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Ocular Microbiota

Taha AYYILDIZ[1]

[1] Department of Ophthalmology, Medical Faculty, Ahi Evran University, Kırşehir, Turkey.

SUMMARY

One of the most prominent of recent developments is the understanding of human microbiota. Microbiota is a group of microbes settled in a specific area, while microbial describes the total genome of these bacteria. The 10 trillion germs, equivalent to 10 times the total number of cells in the human body, live only in the intestines. The ocular surface is in constant contact with the external environment and has been shown to have a unique flora. The diversity, detection methods, and influence of this fluorophore on ocular immunity, personality, and extrinsic factors have been investigated and improved. Specific treatment of patients, planning of systemic and topical antibiotics, and perhaps ocular flora transplantation are the aims of future studies.

Key words: Ocular microbiota, ocular surface, microbiologic analyses

INTRODUCTION

Various mucosal surfaces such as the gastrointestinal tract, oral mucosa, and respiratory tract have been colonized by microorganisms. Changes in microbiology may lead to infection and inflammation (1-3). The ocular surface includes mucosal surfaces such as bulbar conjunctiva, palpebral conjunctiva, and conjunctival fornices. Similarities and differences with other mucosal flora of the ocular surface are under investigation (4).

It is important to define the ocular surface microbiology because infections such as endophthalmitis and infectious keratitis arise from the ocular surface. Factor detection is challenging, and culture positivity is below 60% (5,6). Traditional bacterial culture methods have been shown to have a culture-positive postoperative endophthalmitis of 50%–70% (7,8). However, a new method called biome representational in slico karyotyping (BRISK), which is a metagenomic method of analyzing DNA-based life forms, has shown torque teno virus (TTV) positivity in all culture-negative endophthalmitis (9). This suggests that TTV is a member of the ocular surface flora and bacterial and viral members of the ocular surface flora should be extensively investigated.

Only a subspecies design could be developed in studies conducted on ocular surface flora using traditional culture methods (10,11). Significant differences were found between the results of microbiological analysis of ocular fluorescence and results of conventional culture methods using metagenomic approaches (12-14). In a study using traditional culture methods, only Staphylococcus and Bacillus species were detected. The study showed that the application of 16S rDNA gene polymerase chain reaction (PCR) method could detect five subcapsules including bacteria that could not be cultured (13). Dong et al. found that 59 species of bacteria were present when administering 16S rDNA gene sequencing to the ocular surface (12). Although the diversity of ocular microbiotics has been shown in these studies, the disadvantages are the inadequate number of patients and the inability to detect factors such as viruses and fungi. In another study on asymptomatic volunteers based on viral PCR, herpes simplex virus 1, hepatitis B virus, and even hepatitis C virus were detected in tears (15). This suggests

Correspondence:
Taha AYYILDIZ
Ahievran Mahallesi, Yavuz Sultan Selim Bulvarı, Aybars Apartmanı Daire 9, Kırşehir Merkez 40100, Türkiye.
e-mail: obirtahadir@hotmail.com

that ocular surface viruses are residents of ocular flora. Hence, it is important that only the ocular flora analysis be done on the bacteria that may be missing.

This review explored traditional culture, 16S rDNA gene qPCR and sequencing, and BRISK methods used for ocular flora detection.

METHODS

Traditional culture method

The microbiota cultured on the ocular surface can be determined using swab samples from conjunctiva, capsules, and tears (16-19). Sticks of cotton or calcium alginate may be used for conjunctival swab specimens. Generally, after wetting, the stick is driven toward lower conjunctival fornix or upper conjunctival fornix and then enriched with vitamins and hematin, brain heart infusion agar, thioglycolate agar, chocolate agar, bloody agar, Sabouraud agar (for mushroom), Drigalski agar (for Enterobacteria and some fermentation-negative enteric bacilli isolation and differentiation), Thayer-Martin agar (isolation of selective Neisseria species), Schaedler or phenylethyl alcohol blood agar (for obligatory anaerobic bacteria), kanamycin (for gram-negative bacteria), eosin methylene blue, or MacConkey agar–vancomycin blood agar (isolation of obligatory anaerobic gram-negative bacilli).

The overall conclusion is that coagulase-negative staphylococci are the most common bacteria isolated from the conjunctiva, cap, or tears and are positive in about half of the cases (16-18). The next most common isolated microorganisms are Propionibacterium and diphtheroid bacteria (mostly Corynobacterium). Culture negativity varies between 9% and 87% in conjunctival swabs and 0% and 48% in lid swabs (11,18, 20, 21). The differences are thought to be influenced by sample collection, transport, and culture conditions. A study conducted in India, demonstrated a twofold increase in the frequency of detection of S. epidermidis and S. aureus in conjunctival swab samples taken immediately after 8 h of sleep (22). It has been shown that colonies per swab are mostly below 80, 200, and 10 in conjunctival, valvular, and tear cultures, respectively (21,23,24).

DNA Purification

Genomic conjunctival swabs of the eye are made using the DNeasy blood and tissue kit (Qiagen, Inc., Venlo, the Netherlands). The DNA is put into 30 µL of elution buffer and then stored at –20°C. The DNA analysis is performed using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, MA, USA). Next, the 16S metagenomic analysis or BRISK method is used.

TTV Quantitative PCR

Pan bacterial PCR analysis is performed using 16S ribosomal RNA polymers (DNA Technologies, CA, USA).9 The primer sequences used are 5'-GAGGAAGGTGGGGATGACGT-3 'and 5'-AGGCCCGGGAACGTATTCAC-3'. HotStarTaq DNA polymerase is used for PCR. 100 ng of genomic DNA is used for each reaction. The master mix contains 10× buffer, Taq polymerase, deoxynucleotide triphosphate, and primers. This mixture is treated for 5 min with 8-methoxypsoralen (25 µg/mL) and ultraviolet radiation (Bio-Rad GS gene linker, ultraviolet chamber; Bio-Rad Laboratories Inc., CA, USA). Amplification is done using the MasterCycler gradient (Eppendorf, Hamburg, Germany). The cycle includes 10 min of denaturation at 94°C, 30 cycles of denaturation at 45°C for 30 s, a 30-s cohesion at 58°C and 1-min synthesis at 72°C. The final extension is 10 min at 72°C.

The PCR mix contains 0.8 µL forward and reverse primers (0.4 µL each), 10 µL ABsolute Blue qPCR SYBR low ROX Mix (Thermo Fisher Scientific), and 1 µL unmixed genomic DNA. The 16S DNA PCR analysis was performed with 8-methoxypsoralen without the master mix template and with 5 min of treatment with ultraviolet. The final reaction volume is 20 µL. At the end of the process, 1×10^{1} to 10^{8} copies per milliliter are obtained with the corresponding plasmid (16S, TTV, and actin) on specific regions of the complementary DNA of the cloned gene. Quantitative PCR routinely identifies 10 copies/mL of the sample in each complementary control DNA. The process is completed after 2 min at 50°C and 10 min at 95°C, and then 15 s at 95°C and 1 min at 60°C.

BRISK

BRISK is a DNA sequencing technique (25). The total DNA sample is divided into 33 fragments around the DNA sequences of type 2b DNA restriction enzyme BsaXI and ACNNNNNCTCC. These 33 fragments are multiplexed in a multiple parallel DNA sequencing

platform. Each sequence frame includes >3 × 10^7 sequences per sample or > 1 × 10^6 sequences per sequence. Each sequence is compared with the registered samples in the database. All human sequences are mapped. The nonhuman sequences are also compared with the bacterial, parasite, fungal, and viral sequences recorded in the database.

Contact lens–ocular microbiota association

Various studies have mostly focused on cultivating bacteria that stick on contacts (26,27). Many different polymer contact lenses are in daily use. Some are disposable on a daily basis, while some are long-lasting contact lenses, which can be retained for a period of 1 month. The bacterial cultures most commonly performed in contact lens wearers were coagulase-negative staphylococci, Propionobacterium, and Corynebacterium. No difference was detected in terms of different contact lens polymers and usage patterns. Kültür negatiflik oranı da %9-84 arasında değişmektedir (26,27). The participant was positive for approximately 123 colonies after removing contact lenses and washing the hands with tap water. Touching with the fingers significantly reduced the number of germs on the lens (28). In asymptomatic contact lens users, A study of more than 300 colonic bacilli per lens showed that the removal of contact lens under aseptic conditions in asymptomatic contact lens users might not be effective (29).

A study on microbiotics of conjunctiva and eyelids of long-term contact lens users showed that the conjunctiva and flap flora remained unchanged, but colonization with pathogenic bacteria such as gram-negative bacilli was found on these two surfaces (21). On the contrary, daily contact lens wearers showed an increased incidence of coagulase-negative staphylococcal colonization in conjunctiva and eyes. A study evaluating conjunctival lenses of old lens wearers, new lens wearers, and non-wearers demonstrated no difference in the other two groups of older lens users with increased bacterial colonization (30).

Age–microbiota association

The evaluation of the conjunctival culture samples taken immediately after birth of the newborns revealed that the bacterial composition was similar to that of the cervix (31-33). A birth through a cesarean section was examined for bacteria immediately after birth. However, S. epidermidis, E. coli, and S. aureus were observed most frequently in conjunctival swabs made 2 days later (31-33). In another study evaluating conjunctival lid swabs of children aged 8–14 years, the culture positivity was found to be 36% in conjunctival swabs and 54% in valvular swabs, suggesting that the composition resembled that in adults (34).

Comparison of ocular microbiota with other mucosal surfaces

Oral cavity has a wide range of microbiota diversity. Less than 100 colonies are observed in microliters of tears, whereas in saliva this number is around 10^7–10^8 (24). The most common bacteria found in oral cavity cultures are Streptococcus, Actinomyces, Veillonella, and Bacteroides (35). In a study in which swabs from the external auditory canal were cultured, the most frequently identified bacteria were staphylococci (S. auricularis), followed by Corynebacteria (36).

Given the wide variety and number of flora in the oral cavity and intestines, it can be concluded that the number and variety of ocular surface flora are low. Also, the bacterial density cultured from the conjunctival swabs is also less than that in the flap swabs.

Relation between ocular microbiota and ocular surface disease

Ocular microbiota may also be altered because lactotransferrin, lysozyme, polymeric immunoglobulin receptor, and lacritin have bactericidal components in tears, and the tear composition changes in the dry eye. Some studies have shown a more intense bacterial burden in the eyes of patients with Sjogren's syndrome than in the eyes of healthy volunteers (13,37). Genetic analyses may reveal details of ocular surface problems and ocular microbiota associations.

Relation of ocular immunity with ocular microbiota

Commensal microbes on the mucosal surfaces such as the intestine prevent the colonization of pathogenic microbes (38). However, this is not the case for ocular flora because the ocular surface is paucibacterial. Therefore, the effect of ocular fluorosis on ocular surface preservation is different from that on other mucosal surfaces. Although ocular microfilaments are thought to inhibit the

colonization of pathogenic agents by activating local immunity, the lack of number and diversity of ocular flora suggests that immunodetection mechanisms may be inadequate.

Immunoglobulin A (IgA) is the main antibody of the ocular surface (39). IgA also exhibits antiinflammatory activity by neutralizing viruses and bacteria, as well as stimulating interleukin 10 release and affecting maturation of dendritic cells (40). IgD and IgM antibodies are first carried on the surface of bone marrow B lymphocytes, and other antibodies achieve somatic hypermutation and class exchange pathways in the germinal centers of secondary lymphoid tissues (41). The IgA performs class exchange in two distinct ways: T-lymphocyte-dependent and T-lymphocyte-independent (42). The T-lymphocyte-dependent pathway is through the interaction of antigen-specific B lymphocytes with CD4+ T lymphocytes. This interaction occurs in the eye conjunctiva and lacrimal glands (43). It is important for normal rats to reach IgA and IgM levels of germ cell-free rats in the lacrimal glands 4 weeks after exposure to environmental factors, suggesting that the local immunity may interact with the ocular flora leading to a direct effect (44). In another study conducted on rats, oral antibiotic treatment reduced secretory IgA levels in tears (45). Immediately after birth, unaffected lacrimal B lymphocyte maturation in rats before opening the eyelids suggests that secretory IgA secretion can be regulated by other mucosal surfaces such as the intestine and nasal mucosa (46).

Unlike the T-lymphocyte-dependent pathway, microbiota in the lungs and intestines is known to mediate the class change in immunoglobulins over the Toll-like receptor (TLR) (47,48). TLR activation leads to IgA class change by stimulating the release of B-lymphocyte-activating factor in epithelial and dendritic cells. The T-lymphocyte-independent pathway is important in protecting against intestinal pathogenic microorganisms. The effect of the ocular surface fluorescence on immunity is also via the T-lymphocyte-independent pathway.

This review aimed to compare the traditional culture methods for ocular microbiota with modern analysis methods such as 16S rDNA and BRISK, and also explore the interaction between ocular surface and ocular flora in the context of available studies. Fecal-transplantation-like ocular flora transplantation, personalized treatments, and ophthalmic microbiotics when planning antibiotic therapy are important to predict further plans in this regard.

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