interpretation of clinical culture results and in management of potential pathogens colonizing the ocular surface. It is also of particular concern when planning surgery since sterility at the time of surgery presumably decreases the frequency of postoperative infection.

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