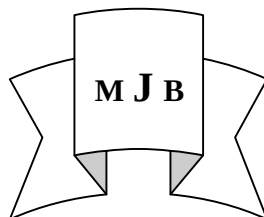


Normal Conjunctival Flora In Iraqis

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Abstract

The conjunctival sac is practically never free from microorganisms. In order to define the population of normal conjunctival flora, in Iraqis, 627 conjunctival swabs were obtained from healthy subjects and cultured on ordinary microbial culture media (blood, Mac Conkey and chocolate agar plates). The growth results were analyzed. The yield was 39% positive cultures, 34.4% *Staphylococcus epidermidis*, 2.4% *Diphtheroids*, 1.6% *Staphylococcus aureus*, 0.3% *Candida*-mixed with *S.epidermidis* and 0.16% *Bacillus* spp. Although the results were in consistence with most of the results in the textbooks and published papers, we recommend a more elaborative study to focus on other microbial flora such as the an aerobes or the facultative aerobes that would not be isolated in the traditional work.

البكتيريا غير المرضية لملتحمة العين عند العراقيين

الخلاصة

لغرض التعرف على البكتيريا غير المرضية لملتحمة العين في العراق أخذت ٦٢٧ مسحة من أغشية الملتحمة لأعين أشخاص سالمين من أي مرض عيني وزرعت في الأوساط الزرع البكتيرية التقليدية. كانت النتائج مقارنة لأكثر الدراسات المنشورة بأغلبية للمكورات العنقودية.

Introduction

The conjunctiva is a thick translucent mucous membrane. Its palpebral portion lies over the posterior portion of the eyelids and is firmly attached to the tarsal plate, while the bulbar portion lies loosely over the anterior globe with firm adherence to the limbus. The conjunctiva has a layer of submucosal substantia propria that consists of a superficial adenoid portion containing mainly connective tissue(1).

Although the conjunctival sac is directly exposed to the external world and is practically never free from microorganisms, it is remarkably resistant to infection(2,3). Tears, in combination with eyelid action, mechanically flush the ocular surface to reduce an ever

present bacterial load. Further, the tears contain immunoglobulins and components of the complement pathways namely lactoferrin, lysozymes and B-lysin that help kill microorganisms and decrease their adherence to the ocular surface(3,4). In healthy subjects the surface tissues are readily colonized by certain microbial species. The mixture readily found at any anatomical site is referred to as "normal flora"(4). Colonization of the eyelid and conjunctiva by normal microbial flora contributes to the defense of ocular surface. Indigenous bacterial flora inhibit the establishment of foreign, potentially pathogenic bacteria by elaborating

antibacterial substances and by competing for space and nutrients(4,5,6).

The conjunctiva harbors few and sometimes no microorganisms(7).The predominant isolates recovered from the eyelids and conjunctivae are Staphylococci(more than 60%;mostly S.epidermidis),Corynebacterium spp.and Propionibacterium acnes(3,4,5,6).Non hemolytic Streptococci, Neisseriae and gram negative bacilli resembling Haemophilus (Moraxella species) are also frequently present(7,8).In the neonatal periods the predominant bacteria are Staphylococcus species,E.coli and Streptococci. In children the predominant bacteria are hemolytic Streptococcus, Pneumococcus and Haemophilus.In adults more than fifty years the Diphtheroids type increases and the mixed type of normal microbial conjunctival flora could be seen with a decrease number of sterile conjunctiva(7).

Sometimes the conjunctivae remain sterile, but many people have normal microbial flora. There are no differences of the flora between sexes and right or left eyes(9).Table 1 summarizes the types of normal flora of the eye surface(1,4,5,6).

A wide variety of disorders can overcome the normal defenses of the eye and ocular adnexae.Our challenge as ophthalmologists is to recognize and remedy the situation where the eyes defense systems have failed or over reacted(1).Several reports have demonstrated that conjunctival micro flora are related to postoperative infections or infections where the corneal integrity is broken(9).For these reasons a good reference is needed defining such microbial flora. The objective of our study was to define the population of conjunctival normal flora in Iraqis.

Patients and Methods

A total number of 627 conjunctival swabs were collected from attendants of the ophthalmological consultation unit at al Kadhimya teaching hospital/Baghdad over the

period between June 2002 to March 2004.Swabs were taken from the lower conjunctival fornices of subjects respecting the following exclusion criteria:

1. red eye for any reason.
2. conjunctival discharge.
3. diabetic patients.
4. patients on any type of topical medications.
5. current or previous contact lens wear.

Sterile cotton swabs were scraped in the lower conjunctival sac and immediately transferred to the microbiology lab.at Al Nahrain medical college to be inoculated on three culture media (blood,chocolate and Mac Conkey agar plates).The plates were incubated at 37°C over night,cultures were examined the next day and gram staining with further identification tests were carried out (coagulase,sugar fermentation and mannitol fermentation tests) to record the final results.Data were analysed and presented using the simple mathematical methods of frequency percentage of occurrence.

Results

627 eyes were swabbed and considered as the population of our study.350 eyes(55.8%) were of males and 277(44.4%) were of females. Mean age of the participants was 42 years with a range of 6-8 years.

Bacteriological examination of the samples revealed the results in table 2.

Discussion

The normal microbial flora of the eye lid and conjunctiva has already been described. The heterogenous bacterial collection is controlled both by its own mechanisms(nutrient competition, metabolic inhibition and enzyme production) and by the host anatomic barriers and immunological system(9).

Knowing the nature of our patients conjunctival flora is of importance because it is one of the main sources of intraocular contamination during surgery(10),and some of these microflora;namely coagulase negative staphylococci;are now recognized as opportunistic pathogens(11).

61% of our swabs revealed no bacterial growth and 39% gave positive cultures.Suzanne's positive cultures were 30%(12),Mc Natt reported 61% positive cultures(13),Cecilia Tobias reported 61.5% positive cultures(9),Martin's reported 73% positive cultures(14)and Perkin's positive isolates were 90.6%(15).Although in different literatures a variety of bacteria was cultivated the number is small in most studies. Negative cultures can be increasingly noticed after topical or systemic antibiotics use.

The microbial pattern of our model is shown in table 2.Many authors stated that the predominant microbial flora of the conjunctiva is diphtheroids(*Corynebacterium xerosis*) followed by *Staphylococcus epidermidis*(2). Erdogan found a predominance of *S.epidermidis* in his series (23.3%)(16).Perkin's also reported the same organism to predominate in his study(69.8%),he also found 12.5% *S.aureus* ,26% *streptococcus sp.*,22% *micrococcus sp.*,10.4% Gram negative rods , 7.3% diphtheroids,4.2% *Bacillus sp.* And his cultures were negative for candida(15).In Mac Natt study *S.epidermidis* was also the predominant aerobic microorganism and *Propionibacterium acnes* predominates as anaerobic microorganism(49.5%).Fungi were rarely found(13).Jensen mentioned that *S.albus* and diphtheroids were found in 80% and 60% of his swabs respectively(17).In Cecilia's work, the most common microorganism isolated were coagulase negative staphylococcus and corynebacterium sp.(9).Suzanne reported that although *Staphylococcus* species were isolated most frequently from the conjunctiva of normal subjects,significantly more diphtheroids were

isolated from those labeled as former contact lens wearer(12).

Although the results of the above mentioned studies depart a little bit from ours again due to the inconsistencies in microbial sampling techniques;subject selection and other aspects of methodology, the predominant microorganisms were the same being *Staphylococcus epidermidis* and *Corynebacterium*.

It is thought that in the conjunctival sac there is a synergistic relationship between diphtheroids (*corynebacterium*) and coagulase negative staphylococcus species(12)that is why almost all studies refer to them as the dominators.

Our data were insufficient to correlate the microbiology to age,sex and residency of the subjects examined.

It is important to mention that modification of ocular flora of a given population depends on seasonal variations , temperature, host age, environmental exposure, additional ocular trauma, surgical procedures and local or systemic immunosuppressant. Modifying the ocular flora alter the normal residing flora and promote the growth of strains with increased antimicrobial resistance and pathogenicity (5,6,9).

In conclusion, our findings suggest that the conjunctival sac is either sterile or normally contains small number of bacteria and the origin of these bacteriae may be the skin of the eyelids as they resemble skin normal flora,some of them can be potentially pathogenic in the eye.

More elaborative analysis of the conjunctival flora can be obtained using both liquid and solid microbial culture media to increase the chances of isolate recovery. Brain heart infusion broth is recommended by the microbiologists as a highly nutritive culture media that allows growth of especially fastidious organisms such as Gram positive and negative cocci and fungi. In addition it has different levels of oxygen that allow the

recovery of compulsory and non compulsory aerobic bacteria and even anaerobic bacteria(9)the part we miss in our study and therefore is recommended to be considered in future studies to bring into focus other hidden microbial flora.

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TABLE 1 Normal flora of the eye surface(5).

S.epidermidis	+++	
S.aureus	++	
Diphtheroids	++	
Propionibacterium acnes	++	
Streptococcus species		+
Neisseria species	+	
Enteric gram negative bacteria	+/-	
H.influenza	+/-	
Moroxella	+/-	
Yeast	-	
Filamentous fungi	+/-	
Demodex species	-	

TABLE 2 Normal conjunctival flora in this study.

<u>Microorganism</u>	<u>Frequency(%)</u>
Negative culture	383 (61%)
S.epidermidis	216 (34%)
Diphtheroids	15 (2%)
S.aureus	10 (1,5%)
Candida(mixed with S.epidermidis)	2 (0.3%)
Bacillus species	1 (0.15%)