Evaluation of normal ocular bacterial flora with two different culture media

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ABSTRACT • RÉSUMÉ

Background: The purpose of this study was to determine if the use of broth culture medium is efficient in investigating bacterial flora of the normal eyelid and conjunctiva.

Methods: Samples from the conjunctiva and eyelid of healthy patients of various ages who were undergoing ocular surgeries were obtained and cultured at 3 periods: before topical antibiotic prophylaxis, in the postoperative period during topical antibiotic treatment, and 15 days after discontinuation of antibiotic use. Samples were inoculated into both brain heart infusion broth and blood agar plate, and the growth results of both media were analyzed.

Results: Brain heart infusion broth medium showed a significantly higher bacterial growth of gram-positive cocci in most periods. The solid blood agar medium had a higher recovery of gram-positive bacilli before prophylaxis only in the older patients.

Interpretation: Our results show that a more complete analysis of eyelid and conjunctival flora can be obtained using both liquid and solid media to increase the chances of isolate recovery. The inclusion of liquid media in this analysis was even more relevant in the period of concomitant use of antibiotic treatment.

Contexte : La présente étude a pour objet d'établir l'efficacité du bouillon de culture pour étudier la flore bactérienne de la paupière et de la conjonctive normales.

Méthodes : Des échantillons de conjonctive et de paupière de patients en santé d'âges variés qui subissaient une chirurgie oculaire ont été prélevés et cultivés à 3 périodes : avant la prophylaxie antibiotique topique, pendant la période post-opératoire de traitement antibiotique topique et 15 jours après l'utilisation de l'antibiotique. Les échantillons ont été inoculés soit dans un bouillon d'infusion de cerveau et de cœur, soit dans une boîte de gélose au sang, et l'on en a analysé la croissance.

Résultats : Le bouillon d'infusion de cerveau et de cœur a produit une croissance bactérienne significativement plus élevée de coccus gram-positifs à la plupart des périodes. Le milieu solide de gélose au sang a donné une récupération plus élevée de bacilles gram-positifs avant la prophylaxie, mais seulement chez les patients âgés.

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Interprétation : Nos résultats démontrent qu'on peut obtenir une analyse plus complète de la flore de la paupière et de la conjonctive en utilisant les milieux liquides et solides pour accroître les chances de récupération des isolats. L'inclusion de milieux liquides dans l'analyse s'est avérée encore plus pertinente lors de la période d'utilisation concomitante du traitement antibiotique.

The normal microbial flora of the eyelid and conjunctiva has already been described.[1–6] This heterogeneous bacterial collection is controlled both by its own mechanisms (nutrient competition, metabolic inhibition, and enzyme production) and by the host (anatomic barriers and the immunological system).[6]

Coagulase-negative *Staphylococcus, Staphylococcus aureus,* and *Corynebacterium* species are the most commonly isolated ocular flora microorganisms in the eyelid and conjunctiva.[1–4,7] Improved methods to isolate and identify anaerobic bacteria and fungal flora from clinical specimens has shown the presence of these agents as normal eyelid and conjunctival flora.[5,8]

Modification of ocular flora depends on seasonal variations, temperature, host age, and environmental exposure. Additionally, ocular trauma, surgical procedures, and local or systemic immunological system can modify the ocular flora.[5] Reduction in number of positive cultures after topical or systemic antibiotic use has also been described.[1,2,9,10]

Different kinds of culture media can be used to isolate microorganisms from ocular samples, and using several kinds at the same time can increase the chance of bacterial recovery. The most frequently used media in ophthalmological clinical laboratories are solid media types, such as sheep blood agar, chocolate agar, and Sabouraud dextrose agar, or liquid media types, such as brain heart infusion (BHI) broth and thioglycollate broth. Particularly when using liquid media, investigators must always be alert to the possibility of contamination during the inoculation process because the evaluation of contamination is not easy.[11,12]

BHI is a highly nutritive culture medium that allows growth of especially fastidious organisms such as gram-positive cocci like *Streptococcus* and *Staphylococcus*, gram-negative cocci like *Meningococcus*, and fungi like *Histoplasma capsulatum* and *Blastomyces dermatitidis*, among others.[13,14] Besides its nutritive characteristics, BHI as a liquid medium has different levels of oxygen that allow the recovery of compulsory and noncompulsory aerobic bacteria or even anaerobic bacteria.[14]

The aim of this study was to compare brain heart infusion with sheep blood agar for use in the investigation of eyelid and conjunctival flora.

Methods

Conjunctival and eyelid samples were obtained from healthy patients who had undergone uneventful surgery. They were divided into 2 groups based on the type of surgery: group 1 underwent cataract surgery through phacoemulsification, and group 2 had refractive surgery, either photorefractive keratectomy (PRK) or laser-assisted in situ keratomileusis (LASIK). Povidone-iodine eye drops were used preoperatively in all operated eyes. All procedures were performed by the same surgeon.

Samples were obtained before antibiotic prophylaxis (period A), in the postoperative period while using a topical antibiotic (period B), and 15 days after antibiotic use had ended (period C). Patients were randomly assigned to use either 0.3% lomefloxacin or 0.3% tobramycin. One drop of antibiotic was applied to the operated eye 4 times daily for 1 week. Patients allergic to any component of the medications and those who were taking other topical medications were excluded.

Specimens were obtained by scraping a sterile cotton swab in the lower conjunctival sac and eyelid border of the operated eye and were inoculated into BHI broth and 5% sheep blood agar plates. Specimens were incubated at 37°C and examined daily for bacterial growth for 1 week and again after 1 month.

Samples for microbiological evaluation obtained from the conjunctiva and eyelid were submitted to culture media and microorganism identification. Cultures presenting growth of bacteria in at least 2 strains in any plate or changes in color or turbidity of the broth media were considered positive. Liquid broth considered positive was subcultured into blood agar plates to identify the microorganism. All coagulase-