

## ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM CONJUNCTIVAL CONTENTS

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### Abstract

**Introduction.** Opportunistic pathogens, which normally vegetate on the conjunctival mucosa, are involved in the development of perioperative infectious complications in ophthalmic practice. When examining the contents of the conjunctival sac of healthy individuals by the culture technique, coagulase-negative staphylococci, which are always present on the eyelid skin, are most often isolated in small quantities. Bacteria of this genus belong to opportunistic pathogens, since with mechanical, chemical or thermal injury to the skin or mucous membranes, they are actively engaged in the development of an inflammatory reaction.

**Aim.** The purpose of the work was to determine the prophylactic and therapeutic efficacy of antimicrobial agents widely used in ophthalmology by studying the sensitivity of coagulase-negative staphylococci isolated from the conjunctival contents to the mentioned agents.

**Materials and methods.** Bacteriological examination of the conjunctival content of 20 healthy individuals and 10 individuals with signs of post-traumatic conjunctivitis was carried out, 17 strains of *S. epidermidis* were isolated.

**Results.** There were no significant differences in the level of susceptibility to the studied agents of *S. epidermidis* strains isolated from the surface of a healthy conjunctiva compared to strains isolated from the injured mucosa. Both were highly resistant to the most long-used drugs Sulfacil sodium and levomycetin. The mean MIC of tobramycin for isolates obtained from intact conjunctiva amounted to  $8.3 \pm 3.4$  µg/ml and was significantly lower than that of the antiseptic miramistin ( $20.8 \pm 4.2$  µg/ml). The obtained indices of the antistaphylococcal activity of decametoxin suggest a high prophylactic and therapeutic activity of the eye drops. After all, the concentration of the antiseptic in eye drops accounts for 200 µg/ml, which is 20 times more than the highest determined MBC for coagulase-negative staphylococci.

**Conclusions.** The study results of the sensitivity of the obtained isolates to antimicrobial agents showed a low level of efficacy of Sulfacil sodium and levomycetin, doubtful sensitivity to levofloxacin and miramistin and a high level of antistaphylococcal activity of decametoxin and tobramycin.

**Keywords:** ophthalmic perioperative infectious complications, conjunctival microflora, sensitivity to antibiotics and antiseptics

### INTRODUCTION

The mucous membrane of the eye, as well as other mucous membranes of the human body, has its own microecosystem, the formation of which begins immediately after birth. It is characterized by stability and includes mainly cutaneous microflora, which is not able to cause an inflammatory reaction in the absence of damage to the mucous membrane by external factors [1].

The normal conjunctival microbiome includes representatives of the *Staphylococcus*, *Corynebacterium*,

*Streptococcus*, *Propionibacterium* and *Micrococcus* genera. These types of bacteria have a commensal relationship with the human body, are found on the conjunctiva in small quantities and assist in maintaining local homeostasis [2]. The vital activity of the eye microbiome strengthens the ocular immune barrier, stimulating the production of immune effectors, including complement and IgA, and prevents colonization of the mucous membrane by pathogenic bacterial species [3].

When examining the contents of the conjunctival sac of healthy individuals by the culture technique,

coagulase-negative staphylococci, which are always present on the eyelid skin, are most often isolated in small quantities. The required presence of the genus *Staphylococcus* in the «core» of the ocular microbiome has been confirmed by modern molecular genetic methods [4]. Meanwhile, bacteria of this genus belong to opportunistic pathogens, since with mechanical, chemical or thermal damage to the skin or mucous membranes, they are actively engaged in the development of an inflammatory reaction. Staphylococci often trigger issues for contact lens wearers, as they are common causative agents of infectious keratitis. The risk of complications of staphylococcal etiology always arises in cases of surgical interventions in the ocular area [5, 6].

For the prevention and treatment of purulent inflammation of the eye, drops are used, the main active ingredients of which are antibiotics of various chemical groups or antiseptics. However, the WHO recognizes the spreading of antibiotic resistant strains of a wide range of opportunistic pathogens, including staphylococci, as a global threat to human health [7]. Therefore, in modern conditions, it is only through permanent local monitoring of the level of sensitivity of opportunistic bacteria to antimicrobial agents that it is possible to determine the rational tactics for the prevention and treatment of diseases of bacterial etiology for each medical institution.

### AIM

The purpose of our work was to determine the level of sensitivity to antimicrobial agents widely used in ophthalmology, isolates of coagulase-negative staphylococci extracted from the contents of the conjunctival sac of healthy individuals, as well as from the mucous membranes of those with purulent inflammatory lesions.

### MATERIALS AND METHODS

An immediate bacteriological study of the contents of the conjunctival sac was carried out in 20 healthy individuals who sought help for surgical vision correction in the ophthalmological department, as well as 10 patients who were undergoing inpatient treatment for thermal trauma of the face with eyelid injury and signs of inflammation of the conjunctiva.

In order to isolate pure cultures, the contents of the conjunctival sac were collected with a disposable sterile plastic bacteriological applicator, placed in a test tube with a SARSTEDT AG & Co Germany transport medium and delivered to the bacteriological laboratory.

The test material was inoculated by the semi-quantitative cup plate method [8] on Columbia agar with 5% sheep blood and mannitol salt agar for the isolation and differentiation of staphylococci (GRASO biotech,

Poland). For further study of biological characteristics, isolated pure bacterial cultures were cultivated on special nutrient media corresponding to the bacterial species (BHI, glucose BHI, Sabouraud agar, etc.).

Species identification of microorganisms was carried out taking into account the morphological and tinctorial properties of microorganisms based on the results of microscopy of the preparation stained by Gram, cultural properties; results of biochemical typing. Biochemical typing was performed on STAPHYtest 24 diagnostic panels by PLIVA – Lachema a.s. Brno, Czech Republic.

Preliminary susceptibility identification of isolated strains of the genus *Staphylococcus* to amoxicillin, amoxicillin/clavulanate, levomycetin, ofloxacin, ciprofloxacin, levofloxacin, tobramycin and trimethoprim was carried out by the disc diffusion method (DDM) using standard indicator discs. A quantitative study of the level of antibiotic resistance with the determination of the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of the studied isolates, which were classified as moderately sensitive or resistant by DDM, and the study of sensitivity to the antiseptics miramistin and decametoxin was carried out by the method of two-fold serial dilution of the drug in a liquid nutrient medium [9].

Statistical analysis of the obtained results was carried out using the standard «Microsoft Excel 2019» program package. The obtained samples were analyzed for distribution regularity and the main indices were calculated: mean value (M), standard deviation of the mean value ( $\sigma$ ), p-value (p). To compare data groups, the criteria of F-TEST, T-TEST were determined. The difference significance of the obtained results (deviation of the null hypothesis) was confirmed with the condition that  $p \leq 0.05$ .

**Analysis of the Obtained Data:** bacteriological examination of the contents of the conjunctival sac of healthy individuals undergoing preoperative examination by culture method showed the absence of bacterial microflora in 50% of the examined. 10 strains of microorganisms attributed to the species *Staphylococcus epidermidis* according to the totality of morphological and biochemical characteristics were isolated in the rest of the patients examined in this group. The density of mucosal colonization by staphylococci in this group ranged from 10 to 100 colony-forming units per  $\text{cm}^2$  (CFU/ $\text{cm}^2$ ).

All examined patients with periorbital traumas had bacterial microflora present in the secretions of the mucous membrane of the eye. Among the 10 subjects, 3 (30%) had gram-negative bacteria of the species *Acinetobacter baumannii* and *Proteus vulgaris* present, the rest of the patients (70%) had *S. epidermidis* isolated. The quantitative content of staphylococci in the conjunctival secretions of this group of patients was

higher than in patients of the first group and ranged from 103 to 104 CFU/cm<sup>2</sup>.

A preliminary study of the susceptibility of isolated strains of *S. epidermidis* to antibiotics using DDM found that 4 out of 10 isolates obtained from the subjects who did not have periorbital traumas showed sensitivity to all 8 antimicrobial agents used in the study. The remaining strains isolated from patients in this group, as well as 7 isolates obtained from patients with periorbital injuries, were

resistant or showed moderate sensitivity to amoxacillin, levomycetin, trimethoprim, as well as to aminoglycoside and fluoroquinolone drugs. It is these strains that we used to quantify the level of sensitivity to antibiotics and antiseptics, most commonly used in ophthalmology for the prevention and treatment of microbial eye lesions. The mean values ( $M \pm m$ ) of the minimum inhibitory and bactericidal concentrations of the six most commonly used drugs in ophthalmology are shown in Table 1.

Table 1

### Quantitative characteristic of *S. epidermidis* isolates sensitivity level to antibiotics and antiseptics ( $M \pm m$ , $\mu\text{g/ml}$ )

Agent	Index ( $\mu\text{g/mL}$ )	Origin of isolates (quantity)	
		Healthy conjunctiva (6)	Inflamed conjunctiva (7)
Sulfacyl sodium	MIC	125000 $\pm$ 33333,3	128571,4 $\pm$ 30612,3
	MBC	>150000	>150000
Levomycetin	MIC	>500	>500
	MBC	>500	>500
Levofloxacin	MIC	121,1 $\pm$ 35,1	112,7 $\pm$ 24,8
	MBC	195,2 $\pm$ 45,5	245,5 $\pm$ 47,9
Tobramycin	MIC	8,3 $\pm$ 3,4	4,4 $\pm$ 2,9
	MBC	10,2 $\pm$ 2,4	7,9 $\pm$ 3,1
Miramistin	MIC	20,8 $\pm$ 4,2	8,9 $\pm$ 2,5*
	MBC	33,3 $\pm$ 6,4	25,0 $\pm$ 7,1*
Decamethoxine	MIC	2,4 $\pm$ 1,5	2,0 $\pm$ 0,5
	MBC	7,8 $\pm$ 2,0	7,2 $\pm$ 1,7

Note: \* – the index is statistically significantly different from that of *S. epidermidis* isolates obtained from a healthy conjunctiva ( $p < 0,05$ ).

Analysis of the obtained results did not reveal significant differences in the sensitivity level of *S. epidermidis* strains isolated from the surface of a healthy conjunctiva to the studied drugs, compared to the strains isolated from the injured mucosa. Both were highly resistant to the most long-used drugs Sulfacyl sodium and levomycetin. Certain differences were observed with other studied agents, which were often statistically unreliable and did not allow to notice patterns of tendencies towards regularity. Thus, in terms of levofloxacin MBC, isolates obtained from the injured mucosa (245.5 $\pm$ 47.9  $\mu\text{g/ml}$ ) were less sensitive compared to strains isolated from healthy conjunctiva (195.2 $\pm$ 45.5  $\mu\text{g/ml}$ ). However, isolates from intact conjunctiva were less sensitive in terms of tobramycin MBC (10.2 $\pm$ 2.4  $\mu\text{g/mL}$  and 7.9 $\pm$ 3.1  $\mu\text{g/mL}$ , respectively). Depending on the origin of *S. epidermidis* isolates, the MIC and MBC values of miramistin differed statistically significantly. At the same time, strains isolated from the injured mucous membrane were distinguished by higher sensitivity to the action of the antiseptic.

Regardless of the isolates origin, the higher antistaphylococcal efficacy of tobramycin compared to levofloxacin is noticeable. After all, death of the studied strains was observed at concentrations of tobramycin close to 10  $\mu\text{g/ml}$ , while levofloxacin achieved the same effect at concentrations close to 200  $\mu\text{g/ml}$ .

Comparison of the antimicrobial activity of the antiseptics miramistin and decamethoxin causes us to

acknowledge the superiority of decamethoxin: the lowest of the determined indices of the MBC of miramistin was 25.0 $\pm$ 7.1  $\mu\text{g/ml}$ , whereas for decamethoxin it was more than three times lower, and equated to 7.2 $\pm$ 1.7  $\mu\text{g/ml}$ .

## DISCUSSION

The choice of effective means of prevention and treatment of microbial eyelid lesions in the context of the global spread of antibiotic resistance phenomenon is a challenging task, and the empirical use of antimicrobial agents in ophthalmology, as well as in clinics with other specializations, only accelerates the spread of multidrug-resistant strains of pathogens [10, 11].

The above results of bacteriological studies of the conjunctival contents of individuals preparing for ophthalmic surgery showed that the ocular mucous membrane in half of the examined was colonized with *S. epidermidis*, which can complicate the course of the postoperative period and negatively affect the final result of treatment. That is, effective preoperative debridement with antimicrobial agents at the point of intervention is essential. However, 60% of the isolated strains are resistant to certain antimicrobial agents, which necessitates the need for laboratory determination of an effective drug for the prevention of complications before preventive measures begin.

Quite expectedly, the conjunctiva of patients with signs of post-traumatic inflammation turned out to be

more densely populated with microflora of a wider species spectrum. At the same time, 100% of the isolated strains of *S. epidermidis* could not be classified as nonresistant, and the empirical use of antimicrobial agents would not guarantee the treatment effectiveness.

Bacteria of the genus *Staphylococcus*, due to their resistance increase to antibiotics, have long been a significant issue for specialists in numerous medical fields [12, 13]. An increase in antibiotic resistance of strains involved in the development of inflammatory diseases of ocular mucous membrane has also been reported [14]. All isolates of this kind obtained in our study turned out to be absolutely resistant to the eye antiseptic of the sulfonamide series of sulfacil sodium with extensive experience in practical application, as the twice diluted working solution of the drug did not have any bactericidal effect on them. Another drug that has long been used in ophthalmic practice – the antibiotic levomycetin – did not have an adequate effect on the studied strains. At the maximum studied concentration (500 µg/ml), the levomycetin solution did not even provide a bacteriostatic effect.

Compared to these antimicrobial agents, levofloxacin has certain advantages, as its antimicrobial action is much weaker, and 245.5±47.9 µg/ml of the drug was enough to achieve a bactericidal effect. It should be noted that according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), isolates of coagulase-negative staphylococci, for which the minimum MIC of the drug is >1 µg/ml, are considered resistant to levofloxacin [15]. However, in ophthalmology, a levofloxacin 0.5% solution (5000 µg/ml) is used and a 20-fold higher concentration of the working solution, compared to the average MBC, should provide a sufficient level of efficiency. Nonetheless, in the world ophthalmology practice there is a clear increase resistance tendency of the ocular microflora, including staphylococci, to fluoroquinolone preparations. Therefore, most researchers consider it inappropriate to empirically use these drugs for prophylactic or therapeutic purposes [16, 17].

According to the results of a multicenter study in 19 clinics of European countries, 25 years ago, there was an increase resistance tendency of coagulase-negative staphylococci to tobramycin. The proportion of resistant strains ranged from 15% to 60% depending on the clinic [18]. The results of our studies do not confirm the spread of this threatening tendency to the strains circulating in our region, since only a few of them showed moderate resistance to the drug. The mean MIC of tobramycin for isolates obtained from intact conjunctiva amounted to 8.3±3.4 µg/ml and was significantly lower than that of the antiseptic miramistin (20.8±4.2 µg/ml).

Bacterial resistance to synthetic biocides is known to be formed much more slowly than to antibiotics and does not achieve a high level [19]. Miramistin is a biocide

of a series of quaternary ammonium compounds and is successfully used in medical practice. But, given the above data, the effectiveness of the drug for prophylactic purposes is questionable. During the preoperative debridement of the conjunctival sac, it is desirable for the mucosa to be completely cleansed from any microflora, that is to achieve a bactericidal effect. The average MBC of miramistin for staphylococcal strains isolated from intact conjunctiva is 33.3±6.4 µg/ml, and the eye drops used hold 100 µg/ml of the drug. Provided that the drug is washed out with tear fluid, the possibility of achieving a rapid sterilizing effect of the mucosa is questionable.

The obtained indices of the antistaphylococcal activity of decametoxin suggest a high prophylactic and therapeutic activity of the eye drops. After all, the concentration of the antiseptic in eye drops accounts for 200 µg/ml, which is 20 times more than the highest determined MBC for coagulase-negative staphylococci. In addition, decametoxin is known to effectively destroy antibiotic-resistant species of gram-negative bacteria, some of which have been isolated from patients with an injured eye [20, 21].

## CONCLUSIONS

Based on the results of bacteriological studies of the conjunctival content, it is possible to predict that eye drops of decametoxin 0.02% and tobramycin 0.3% will have high prophylactic action when used before ophthalmic surgical interventions and therapeutic action in the treatment of bacterial conjunctivitis.

**Future research perspectives.** A study of a wide species spectrum of ocular pathogens sensitivity to decametoxin should be conducted. Further studies need to substantiate the composition of eye drops with prolonged antimicrobial action.

## FUNDING AND CONFLICT OF INTEREST

The authors declare no conflict of interests. Source of funding – self-funded.

## COMPLIANCE WITH ETHICAL REQUIREMENTS

While conducting the research, the authors adhered to all relevant ethical standards. The study did not use personal data of patients, animals or humans.

## AUTHOR CONTRIBUTIONS

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## Резюме

### ЧУТЛИВІСТЬ ДО ПРОТИМІКРОБНИХ ЗАСОБІВ КОАГУЛАЗОНЕГАТИВНИХ СТАФІЛОКОКІВ, ВИДІЛЕНИХ З КОН'ЮНКТИВАЛЬНОГО ВМІСТУ

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**Вступ.** У розвитку періопераційних інфекційних ускладнень у офтальмологічній практиці приймає участь умовно-патогенна мікрофлора, яка у нормі вегетує на слизовій кон'юнктиви. При дослідженні вмісту кон'юнктивального мішка здорових людей культуральним методом найчастіше у невеликій кількості виділяють коагулазонегативних стафілококів, які завжди присутні на шкірі повік. Бактерії цього роду належать до умовно-патогенних мікроорганізмів, оскільки при механічному, хімічному чи термічному пошкодженні шкіри чи слизових беруть активну участь у розвитку запальної реакції.

**Мета.** Метою роботи було визначити профілактичну та терапевтичну ефективність широко вживаних у офтальмології протимікробних препаратів шляхом дослідження чутливості до них коагулазонегативних стафілококів, виділених з кон'юнктивального вмісту.

**Матеріали та методи.** Проведено бактеріологічне дослідження кон'юнктивального вмісту 20 здорових осіб та 10 осіб з ознаками посттравматичного кон'юнктивіту, виділено 17 штамів *S. epidermidis*.

**Результати.** Істотних відмінностей у рівні чутливості до досліджених препаратів штамів *S. epidermidis*, ізольованих з поверхні здорової кон'юнктиви, у порівнянні зі штамми, виділеними із пошкодженої слизової, не встановлено. І ті, і інші були високорезистентними до найбільш давно вживаних препаратів сульфацилу натрію та левоміцетину. Середній показник МІК тобраміцину для ізолятів, виділених з неушкодженої кон'юнктиви, дорівнював  $8,3 \pm 3,4$  мкг/мл і був значно нижчим, ніж у антисептика мірамістину ( $20,8 \pm 4,2$  мкг/мл). Отримані показники протистафілококової активності декаметоксину дозволяють припустити високу профілактичну та терапевтичну активність очних крапель препарату. Адже, концентрація антисептика у очних краплях 200 мкг/мл, що у 20 разів більше, ніж самий високий визначений показник МБЦК для коагулазонегативних стафілококів.

**Висновки.** Результати дослідження чутливості отриманих ізолятів до протимікробних засобів показали низький рівень ефективності сульфацилу натрію та левоміцетину, сумнівну – левофлораксину та мірамістину, високий рівень протистафілококової активності декаметоксину і тобраміцину.

**Ключові слова:** офтальмологічні періопераційні інфекційні ускладнення, мікрофлора кон'юнктиви, чутливість до антибіотиків і антисептиків

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