Bacteriological Profile of Conjunctiva Bacterial Flora in the Northeast of China: A Hospital-Based Study

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Research Article

Keywords: Conjunctival bacterial flora, gender, seasonal changes, northeast of China

Posted Date: November 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-963642/v1>

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Abstract

Purpose: To investigate the distribution and influencing factors of pre-operative conjunctival flora from patients undergoing penetrating ocular surgeries in northeast of China.

Methods: An observational and cross-sectional study design was used. In 305 eyes of 305 patients without infective eye diseases who underwent ocular surgeries in the First Affiliated Hospital of Harbin Medical University between May 2018 and May 2019, conjunctival sac scrapings were collected on the day before surgery.

Results: The mean age of participants was 60.73 ± 14.23 years, with the majority being female, married and unemployed with at least primary school education. The positive culture rate was 48.20% (147/305 eyes), and a total of 191 bacterial strains were isolated, 22.45% (33/147) positive samples had two or more strains isolated. The most commonly isolated bacteria was *Staphylococcus epidermidis* (64.92%), surpassing *Staphylococcus aureus* (5.76%). The culture positive rate of young (18-40 years) group was different between the females (26.67%) and the males (69.23%) ($P = 0.024$), but in middle aged group and the elderly group, the rates between the sexes were similar with increased trend. Patients visited in summer or autumn presented a higher positive rate than other seasons. The hypertensive women had a higher rate than hypertensive men (58.14% vs. 40%, $X^2 = 5.8662$, $P = 0.0154$).

Conclusions: In northeast of China, coagulase-negative *Staphylococcus* were the most common pre-operative conjunctival bacteria. The hypertensive female patients, elderly patients, or operated patients in summer and autumn should pay more attention to perioperative treatment.

Trial registration number: ChiCTR2100044659

1. Introduction

The conjunctiva is the mucous membrane that lines the internal aspect of the eyelids and extends to the orbital globe, and the conjunctival sac is the space bound between the palpebral and bulbar conjunctiva¹. Given its continuous exposure to the outside environment, the normal commensal flora and potential pathogens can be detected in the conjunctival sac². The normal commensal flora can protect the host either by occupying the potential colonisation positions of pathogens or producing antimicrobial products, or by stimulating an immune response that protect the host against infection². However, random or surgical penetrating trauma can promote the migration of resident bacterial flora into the eye, and leading to endophthalmitis^{3,4}. Therefore, understanding and monitoring the distribution of conjunctival bacteria are important in pre-, peri- and postoperative management.

Coagulase-negative staphylococci (CoNS) and *corynebacterium sp.* are the two most commonly found bacterial flora of the normal conjunctiva^{5,6}. Due to climate changes and local dietary habits between the areas, geographic location influence the distribution of conjunctival flora. A study compare the conjunctival microbiome among three major Chinese cities (Guangzhou, Wenzhou, and Beijing), thousands of kilometers apart from each other within different climate, and Beijing conjunctival samples displayed elevated diversity and abundance compared to other cities⁷. There are numerous causative factors linked with changes in the composition of ocular surface flora, including ocular diseases (dry eye⁸ and infectious keratitis), systemic diseases (diabetes⁹ and hyperlipidemia¹⁰), alcoholism¹¹ and patients age¹². Investigating the conjunctival flora in specific population has long been a question of great interest in a wide range of fields. Heilongjiang province locates in northeast of China, the weather is dry and cold, no study of conjunctiva flora in this area has been reported.

An objective of this study was to investigate the bacterial flora of conjunctival sac in patients scheduled for ocular surgeries, and to explore the influence factors of culture-positive or varied numbers of bacteria of patients before surgery in a city locates in the northeast of China.

2. Materials And Methods

2.1. Study subjects

This was a observational and cross-sectional study enrolled 305 eyes from 305 patients undergoing eye surgery recruited from the First Affiliated Hospital of Harbin Medical University between May 2018 and May 2019. Inclusion criterias included: consenting patients suffered from cataract, diabetic retinopathy, pterygium, strabismus, macular hole or epimacular membrane, retinal detachment and silicone oil-filled eye. Patients were excluded if they had active ocular inflammation (dry eye, blepharitis, conjunctivitis chronic dacryocystitis, and keratitis), nasolacrimal duct obstruction, ocular trauma, history of contact lens wear, topical or systemic administration of antimicrobial or immunosuppressive agents in the past 3 months and use of eyedrops in either eye within 3 days. This study was approved by the First Affiliated Hospital of Harbin Medical University Ethical Committee (No.201848) and strictly followed the Helsinki Declaration. All patients provided written

informed consent before participating in the study. Study registration Chinese Clinical Trial Registry (registration number is ChiCTR2100044659).

2.2. Specimen Collection From The Conjunctiva Sac

The culture samples were obtained using a previously described method¹³. The specimens were collected from the lower conjunctival cul-de-sac of surgical eyes without applying a topical anesthetic as follows, with details below. The periocular skin and eyelid margins were firstly cleaned with sterile normal saline. Pulling the lower lid with a cotton swab to expose the lower conjunctival fornix. Asking each subject gazed upward, a sterilized swab moistened with a drop of sterile normal saline, gently wiping the lower fornix conjunctiva from medial to lateral side. Asking the individuals should not blink during the procedure in order to avoid touching the lid margins. Then the samples were inoculated into thioglycolate broth (Oxoid, England) immediately.

2.3. Bacterial Culture, Isolation And Identification

Thioglycolate medium was used as the enrichment culture, and specimens were incubated in 5% CO₂ at 35°C for 48h. The bacteria, if any, would be immediately streaked on into 5% sheep blood (JinZhang technology, China) and chocolate agar plates(JinZhang technology, China) and incubated at 35°C for another 24h. The identification of bacteria species was carried out by analytical profile index (API) panels (BioMérieux, France).

2.4. Statistical Analysis

Sociodemographic and clinical characteristics for the study population were summarized with frequencies and percentages for category variables and mean value and standard deviation for continuous variables. We used variance analysis and chi-square test to compare the positive rate difference. We calculated Spearman rank correlation coefficients between the total bacterial count and patient characteristics. Univariate logistic analysis was conducted to analysis the relationship between the positive rate and patient characteristics. All statistical analysis was performed in the SPSS software(v20.0, SPSS Inc, Chicago, IL,USA). $P < 0.05$ was considered a statistical difference.

3. Results

3.1 Baseline characteristics

305 conjunctivals samples were collected from operative eyes of 305 patients enrolled in the study. The average participants age was 60.73 years $\pm$ 14.23 SD years (range 18 to 89). The positive culture rate from patients was 48.20% (147/305 eyes). Other sociodemographic and clinical characteristics of the patients were summarized in Table 1.

Table 1
Demographic and clinical characteristics of the enrolled patients (n=305)

	Culture-negative patients	Culture-positive patients	Overall	F/χ^2	P
Patients, n	158	147	305		
Age, year					
mean±SD	59.92±15.05	61.60±13.27	60.73±14.23	1.06	0.3032
n(%)					
≤40	15(9.49)	13(8.84)	28(9.18)	2.4957	0.2871
40-65	87(55.06)	69(46.94)	156(51.15)		
≥65	56(35.44)	65(44.22)	121(39.67)		
gender, n(%)					
F	91(57.59)	87(59.18)	178(58.36)	0.0791	0.7785
M	67(42.41)	60(40.82)	127(41.64)		
Education, n(%)					
No formal education or Primary	38(24.05)	47(31.97)	85(27.87)	2.5171	0.2841
Secondary	69(43.67)	60(40.82)	129(42.30)		
Tertiary	51(32.28)	40(27.21)	91(29.84)		
Job, n(%)					
Worker	20(12.66)	30(20.41)	50(16.39)	4.2992	0.2309
Farmer	30(18.99)	30(20.41)	60(19.67)		
Clerk	24(15.19)	16(10.88)	40(13.11)		
Unemployed	84(53.16)	71(48.30)	155(50.82)		
Marriage, n(%)					
Married	26(16.46)	24(16.33)	50(16.39)	0.0009	0.9757
Single or Widowed	132(83.54)	123(83.67)	255(83.61)		
HBP, n(%)					
No	94(59.49)	80(54.42)	174(57.05)	1.4945	0.4737
<10 years	39(24.68)	36(24.49)	75(24.59)		
≥10 years	25(15.82)	31(21.09)	56(18.36)		
DM, n(%)					
No	103(65.19)	101(68.71)	204(66.89)	0.4336	0.8051
<10 years	27(17.09)	23(15.65)	50(16.39)		
≥10 years	28(17.72)	23(15.65)	51(16.72)		
smoke, n(%)					
No	123(77.85)	107(72.79)	230(75.41)	1.2735	0.529
<20 years	13(8.23)	17(11.56)	30(9.84)		
≥20 years	22(13.92)	23(15.65)	45(14.75)		
drink, n(%)					
No	134(84.81)	125(85.03)	259(84.92)	0.003	0.9565

	Culture-negative patients	Culture-positive patients	Overall	F/χ^2	P
Yes	24(15.19)	22(14.97)	46(15.08)		

3.2 Isolation Rate And Bacterial Isolates

The positive culture rate from patients was 48.20% (147/305 eyes). Of the 147 samples with positive culture results, 33(22.45%) had mixed cultures with two to three strains isolated. Altogether, 31 types of bacteria were identified. The most common microorganism was *Staphylococcus epidermidis* (64.92%), followed by *Staphylococcus aureus* (5.76%) and *Staphylococcus xylosus* (5.24%). Isolation rate of Gram-negative bacteria was 8.38%, and three most isolates were *Citrobacter freundii* (1.57%), *Burkholderia cepacia* (1.57%) and *Pseudomonas aeruginosa* (1.05%). The microorganism profiles isolated from the conjunctival sac were shown in Table 2.

Table2 Bacterial isolates from the conjunctiva of patients (n= 191)

Bacteria	No.isolates	Percentage(%)
Gram-positive	175	91.62%
<i>Staphylococcus epidermidis</i>	124	64.92%
<i>Staphylococcus aureus</i>	11	5.76%
<i>Staphylococcus xylosus</i>	10	5.24%
<i>Corynebacterium species</i>	7	3.66%
<i>Klebsiella oxytoca</i>	3	1.57%
<i>Staphylococcus lugdunensis</i>	3	1.57%
<i>Staphylococcus sciuri</i>	2	1.05%
<i>Staphylococcus lentus</i>	2	1.05%
<i>Bacillus cereus</i>	2	1.05%
Others(<i>Staphylococcus hominis</i> , <i>Enterococcus faecalis</i> , <i>Kocuria roseus</i> , <i>Staphylococcus cohnii urealyticum</i> , <i>Staphylococcus warneri</i> , <i>Rhodococcus</i> , <i>Staphylococcus caprae</i> , <i>Micrococcus luteus</i> , <i>Staphylococcus capitis</i> , <i>Kocuria varians</i> , <i>Bacillus halmapalus</i>)	11	5.75%
Gram-negative	16	8.38%
<i>Citrobacter freundii</i>	3	1.57%
<i>Burkholderia cepacia</i>	3	1.57%
<i>Pseudomonas aeruginosa</i>	2	1.05%
Others(<i>Enterobacter aerogenes</i> , <i>Delftia acidovorans</i> , <i>Brevendimonas vesicularis</i> , <i>Proteus</i> , <i>Brevendimonas diminuta</i> , <i>Stenotrophomonas maltophilia</i> , <i>Enterobacter cloacae</i> , <i>Mannheimia haemolytica</i>)	8	4.19%

3.3 Correlation Between The Culture-positivity Rate And Clinical Factors

Firstly, the separate impact of the age or gender on culture-positivity rate were analyzed. As shown in Table 3, the frequency of culture-positive in the conjunctiva in females was higher than that in males (48.88% vs. 47.24%, $\chi^2 = 0.0791$, $P = 0.7785$). According to age, the patients were divided into three groups of 18-40 years old (young), 41-65 years old (middle aged), and >65 years old (elderly). The positive culture rates were 46.43% (13/28), 44.23% (69/156) and 53.72% (65/121), respectively. Although not statistically significant ($\chi^2 = 2.4957$, $P = 0.2871$), the frequency of culture-positive in the conjunctival of elderly patients was higher than that in young patients.

Further, we analyzed the interactive impact of the age and gender on culture-positivity rates. As shown in Table 4, in young (18-40 years) group, the frequency of culture-positive in the conjunctival in male patients (69.23%) was much higher than that in female patients (26.67%) ($\chi^2 = 5.072$, $P = 0.024$). Between the young group and the middle aged group, the frequency of culture-positive in the conjunctival of male patients showed a trend towards reduced at first, and then the incidence increases with advancing age in elderly group (Additional file 1). But the results was totally different for female patients, the frequency of culture-positive in the conjunctival of female patients increased significantly along

with age, the isolation rate of the elderly group(55.41%) was higher than other groups (55.41% vs.47.19% vs. 26.67%, $\chi^2 = 4.3247$ $P= 0.1151$). Therefore, we believe that age and gender have a synergistic effect on the bacterial isolation of patients.

Table 3
Bacteria frequency in different age groups and gender groups

	gender		χ^2	P	age			χ^2	P
	Female	Male			≤40	40-65	>65		
Culture-negative	91(51.12)	67(52.76)	0.0791	0.7785	15(53.57)	87(55.77)	56(46.28)	2.4957	0.2871
Culture-positive	87(48.88)	60(47.24)			13(46.43)	69(44.23)	65(53.72)		

Table 4
The isolation rates of conjunctival samples in each group

Age, year	age≤40, n(%)		χ^2	P	Age=40-65, n(%)		χ^2	P	Age>65, n(%)		χ^2	P
	Culture-negative	Culture-positive			Culture-negative	Culture-positive			Culture-negative	Culture-positive		
Female	11(73.33)	4(26.67)	5.072	0.024	47(52.81)	42(47.19)	0.736	0.390	33(44.59)	41(55.41)	0.217	0.640
Male	4(30.77)	9(69.23)			40(59.70)	27(40.30)			23(48.94)	24(51.06)		

Harbin locates in the northeast of China, which has a predominantly dry climate. It had lower temperature than the other parts of China. According to the internationally recognised astronomical seasons, spring was defined as March 21 to June 21, summer as June 21 to September 22, fall as September 22 to December 21, and winter as December 22 to March 20 of the following year¹⁴. The culture positivity rate was higher in patients who visited in summer(49.15%) and autumn(56.72) compared to those visited in spring(41.03%) and winter(45.24%) (Additional file 2). Although it was not statistically significant($P= 0.0605$, $OR(95\%CI)= 1.88(0.97,3.65)$), the difference of culture positive rate between autumn and spring still was the largest.

In this study, smoking history was available in 75 patients. Although not statistically significant, the culture-positive rate of patients with a smoking history(53.33%) was higher than those who never smoked (46.65%)($\chi^2 = 0.8866$, $P= 0.6419$). The history of alcohol consumption did not affect the culture-positive rate($\chi^2 = 0.0006$, $P= 0.9807$) (Additional file 3).

In this project, history of hypertension was present in 131 patients, diabetes mellitus in 101 patients. The culture-positive rate of patients with hypertension (51.15%) was higher than those without hypertension (45.98%)($\chi^2 = 1.5207$, $P= 0.4675$). Further, we analyzed the interactive impact of hypertension and gender on culture-positivity rates. The culture positive rate were higher among hypertensive women (58.14%, 50/81) than hypertensive men (40%, 20/50)($\chi^2 = 5.8662$, $P= 0.0154$). But for diabetes mellitus patients, the culture-positive rate was 45.54%, lower than those patients without diabetes mellitus(49.51%)($\chi^2 = 0.4297$ $P= 0.8067$)(Supplement file2).

3.4 Correlation between the numbers of detected species and clinical factors

There was a positive correlation between the numbers of detected species and gender($r_s^* = 0.17921$, $P= 0.0299$). Male patients(1.367 ± 0.581) had more bacteria species than female patients(1.195 ± 0.478)(Table 5). Patients admitted to hospital in summer and autumn were likely to have more bacterial strains than those in spring and winter. However, no significant correlation was found between the numbers of detected species and seasons, patients age, educational status, history of smoking and drinking, hypertension and diabetes mellitus (all $P > 0.05$).

Table 5 Correlation between the numbers of detected species and clinical factors

	Numbers of detected species	r_s^*	P
Season		0.01083	0.8964
Spring	1.125± 0.336		
Summer	1.345± 0.579		
Autumn	1.368± 0.633		
Winter	1.053± 0.229		
Age, year		-0.10577	0.2023
-40	1.154± 0.376		
40-65	1.348± 0.564		
65-	1.200± 0.506		
gender, n(%)		0.17921	0.0299
F	1.195± 0.478		
M	1.367± 0.581		
Education, n(%)		0.10181	0.2198
No formal education or Primary	1.149± 0.416		
Secondary	1.350± 0.577		
Tertiary	1.275± 0.554		
Job, n(%)		-0.01769	0.8316
Worker	1.333± 0.661		
Farmer	1.200± 0.484		
Clerk	1.375± 0.500		
Unemployed	1.239± 0.492		
Marriage, n(%)		0.02598	0.7548
Married	1.208± 0.415		
Single or Widowed	1.276± 0.548		
HBP, n(%)		-0.00788	0.9245
No	1.250± 0.490		
≤10 years	1.333± 0.632		
≥10 years	1.226± 0.497		
DM, n(%)		0.00768	0.9265
No	1.257± 0.523		
≤10 years	1.348± 0.573		
≥10 years	1.217± 0.518		
smoke, n(%)		-0.03684	0.6578
No	1.271± 0.524		
≤20 years	1.294± 0.588		
≥20 years	1.217± 0.518		
drink, n(%)		-0.00650	0.9377
No	1.272± 0.544		

Yes

1.227± 0.429

*Spearman rank correlation coefficient.

4. Discussion

Conjunctival sac flora play an crucial role in eye diseases, such as meibomitis, microbial keratitis, and endophthalmitis in the traumatic or surgical conditions. Exploring the conjunctival sac flora in healthy people not only helps us realise the ocular physiology function, but assists to investigate the infectious diseases etiology and therapy protocol. Multiple factors, including environmental exposure, ocular trauma, age and changes in local immunity^{15–17}, affect the normal conjunctival microbial flora. To the best of our knowledge, our study was the first to reveal the the distribution of conjunctival microbial flora of patients before ocular surgery in Harbin, which locates in the northeastern part of China. We also investigated the systemic clinical factors and environmental factors, which influence the positive bacterial culture in the conjunctival sac of patients. Younger ages of male patients, older ages of patients, female patients with hypertension and patients visited in high temperature seasons such as summer or autumn were four factors which showed highly correlated with the positive bacterial culture rate. This study provides the guidance for the clinicians to select more effective ocular prophylactic modalities for treatment.

In this study, the overall positive culture rate was 48.20%, in the lower middle of the previous results of 36.0–81.7%^{17,18}. Differences are likely heavily influenced by collection, transportation and culture conditions. To improve culture positive rate, we put the conjunctival swab samples directly into thioglycolate broth instead of 5% sheep blood or chocolate agar plates. Normal conjunctival flora is mainly comprises of coagulase-negative staphylococci (CoNS), which also was the most frequently isolated clinical organisms in our study. In our results, the *Staphylococcus epidermidis* was the most frequently isolated organism (64.92%), followed by *Staphylococcus aureus* (5.76%) and *Staphylococcus xylosus* (5.24%). This finding was consistent with those previously published that CoNS was the most frequently isolated ocular bacteria of patients undergoing eye surgery^{19,20} and patients with postoperative endophthalmitis²¹. Gram negative bacteria only were found in a small portion, with *Citrobacter freundii* and *Burkholderia cepacia* being most frequent (1.57%), *P. aeruginosa* (1.05%) also was isolated.

Age can affect the rate of conjunctival sac flora culture in different gender patients. In our study, for the male patients, the frequency of culture-positive in the conjunctival showed a trend towards reduced at first, and then the incidence increases with advancing age in elderly group. The middle aged group of 41–65 years showed the lowest positivity of bacterial isolation (40.30%). Our results were in agreement with the same type of study in Zhengzhou, which locates the central area of China¹³. Their results showed the rate of conjunctival bacterial isolation decreased with the increased of patients age, after the 41–65 years, the rates were increased with age. Although the trend of conjunctival sac flora culture rate in our male patients is the same as that in the above studies, the results in our female patients were diametrically opposite. There was not the turning point, the rate of conjunctival bacterial isolation always increased with age, the young group (18–40 years) presented the lowest positivity of bacterial isolation (26.67%), and the elderly group (>65 years) showed the highest positivity of bacterial isolation (55.41%). Age-associated changes in the normal flora of the body may has relationship with the elevated pro-inflammatory state and changed immune response in elderly people^{22,23}. The results of the study further showed that in each age group, the positive culture rate in male patients was higher than female patients, especially in younger group (≤ 40 years), and the difference was significant. The higher bacteria frequency in younger male people or older people may have clinical meaning to receive more intense prophylactic treatment.

A study said the presence of diabetes mellitus of patients with positive bacterial culture of the conjunctival sac was significantly more than the control patients²⁴. Despite this supportive evidence, conflicting views exist. Martins et al²⁵ found that the diabetes duration had no influence on the rate of positive cultures or the type of flora bacteria. In our results, there were no statistical differences in the rates of positive culture between the diabetic (45.54%) and non-diabetic groups (49.51%) ($\chi^2 = 0.4297, P = 0.8067$).

As we have well known, climatic factors affect the morbidity of conjunctivitis over the year around the world²⁶, so it is also worth studying whether the climate will affect the culture-positive rate and strain distribution of conjunctival. In our results, the total frequency of conjunctival bacteria in summer and autumn was surprisingly higher than in the other seasons, which was a little different another study. E F Rubio²⁶ observed in April, May, and June (spring), the total frequency of conjunctival bacteria was the highest.

Potential limitations of the present study should be mentioned. Firstly, in this study we use the traditional culture method may skew or compromise the growth of cultivable microbiome from the ocular surface and miss some non-culturable microbes. Modified methods, like genetic analysis 16S rRNA sequencing may lead to a much more rapid, precise and complete analysis. But the drawbacks is the technique cannot tell whether the bacteria are alive or not, the bacteria obtained by traditional culture method are living and the dominant bacteria.

Secondly, because the local anesthetic eye drops had strong antimicrobial effects, so we don't use the local anesthetic, which bringing the local pain to the subjects.

In conclusion, in the northeast of China, the most common pre-operative conjunctival bacteria isolated were coagulase-negative *Staphylococcus*. hypertensive female patients, elderly patients, or operated patients in summer and autumn should pay more attention to perioperative treatment.

Declarations

Authors' contributions

Shuo Xu and Hong Zhang conceived the idea of the study. Shuo Xu performed the experiments and acquired clinical samples. Hong Zhang analyzed the data. Shuo Xu drafted the manuscript and Hong Zhang revised the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. U20A20363), National Natural Science Foundation of China (Grant No.81970776) and Natural Science Foundation of Heilongjiang Province, China (Grant No. LH2020H039).

Disclosure of interest

The authors declare no competing interests.

Availability of data and material

All data has been included in the manuscript.

Code availability

SPSS software(v20.0, SPSS Inc, Chicago, IL,USA).

Ethics approval

This study was approved by the First Affiliated Hospital of Harbin Medical University Ethical Committee(No.201848).

Consent for publication

All authors consent to publish this manuscript.

Consent of patients

All patients consent to publish this manuscript, and the statement has been included.

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