doi: http://dx.doi.org/10.19052/sv.3994

Characterization of the transient and resident conjunctival microbiota in young adults

Caracterización de la microbiota conjuntival transitoria y residente de adultos jóvenes

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Received: 08-13-2016 / Accepted: 10-27-2016

ABSTRACT
The Ocular microbiota is mainly found in the conjunctiva and consists of bacteria of the genera *Staphylococcus corynebacterium* spp., *Bacillus* spp., *Neisseria* spp., *Moraxella* spp., and *Streptococcus* spp. This diversity may vary according to seasonal conditions, temperature, age, and environmental exposure. *Objective:* To characterize the diversity of resident and transient conjunctival microbiota in young adults. *Methodology:* A cross-sectional observational descriptive study including 67 young adults, men and women between 18 and 25 years of age, who met the inclusion criteria and agreed to sign the informed consent form. Two samples from the conjunctival sac were collected from each subject, with an interval of one week. These were cultured on blood agar and chocolate agar at 37 °C for 24 hours. Isolated colonies were identified using the VITEK automated system. *Results:* Sixteen genera and 29 different species were identified. The most common genus was *Staphylococcus* (55.6%), followed by *Acinetobacter* (12.0%), *Bacillus* (7.0%), *Pasterella* (4.6%) and *Escherichia* (3.7%). Resident microbiota consisted primarily of the genus *Staphylococcus* and its most representative species were *S. epidermidis* (50.0%) and *S. lentus* (13.3%). *Conclusion:* There is a great diversity of bacteria in the conjunctival flora, greater in the transient than in the resident microbiota; this probably depends on the environment in which the individual lives.

Keywords: ocular microbiota, VITEK, conjunctiva, opportunistic pathogens.

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How to cite this article: Martin Algarra V, Jaimes Niño V, Rodríguez MF. Characterization of the transient and resident conjunctival microbiota in young adults. Cienc Tecnol Salud Vis Ocul. 2017;15(1):37-45. doi: http://dx.doi.org/10.19052/sv.3994

RESUMEN

La microbiota ocular se encuentra principalmente en la conjuntiva y está constituida por bacterias de los géneros *Staphylococcus corynebacterium* spp., *Bacillus* spp., *Neisseria* spp., *Moraxella* spp. y *Streptococcus* spp. Esta diversidad puede variar según las condiciones estacionales, la temperatura, la edad y la exposición ambiental. *Objetivo*: caracterizar la diversidad de la microbiota residente y transitoria de la conjuntiva en adultos jóvenes. *Metodología*: estudio observacional descriptivo de corte transversal en 67 adultos jóvenes, hombres y mujeres entre 18 y 25 años de edad, que cumplieron con los criterios de inclusión y aceptaron firmar el consentimiento informado. A cada sujeto se le tomaron dos muestras del saco conjuntival, con un intervalo de una semana. Estas se cultivaron en agar sangre y agar chocolate a 37 °C por 24 horas. Las colonias aisladas se identificaron mediante el sistema automatizado VITEK. *Resultados*: se determinaron 16 géneros y 29 especies diferentes. El género más frecuente fue *Staphylococcus* (55,6 %), seguido de *Acinetobacter* (12,0 %), *Bacillus* (7,0 %), *Pasterella* (4,6 %) y *Escherichia* (3,7 %). La microbiota residente estuvo constituida principalmente por el género *Staphylococcus* y sus especies más representativas fueron *S. epidermidis* (50,0 %) y *S. lentus* (13,3 %). *Conclusión*: existe gran diversidad de bacterias en la microbiota conjuntival, mayor en la microbiota transitoria que en la residente; probablemente, esto depende del ambiente en el que se encuentra el individuo.

Palabras clave: microbiota ocular, VITEK, conjuntiva, patógenos oportunistas.

INTRODUCTION

The term *microbiota* refers to the population of microorganisms that live on the skin and the mucus of healthy individuals (1). It is mainly constituted by commensal and opportunistic bacteria characterized by moving in different environments where they cause disease and other environments where they don't (2). The majority of the microbiota is stable (resident) which allow to found some patterns in different places. However, the exposure and different individual or group conditions contribute to their remarkable diversity (3).

On the eyes, the microbiota is found mainly in the eyelids and on the conjunctiva since the cornea is practically lacking microorganisms due to its localization and curvature (4). The conjunctiva is the most colonized tissue in the eyes, principally by *Staphylococcus, Corynebacterium, Propionibacterium, Micrococcus, Bacillus, and Streptococcus* (5,6) *Staphylococcus* is the genus most prevalent one in the eyes (81.5% to 32.6%), mainly coagulase negative staphylococci (6-9).

The ocular microbiota can vary according to seasonal conditions, temperature, environmental exposure, hygiene, and age. *S. epidermidis, S. aureus, Diphtheroides, Streptococcus viridans, Bacillus Haemophilus, Bacteroides, Propionibacterium*, and *Lactobacillus* predominate at birth and they are similar to the biota of the cervix, but two days after birth *S. epidermidis, S. aureus*, and *E. coli* are more commonly isolated (4,6,10). In children and in adults, the most frequent ones are coagulase negative staphylococci, *Diphteroides, Propionibacterium*, and *Streptococcus*, being the latest more in children (6,11,12).

It has been reported that according to localization, climatic conditions, and activity, the microorganisms in healthy eyes of individuals that live in rural areas are: *S. epidermidis, S. aureus, S. viridans, Haemophilus, Pseudomona, Actinomyces, Nocardia*, and *E. coli* (13,14).

As it is evident, this population of bacteria is relatively homogeneous in the ocular surface; however, new studies, with molecular techniques, have increased the quantity of genus and species that can be seen in the normal conjunctiva, including another genus like *Pseudomonas, Bradyrhizobium, Acinetobacter, Brevundimonas, Aquabacterium*, and *Sphingomonas.* This species are normal inhabitants

of the environment, the water, and the soil (15). These bacteria—that normally form part of the transient microbiota—have little relevance, only when resident microorganism are change, the transient microorganisms could colonize, proliferate, and produce disease. Their growth also depends on physiological factors, such as temperature, humidity, and nutrients (2,16).

The study of the microorganism in healthy eyes is very important because of the frequency that these agents can cause infections in the ocular surface today (17,18). In Colombia, the microbiological research on eyes is limited, particularly on infections in which high prevalence of coagulase negative staphylococci (77% to 43%), *Corynebacterium* (36% to 5%), *Haemphyllus* (15%), and *Staphyloccoccus aureus* (32% to 30%) in conjunctivitis (19,20). *S. aureus* is the pathogen species of the genus *Staphylococcus*, but it is isolated frequently as part of the conjunctival microbiota. The majority of these bacteria present an increase of multi-resistance to antibiotics regardless of whether being isolated from infections or from the normal microbiota (7-9,18,21-23).

The aim of this research work is to characterize the genus and species prevalent in the conjunctiva of young adults without ocular pathology using the automatized VITECK system. In addition, this research could contribute to the knowledge in the optometry field, in the generation of future projects that will determine antibiotics resistance patterns of this bacteria in our population.

METHODS

Type of study: descriptive cross-sectional study.

Sample: 67 young adults, men and women, between 18 to 25 years old, with a healthy anterior eye segment.

Exclusion criteria: participants with infectious diseases, current contact lenses wearers, use of ophthalmic or systemic medication in the past three weeks. All the individuals included in the study, signed an informed consent after being explained the project aims and its potential risks.

Procedure: A bio-microscopic exam was performed to evaluate the integrity of anterior eye segment. Afterwards, samples of the lower conjunctival fornix were taken from a random eye from each subject with an interval of a week, using a sterilized cotton swab moistened with sterilized physiological saline solution, without local anesthesia.

Culture: The samples were cultured immediately in blood and chocolate agar (Oxoid) at 37°C for 24 hours. Gram staining was performer in order to identify each colony and were cultured again in tripticase agar (Oxoid) under the same conditions.

Microbial identification: The isolated cultures were suspended in 3.0 mL of sterile saline solution at 0.45%, 7.0 pH (Biomerieux, Marcy l'Etoile, France) previously calibrated in a 12×70 mm clear plastic test tube until it reached a concentration of 1.5×10^8 bacteria corresponding to 0.5 McFarland turbidity, according to the requirements on each card. The concentration was determined using the VITEK® 2 DensiCHEK™ (Biomerieux, Marcy l'Etoile, France) previously calibrated. Finally, a tube with the corresponded card was placed in the cassette of the Viteck® 2 Compact equipment (Biomerieux, Marcy l'Etoile, France) for the genus and species identification. For the Gram positive bacteria the Vitek® 2-GP cards (Reference 21342), and Vitek® 2-BCL cards (Reference 21342) were used and for the Gram negative ones, the Vitek® 2-GN cards (Reference 21341) were used.

RESULTS

A sample of 67 undergraduate students from University of La Salle participated in the study. The 47% of the sample were females; the average age of all participants was 20 years old.

Bacteria colony growth was obtained in 80.6% of the individuals. There was no growth in 13 participants (19.4%) in any of the samples taken for a total of 113 positive cultures (59 in the first sampling and 54 in the second one) from which 4% (5/113) were not identified. The 54% (62/113) were Gram-positive cocci, 31.9% (36/113) Gram-negative rods, and 8% (9/113) Gram-positive rods.

Diversity of isolated genus in the conjunctiva

In this study, 16 genus and 29 species were identified. In the majority of participants, only one type of bacteria was identified. In only 23.9% (16/67) of cases, more than one bacterium was identified, with maximum three different bacteria per eye. The most common bacterial isolates were *Staphylococcus* (55.6%), *Acinetobacter* (12.0%), *Bacillus* (7.0%), *Pasterella* (4.6%), *and Escherichia* (3.7%). According to the microorganisms' predominant natural habitat, 66.7% of identified isolated bacteria form part of the skin and mucus microbiota, of which 6.5% were of the *Enterobacteriaceae* family. The 25.9% of the genus is more frequently isolated from the soil, the air or the water and 7.4% were zoonotic bacterial genus (Figure 1).

The *Staphylococcus* genus is one of the major diversity species; coagulase negative staphylococci was the most frequent (95.0%) among which, *S. epidermis* was the most representative species (50%), *S. lentus* was the second most isolated species (13.3%). Species like *S. caprae* (1.7%). and *S. intermedius* (3.3%), are commensal and opportunistic pathogen of skin and mucus of animal that rarely affects humans. *S. aureus* represented 5.0% of the isolated species from this genus (Table 1).

Resident and transient ocular Microbiota

The genus found in both samples on the same individual was considered as resident microbiota. *Staphylococcus* was isolated in 21 individuals in both opportunities; *S. epidermis* coincided in 11 individuals, *S. capitas* in 2 individuals; *S. lentus*, *S. intermedius*, *S. warneri*, *S. pasteuri*, and *S. aureus*, *each specie*, in one individual in both

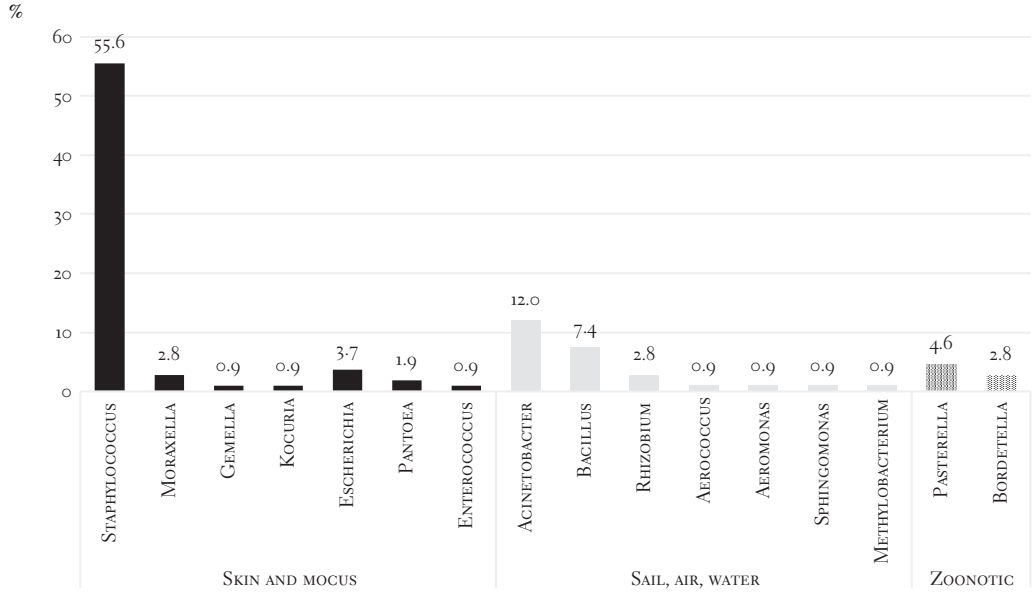

FIGURE 1. **Percentage of genus isolated from the conjunctival samples, classified according to predominant natural habitat of each microorganism**

TABLE 1. **Percentage of species from the *Staphylococcus* genus isolated from the conjunctival samples**

SPECIES OF *STAPHYLOCOCCUS*	%
S. coagulasa negativos	95.0
S. epidermidis	50.0
S. lentus	13.3
S. capitas	11.7
S. aureus	5.0
S. warneri	6.7
S. pasteuri	3.3
S. intermedius*	3.3
S. auricularis	1.7
S. haemolyticus	1.7
S. hominis	1.7
S. caprae	1.7

* Coagulase positive staphylococci

opportunities. *Moraxella lacunata* was identified in 1 individual in both samplings. Three species from *Acinetobacter* genus were identified in 11 individuals: *A. baumannii*, *A. iwoffii*, and *A. ursigii*. The same genus, but with a different species was identified in only one individual.

The other identified bacteria were considered as transient microbiota, because they were isolated only in one sampling with different frequency. Two species were identified from the genus *Bacillus*, in 8 individuals: *B. cereus*. and *B. vallismortis*. *Escherichia coli* was identified in 4 individuals in only one sampling; *Pasterella pneumotropica* was identified in 5 individuals; *Rhizobium radiobacter* and *Bordetella bronchiseptica*, in 3 participants, and *Pantoea agglomerans* in 2. *Enterococcus faecium*, *Kocuria rosea*, *Gemella bergeri*, *Methylobacterium aquaticum*, *Sphingomona paucimobilis*, *Aeromona salmonicida*, and *Aerococcus viridans* were isolated in only one sampling in one patient (Figure 2).

DISCUSSION

According to the literature, the most frequent bacteria present in the conjunctival microbiota that has been identified through the VITEK 2 automated microbiology system was the genus *Staphylococcus* with a percentage of 55.6%, which is similar to that reported in many studies (9,12,14,24). Coagulase negative staphylococci was the most representative (90.0%). *S. epidermis* was the most isolated

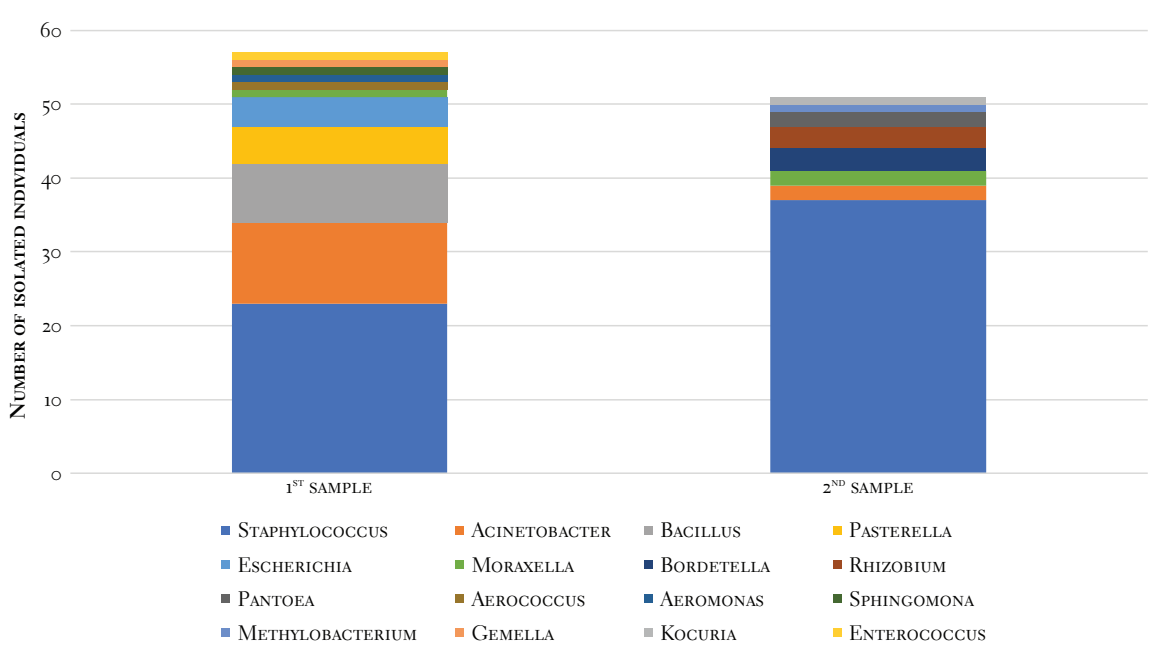

FIGURE. 2. **Diversity of identified bacteria genus in the first and second sampling of the conjunctiva**

(50.0%) with other species *S. warneri*, *S. haemolyticus*, *S. auricularis*, *S. hominis*, and *S. capitas*, previously reported in the conjunctival microbiota (7,24). The high frequency of identification of this genus in two samples from each individual corroborated that it is the principal component of the resident ocular microbiota. *S. epidermis* is the most prevalent specie in the ocular surface, but it also the most common cause of infection in the eyes (17). This bacteria has developed strategies to generate novel phenotypic and genotypic variants through the acquisition of genes that give it resistance to antibiotics and the ability to produce biofilms (25,26). There is a high prevalence of biofilm-forming *S. epidermidis* isolates in the healthy conjunctiva (27), hence the importance of their identification in the ocular microbiota.

The *S. aureus*, a pathogen species of the *Staphylococcus* genus, is the predominant bacteria in the majority of eye infections (28). However, it is found with low frequency (1.5% to 14%) in the conjunctival microbiota (7,9,14) as it was found in the present research work (5%). *Moraxella lacunata* is an opportunistic pathogen of human skin and mucus that can cause invasive infections. It is considered an important eye pathogen for conjunctivitis, queratitis, endofthalmitis, and the main cause of blefaritis in young people (28,29). Likewise, *S. aureus* has been reported as having low frequency in healthy conjunctiva, according to our results (2.8%). Although it was isolated in only two individuals, it must be noticed that in one of them it was isolated in both samplings indicating that it forms part of the resident microbiota.

Four species from the genus Staphylococcus, *S. intermedius*, *S. pasteuri*, *S. lentus*, and *S. caprae* are associated to the animal microbiota (goats, sheep, cats, and bovines) and to the contamination of milk products, but rarely isolated from human samples (30,31). Other two zoonotic species identified in the conjunctiva were *Bordetella bronchiseptica* (pigs and dogs) and *Paterella pneumotropica* (rats and mice). The presence of these bacteria could be justify because 55% of participants were Veterinary Medicine students and/or live with pets (this issue was not analyzed in this study). However, these bacteria were classified as transient, since it was isolated in only one sampling for each individual. Living with animals and the activity performed by the individuals increases the diversity and the quantity of the environmental microbioma that probably influence the community of bacteria that form part of the normal skin and mucus flora (32,33).

A greater diversity of the conjunctival microbiota was identified through the automated system VITEK than through studies that use microbiological conventional methods. 29 species of bacteria were identified, from which only ten are widely reported in the literature. Unexpectedly, many of these opportunistic pathogens and commensals are inhabitants of the gastrointestinal microbiota: *E. coli*, *Enterococcus faecium*, *Pantoea agglomerans*, *Gemella bergeri*, and of the environment (water and soil): *Acinetobacter (A. baumannii, A. iwoffii y A. ursingii)*, *Bacillus (B. cereus y B. vallismortis)*, *Sphingomonas paucimobilis*, *Aeromonas salmonicida*, *Aerococcus viridans*, *Methylobacterium aquaticum*, and *Rhizobium radiobacter*. Bacteria from the gut microbiota have been reported with some frequency in the human conjunctive, specially, *E. coli* (8,14,34). The origin of these bacteria in the conjunctive could be self-contagious or due to bad hygiene habits.

The importance of the environment in the interaction and constitution of the human microbioma is observed in its great diversity as currently reported through molecular techniques. The concept of nucleus and variant microbioma is comparable with that of resident and transient flora. The latter is more independent from other factors such as the environment (35). *Bacillus*, *Aeromonas*, *Sphingomona*, *Aerococcus*, *Methylobacterium*, and *Rhizobium* identified in this research work are commonly found in the soil, the water, the

air, and in *in door* environments (16,36). They are opportunistic pathogens in immune-compromised patients and their presence in the majority of reported cases is of nosocomial origin. In the human microbioma, *Sphingomona, Aeromona and Rhizobium* have been identified by molecular techniques in skin (37), and *Bradyrhizobium*, *Sphingomona and Methylobacterium* in conjunctiva (15). *Bacillus* and *Acinetobacter* have been reported in the healthy conjunctiva using conventional microbiological methods with low frequency (6). In this study, *Acinetobacter* was identified in 13% of the cultures. A similar percentage had only been reported by using molecular techniques (15). *A. baumanni* is an important opportunistic pathogen in nosocomial infections due to its multi-resistance to antibiotics (38).

Except for *Acinetobacter*, all bacteria of environmental origin were classified as transient conjunctival microbiota. The conjunctiva can contain a certain quantity of microorganisms from the skin or the environment, but the tear film proteins such as IgA, enzymes and anti-microbial peptides inhibit the colonization of the majority of bacteria. For this reason, these microorganisms colonize only transiently the conjunctiva, during hours, days or weeks and they vary from one person to another.

The results from the present research work corroborate the diversity of the conjunctival microbiota. More studies are necessary to determine the role these microorganisms play in the health and disease of the ocular surface and what is their resistance to antibiotics.

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