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Actual opportunistic ocular surface microflora and its sensitivity to antimicrobials and bacteriophages in patients with cataracts

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ABSTRACT

BACKGROUND: Species of opportunistic microflora often are the pathogenic agents that causes endophthalmitis in cataract surgery. Frequently microorganisms are characterized by resistance to several antimicrobial medicaments, which limits the ability to choose an effective agent. This problem requires a detailed study and monitoring of the sensitivity of ocular surface microflora.

AIM: To study the species composition of the ocular surface microflora patients before phacoemulsification and to evaluate the antimicrobial activity of antimicrobial medicaments including antiseptics and bacteriophages.

MATERIALS AND METHODS: A total of 60 patients were examined before phacoemulsification. The sensitivity to antimicrobial medicaments and bacteriophages was determined of microorganisms isolated from three loci (conjunctival cavity, eyelid margin, lacrimal ducts).

RESULTS: Among all microorganisms isolated, there was a significant prevalence of *Staphylococcus epidermidis* — 48,4 %. Almost all antiseptics showed high antimicrobial activity. All staphylococci cultures were sensitive to staphylococcal bacteriophage number 2. The smallest proportion of resistant microorganisms to antimicrobial medicaments used in ophthalmology was registered in the group of aminoglycosides.

CONCLUSIONS: Antimicrobial activity of the investigated medicaments was different among different bacterial species. The sensitivity of microflora changes over time, therefore it is appropriate to carry out periodic monitoring and adjust antimicrobial prophylaxis regimens based on the results received.

Keywords: microflora; antiseptics; bacteriophages; antibiotic resistance.

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Актуальная условно-патогенная микрофлора глазной поверхности и её чувствительность к противомикробным препаратам и бактериофагам у пациентов с катарактой

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АННОТАЦИЯ

Актуальность. Представители условно-патогенной микрофлоры часто выступают возбудителями эндофтальмита в катарактальной хирургии. Нередко микроорганизмы характеризуются устойчивостью к нескольким противомикробным препаратам, которая ограничивает выбор эффективного средства. Данная проблема требует проведения мониторинга чувствительности микрофлоры глазной поверхности.

Цель — изучить видовой состав микрофлоры глазной поверхности у пациентов перед факоэмульсификацией и оценить антимикробную активность противомикробных препаратов, в том числе антисептиков и препаратов группы бактериофагов.

Материалы и методы. Обследованы 60 пациентов перед факоэмульсификацией. Определена чувствительность к противомикробным препаратам и бактериофагам микроорганизмов, выделенных из трёх локусов (конъюнктивальной полости, свободного края век, слёзоотводящих путей).

Результаты. Среди выделенных микроорганизмов преобладал *Staphylococcus epidermidis* — 48,2 %. Почти все антисептики показали высокую антимикробную активность. Все культуры стафилококков были чувствительны к стафилококковому бактериофагу № 2. Наименьшая доля резистентных микроорганизмов к противомикробным препаратам, используемым в офтальмологии, отмечалась у группы аминогликозидов.

Заключение. Антимикробная активность исследуемых препаратов в отношении различных видов бактерий отличалась. Чувствительность микрофлоры меняется с течением времени, поэтому целесообразно периодически проводить мониторинг и на основе полученных результатов корректировать схемы противомикробной профилактики.

Ключевые слова: микрофлора; антисептики; бактериофаги; антибиотикорезистентность.

Как цитировать

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BACKGROUND

The normal microbiome of the ocular surface mostly consists of Gram-positive bacteria. Gram-negative bacteria may also be found, but more often in immunocompromised patients and patients with metabolic disorders, for example, diabetes mellitus and chronic alcohol intoxication [1, 2]. A study of microorganisms on the conjunctiva and eyelid surface demonstrates their similar composition, including *Staphylococcus epidermidis*, *Corynebacterium* spp., *Staphylococcus aureus*, *Micrococcus* spp., and *Bacillus* spp. The eyelids (17.9%) had higher colonization level than the conjunctiva (1.4%) [3].

Phacoemulsification is a procedure involving globe incisions, which allow the microorganisms in. Therefore, pre- and intraoperative aseptic techniques are critical to prevent infectious complications [4]. A large epidemiological study shows that opportunistic pathogens are often the causative agents of endophthalmitis in cataract surgery. In 88.6% of cases, the vitreous humor was contaminated with Gram-positive bacteria, with greater percentage of *Staphylococcus* spp., in particular *S. epidermidis* (48.6%). Gram-negative bacteria, such as *Klebsiella oxitoca*, *Enterobacter cloacae*, and *Bacillus* spp., were less common (8.6%) and found at equal incidence. *Aureobasidium* spp. were identified in the remaining cases [5]. Importantly, resistance to moxifloxacin (11.1% to 54.5%, $p = 0.07$), ciprofloxacin (54.5% to 72.7%, $p = 0.659$), and oxacillin (66.7% to 93.3%, $p = 0.13$) increased by 2019 [5]. Fluoroquinolones (FQ) are most widely used to prevent endophthalmitis in cataract surgery. In ESCRS2013, pre- and postoperative instillation of third-generation FQ and treatment of the surgical field with 5% povidone-iodine failed to provide sterility of the conjunctiva in every 10th case [6]. However, there is currently no published data on resistance to povidone-iodine, unlike other antiseptics. Antimicrobial resistance caused by insufficient eradication of microorganisms during the treatment of the surgical field reduces the effectiveness of antibiotic prophylaxis, thereby increasing the risk of endophthalmitis. Antimicrobial resistance, including antiseptics, poses a serious global health challenge in the 21st century. Rising antibiotic resistance is considered one of the biggest threats to human health in the future [4, 7]. Therefore, periodic monitoring of the ocular surface microbiome with antimicrobial susceptibility testing is required to understand current trends in increasing antibiotic resistance and determine the most effective ways to prevent postoperative infections.

The emerging resistance to antimicrobials (antibiotics and antiseptics) can be addressed by bacteriophages, which are successfully used for this purpose in surgery, otorhinolaryngology, and other medical fields [8, 9]. The Federal Clinical Guidelines state that bacteriophages are effective and highly specific biological drugs with antibacterial effect designed to prevent and treat infectious diseases. They eliminate the pathogen without affecting other microorganisms, are indispensable in combating antibiotic resistant infections, and can be used in combination with other drugs [10]. However, only single experimental studies have assessed their use in ophthalmology [11].

The *study aimed* to analyze the ocular surface microbiome and test its susceptibility to antimicrobial drugs, including antiseptics and bacteriophages, before cataract phacoemulsification.

MATERIALS AND METHODS

The study was conducted at the Ophthalmology Clinic of Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation and Saint-Petersburg Pasteur Research Institute of Epidemiology and Microbiology. A total of 60 patients (60 eyes), including 26 men and 34 women, aged 48–85 years were examined. The mean age of the subjects was 71.40 ± 0.99 years.

Preoperative samples for microbiological testing were taken from the following three areas in all patients: the conjunctiva (conjunctival surface), skin-lash margin (meibomian gland secretions), and lacrimal duct (aspirate). The samples were taken using strict aseptic techniques following all sanitary rules by the same clinician.

The samples were delivered to the laboratory on the same day (delay: 1–7 hours) for Gold inoculation on blood agar, salt egg yolk agar, and Sabouraud agar. After incubation at 37 °C for 24 hours, a pure culture was isolated, and the microorganisms were identified by MALDI-TOF (Bruker) mass spectrometry per the EUCAST protocol with an additional extended susceptibility testing to ophthalmologic antimicrobial drugs (AMD) and immunobiological products (bacteriophages) (Table 1). Susceptibility to AMDs and bacteriophages was determined using the disk diffusion method and spot test, respectively.

The following bacteriophages from two manufacturers were evaluated: the Perm branch of Scientific and Production Association “Microgen” JSC, Perm Scientific and Production Association “Biomed” (Sextaphage® polyvalent purified pyobacteriophage, LP-No. (002031)-(RG-RU) dated January 27, 2012; Intestiphage® intesti-bacteriophage No. 1, LP-No. (001999)-(RG-RU) dated October 25, 2011 (renewal date: August 11, 2022); Staphylophage® Staphylococcus bacteriophage No. 1, LP-No. (001973/01)-(RG-RU) dated March 26, 2012; Streptophage® Streptococcus bacteriophage, LP-No. (001974/01)-(RG-RU) dated January 19, 2009), and the N. Novgorod branch of Scientific and Production Association “Microgen” JSC, IMBIO (Piophage® Complex liquid pyobacteriophage, LP-No. (000700)-(RG-RU) dated June 21, 2010 (renewal date: August 11, 2022); Intestiphage® Intesti-bacteriophage No. 2, LP-No. (001999)-(RG-RU)

Table 1. List of antimicrobial medicaments and bacteriophages for sensitivity assessment
Таблица 1. Перечень противомикробных препаратов и бактериофагов для оценки чувствительности

Product class	Product name
Macrolides and azalides	Azithromycin
Second generation aminoglycosides	Tobramycin, gentamicin
Third generation aminoglycosides	Amikacin
Penicillins	Amoxicillin, ampicillin
Combinations of penicillins	Amoxicillin + clavulanate
Tetracyclines	Doxycycline
Second generation fluoroquinolones	Ciprofloxacin, ofloxacin
Third generation fluoroquinolones	Levofloxacin
Fourth generation fluoroquinolones	Moxifloxacin
Oxazolidinones	Linezolid
Second generation cephalosporins	Cefoxitin, cefuroxime
Third generation cephalosporins	Ceftriaxone, ceftazidime
Fourth generation cephalosporins	Cefepime
Amphenicols	Chloramphenicol
Carbapenems	Imipenem, meropenem
Glycopeptides	Vancomycin
Nitrofuran derivatives	Furazidine
Antiseptics and disinfectants	Topical antiseptic No. 1 containing 79% ethyl alcohol and 0.5% chlor-hexidine gluconate solution; skin antiseptic No. 2 containing 70% pro-pyl alcohol and 0.1% didecyldimethylammonium chloride solution; 10%, 5%, 3%, and 1% povidone-iodine; benzyldimethyl-myristoylamino-propyl-ammonium; picloxydine
Immunobiological products (bacteriophages)	Complex pyobacteriophage, polyvalent pyobacteriophage, intesti-bacteriophage No. 1 and No. 2, Staphylococcus bacteriophage No. 1 and No. 2, and Streptococcus bacteriophage

dated October 25, 2011 (renewal date: August 11, 2022); Streptophage® Streptococcus bacteriophage No. 2, LP-No. (001973/01)-(RG-RU) dated March 26, 2012). For microorganisms with known inefficacy of bacteriophages, susceptibility was not determined.

The study revealed monocultures and microbial consortia including two or more species. The isolates were passaged on appropriate differential diagnostic media to identify biochemical properties and test susceptibility to AMDs. A total of 193 samples were obtained from patients. No microbial growth was observed in 22.3% of cases, and a pure culture was isolated in 77.7% of cases.

RESULTS

Range of microorganisms

The following Gram-positive bacteria were identified in 70.9% of cases from three areas: *S. epidermidis* (48.2%), *S. aureus* (3.1%), *Corynebacterium macginleyi* (3.6%), *Staphylococcus lugdunensis* (2.6%), *Staphylococcus hominis* (2.1%), *Staphylococcus warneri* (2.1%), *Streptococcus oralis* (2.1%), *Corynebacterium amycolatum* (2.1%), *Staphylococcus haemolyticus* (1.0%), *Staphylococcus pasteuri* (1.0%), *Corynebacterium mastitidis* (1.0%), *Staphylococcus capitis* (0.5%), *Micrococcus luteus* (0.5%), *Kocuria kristinae* (0.5%), and *Propionibacterium* (0.5%).

Gram-negative bacteria were detected only in 6.8% of samples and included *Enterobacter cloacae* (4.7%), *Moraxella osloensis* (1.6%), and *Acinetobacter pitii* (0.5%).

Thus, over half of bacteria (60.6%) represents various *Staphylococcus* spp., with the largest percentage of Staphylococcus epidermidis. The most common Gram-positive and negative rods were *Corynebacterium* spp. (6.7%) and *E. cloacae* (4.7%), respectively. No growth was reported in 22.3% of samples.

Of the three areas tested in each patient, the skin-lash margin showed the highest contamination (Table 2).

Table 2. Distribution of bacteria by loci

Таблица 2. Распределение бактерий по локусам

Microorganism	Conjunctiva, *n* = 66	Free lid margin, *n* = 62	Lacrimal duct, *n* = 65
Staphylococcus epidermidis	32 (48.5%)	32 (51.6%)	29 (44.6%)
Enterobacter cloacae	4 (6.1%)	4 (6.5%)	1 (1.5%)
Corynebacterium macginley	3 (4.6%)	3 (4.8%)	1 (1.5%)
Staphylococcus aureus	1 (1.5%)	2 (3.2%)	3 (4.6%)
Staphylococcus lugdunensis	3 (4.6%)	1 (1.6%)	1 (1.5%)
Corynebacterium amycolatum	3 (4.6%)	0 (0.0%)	1 (1.5%)
Streptococcus oralis	1 (1.5%)	0 (0.0%)	3 (4.6%)
Staphylococcus warneri	1 (1.5%)	2 (3.2%)	1 (1.5%)
Moraxella osloensis	1 (1.5%)	2 (3.2%)	0 (0.0%)
Staphylococcus hominis	2 (3.1%)	1 (1.6%)	1 (1.5%)
Corynebacterium mastitidis	2 (3.1%)	0 (0.0%)	0 (0.0%)
Staphylococcus haemolyticus	1 (1.5%)	1 (1.6%)	0 (0.0%)
Staphylococcus pasteuri	0 (0.0%)	1 (1.6%)	1 (1.5%)
Staphylococcus capitis	0 (0.0%)	1 (1.6%)	0 (0.0%)
Micrococcus luteus	0 (0.0%)	1 (1.6%)	0 (0.0%)
Kocuria kristinae	0 (0.0%)	1 (1.6%)	0 (0.0%)
Propionibacterium	0 (0.0%)	0 (0.0%)	1 (1.5%)
Acinetobacter pitii	1 (1.5%)	0 (0.0%)	0 (0.0%)
No growth	11 (16.6%)	10 (16.3%)	22 (34.2%)

Resistance of the identified microorganisms to AMDs and bacteriophages

The following results were obtained. *S. epidermidis* showed resistance to the following AMDs: penicillins, macrolides and azalides, cephalosporins, FQs, second generation aminoglycosides, levomycetin (2% cases), and benzyldimethyl-myristoylamino-propylammonium (1% cases).

S. epidermidis was most resistant to complex pyobacteriophage (58%), intesti-bacteriophage No. 1 (60%), Staphylococcus bacteriophage No. 1 (54%), polyvalent pyobacteriophage (46%), unprotected penicillins (44%), 2nd generation FQs (22%), 3rd generation FQs (20%), 4th generation FQs (20%), ceftazidime (20%), and azithromycin (19%) (see Figure).

Percentage of other Staphylococci resistant to AMDs and bacteriophages varied among the types. *S. haemolyticus* was multidrug-resistant to penicillins, 2nd, 3rd, and 4th generation FQs, 2nd, 3rd, and 4th generation cephalosporins, azithromycin, intesti-bacteriophage No. 1, Staphylococcus bacteriophage No. 1, complex pyobacteriophage, and polyvalent pyobacteriophage. All *S. aureus* strains were resistant to penicillins, and some were resistant to levomycetin (33%) and ceftazidime (20%), with all strains being susceptible to all bacteriophages. Azithromycin resistance was observed in 100% of *S. capitis* and 40% of *S. lugdunensis* strains.

S. capitis resistance to intesti-bacteriophage No. 1, Staphylococcus bacteriophage No. 1, complex pyobacteriophage, and polyvalent pyobacteriophage was observed in 100% of cases; *S. lugdunensis* resistance to these bacteriophages was detected in 40% of isolates.

S. pasteuri was completely resistant to penicillins, intesti-bacteriophage No. 1, Staphylococcus bacteriophage No. 1, complex pyobacteriophage, and polyvalent pyobacteriophage. *S. hominis* was susceptible to all bacteriophages and AMDs, except for doxycycline (25%). *S. warneri* was sensitive to all AMDs and resistant (100%) to the following bacteriophags: intesti-bacteriophage No. 1, Staphylococcus bacteriophage No. 1, complex pyobacteriophage, and polyvalent pyobacteriophage.

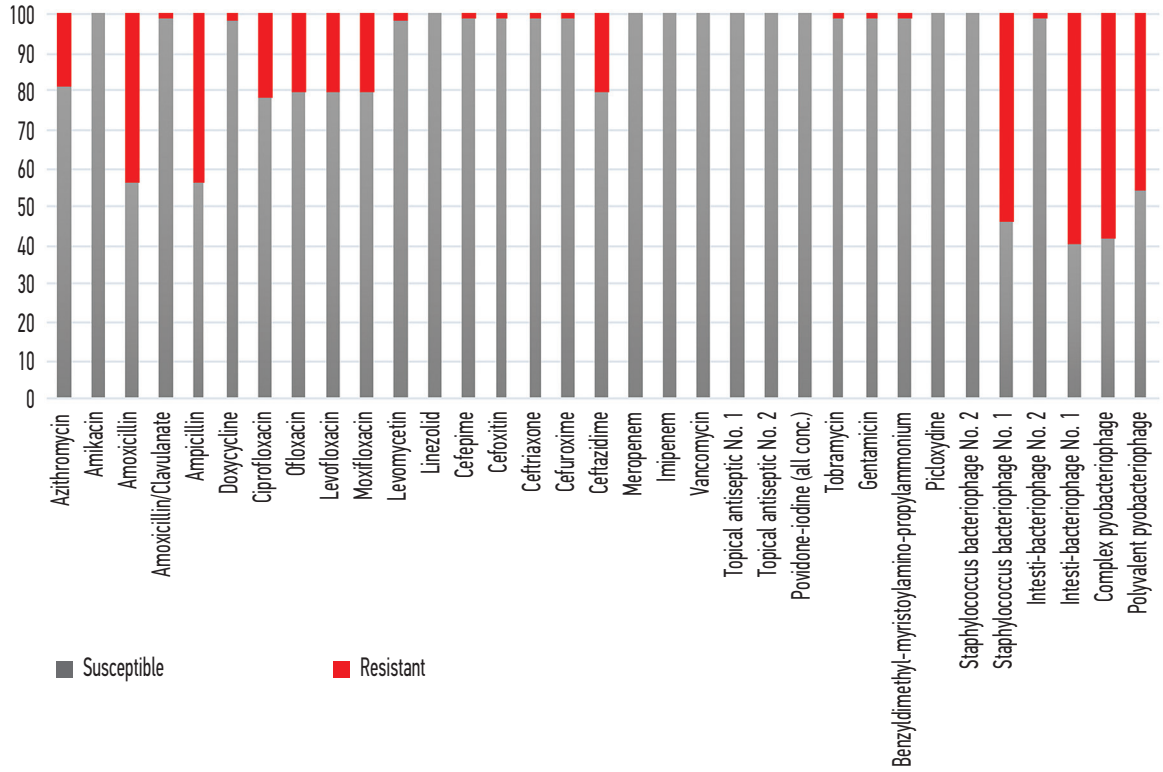

Figure. Sensitivity of Staphylococcus epidermidis to antimicrobial medicaments and bacteriophages
Рисунок. Чувствительность *Staphylococcus epidermidis* к противомикробным препаратам и бактериофагам

S. oralis was completely sensitive to all drugs, except for levomycetin, polyvalent pyobacteriophage, complex pyobacteriophage, Streptococcus bacteriophage, and intesti-bacteriophage No. 1.

Vancomycin resistance was reported in 50% of *Corynebacterium* spp. They were also resistant to FQs, ceftazidime, and levomycetin.

K. kristinae was resistant to FQs, 3rd generation cephalosporins, 2nd generation aminoglycosides, and 1% povidone-iodine.

As with *Propionibacterium*, *M. luteus* was resistant to levomycetin, benzyldimethyl-myristoylamino-propylammonium, and 1% povidone-iodine; however, these cocci were sensitive to all bacteriophages (Table 3).

As for Gram-negative bacteria, *E. cloacae* was resistant to penicillins (78%) and all bacteriophages (100%). *M. osloensis* was resistant to aminoglycosides in 67% of cases. *A. pitii* was multidrug-resistant, including to vancomycin. All Gram-negative bacteria were resistant to 1% povidone-iodine (Table 4).

All cultures showed 100% sensitivity to topical antiseptics (79% ethyl alcohol and 0.5% chlorhexidine gluconate solution; 70% propyl alcohol and 0.1% didecyldimethylammonium chloride solution), 5% and 3% povidone-iodine, and picloxydine. No Staphylococcus strains were resistant to Staphylococcus bacteriophage No. 2.

In most cases, ophthalmic antimicrobial resistance was reported for the following FQs: ciprofloxacin (16.7%), levofloxacin (16.1%), moxifloxacin (16.1%), ceftazidime (16.1%), and azithromycin (15.4%). Intesti-bacteriophage No. 1, Staphylococcus bacteriophage No. 1, complex pyobacteriophage, and polyvalent pyobacteriophage demonstrated low effectiveness against *Staphylococcus* spp. with resistance in 40%–100% of cases.

A. pitii (100%), *C. mastitidis* (100%), *C. amycolatum* (50%), and *C. macginleyi* (17%) were resistant to vancomycin.

The lowest percentage of microorganisms was resistant to aminoglycosides, such as tobramycin (3.4%) and gentamicin (3.4%).

DISCUSSION

The identified microbiome species includes mostly *Staphylococcus* spp., in particular S. epidermidis, which is consistent with the data from most studies [1–3, 12, 13]. Notably, the range of microorganisms is similar to the isolates from the vitreous humor in patients with post-phacoemulsification endophthalmitis [5]. Thus, opportunistic pathogens of the ocular surface should be considered as potential causative agents of postoperative endophthalmitis. Eyelid colonization with *S. epidermidis* (51.6%) is slightly higher than that of conjunctiva (48.5%)

Table 3. Proportion of sensitive (S) and resistant (R) Gram-positive bacteria to antimicrobial medicaments and bacteriophages, %

Таблица 3. Доля чувствительных (S) и резистентных (R) грамположительных бактерий к противомикробным препаратам и бактериофагам, %

Product		*S. epidermidis*	*S. aureus*	*S. warneri*	*S. lugdunensis*	*S. hominis*	*S. haemolyticus*	*S. pasteuri*	*S. capitis*	*C. amycolatum*	*C. mastitidis*	*C. macginley*	*K. kristinae*	*S. oralis*	*M. luteus*	*Propionibacterium*
Azithromycin	S	81	100	100	0	100	0	100	0	–	–	–	100	100	100	–
	R	19	0	0	100	0	100	0	100	–	–	–	0	0	0	–
Amikacin	S	100	100	100	100	100	100	100	100	–	–	–	100	100	100	–
	R	0	0	0	0	0	0	0	0	–	–	–	0	0	0	–
Amoxicillin	S	56	0	100	100	100	0	0	100	–	–	–	100	100	100	–
	R	44	100	0	0	0	100	100	0	–	–	–	0	0	0	–
Amoxicillin + clavulanate	S	99	100	100	100	100	0	100	100	–	–	–	100	100	100	–
	R	1	0	0	0	0	100	0	0	–	–	–	0	0	0	–
Ampicillin	S	56	0	100	100	100	0	0	100	–	–	–	100	100	100	–
	R	44	100	0	0	0	100	100	0	–	–	–	0	0	0	–
Doxycycline	S	98	100	100	100	75	100	100	100	100	100	100	100	100	100	100
	R	2	0	0	0	25	0	0	0	0	0	0	0	0	0	0
Levofloxacin	S	80	100	100	100	100	0	100	100	50	100	100	0	100	100	0
	R	20	0	0	0	0	100	0	0	50	0	0	100	0	0	100
Linezolid	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	–
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–
Cefepime	S	99	100	100	100	100	0	100	100	–	–	–	100	100	100	–
	R	1	0	0	0	0	100	0	0	–	–	–	0	0	0	–
Cefoxitin	S	99	100	100	100	100	0	100	100	–	–	–	100	100	100	–
	R	1	0	0	0	0	100	0	0	–	–	–	0	0	0	–
Ciprofloxacin	S	78	100	100	100	100	0	100	100	50	100	100	0	100	100	100
	R	22	0	0	0	0	100	0	0	50	0	0	100	0	0	0
Ceftriaxone	S	99	100	100	100	100	0	100	100	–	–	–	100	100	100	–
	R	1	0	0	0	0	100	0	0	–	–	–	0	0	0	–
Cefuroxime	S	99	100	100	100	100	0	100	100	–	–	–	100	100	100	–
	R	1	0	0	0	0	100	0	0	–	–	–	0	0	0	–
Imipenem	S	100	–	100	–	100	–	100	100	–	–	–	–	–	100	–
	R	0	–	0	–	0	–	0	0	–	–	–	–	–	0	–
Meropenem	S	100	–	100	–	100	–	100	100	–	–	–	–	–	100	–
	R	0	–	0	–	0	–	0	0	–	–	–	–	–	0	–
Ceftazidime	S	80	80	100	100	100	0	100	100	50	50	100	0	100	100	100
	R	20	20	0	0	0	100	0	0	50	50	0	100	0	0	0
Vancomycin	S	100	100	100	100	100	100	100	100	50	0	67	100	100	100	100
	R	0	0	0	0	0	0	0	0	50	100	33	0	0	0	0
Topical antiseptic No. 1	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Topical antiseptic No. 2	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 (continued) / Окончание таблицы 3

Product		*S. epidermidis*	*S. aureus*	*S. warneri*	*S. lugdunensis*	*S. hominis*	*S. haemolyticus*	*S. pasteuri*	*S. capitis*	*C. amycolatum*	*C. mastitidis*	*C. macginley*	*K. kristinae*	*S. oralis*	*M. luteus*	*Propionibacterium*
10% povidone-iodine	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5% povidone-iodine	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3% povidone-iodine	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1% povidone-iodine	S	100	100	100	100	100	100	100	100	100	100	100	0	100	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	100	0	100	100
Moxifloxacin	S	80	100	100	100	100	0	100	100	50	100	100	0	100	100	100
	R	20	0	0	0	0	100	0	0	50	0	0	100	0	0	0
Tobramycin	S	99	100	100	100	100	100	100	100	100	100	100	0	100	100	0
	R	1	0	0	0	0	0	0	0	0	0	0	100	0	0	100
Levomycetin	S	98	67	100	100	100	100	100	100	50	100	100	100	75	0	0
	R	2	33	0	0	0	0	0	0	50	0	0	0	15	100	100
Ofloxacin	S	80	100	100	100	100	0	100	100	50	100	100	0	100	100	100
	R	20	0	0	0	0	100	0	0	50	0	0	100	0	0	0
Gentamicin	S	99	100	100	100	100	100	100	100	100	100	100	0	100	100	0
	R	1	0	0	0	0	0	0	0	0	0	0	100	0	0	100
Benzyldimethyl-myristoylamino-propylammonium	S	99	100	100	100	100	100	100	100	100	100	100	100	100	0	0
	R	1	0	0	0	0	0	0	0	0	0	0	0	0	100	100
Picloxydine	S	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus bacteriophage No. 2	S	100	100	100	100	100	100	100	100	–	–	–	–	–	100	–
	R	0	0	0	0	0	0	0	0	–	–	–	–	–	0	–
Staphylococcus bacteriophage No. 1	S	46	100	0	60	100	0	0	0	–	–	–	–	–	100	–
	R	54	0	100	40	0	100	100	100	–	–	–	–	–	0	–
Intesti-bacteriophage No. 2	S	99	100	100	100	100	100	100	100	–	–	–	–	0	100	–
	R	1	0	0	0	0	0	0	0	–	–	–	–	100	0	–
Intesti-bacteriophage No. 1	S	40	100	0	60	100	0	0	0	–	–	–	–	0	100	–
	R	60	0	100	40	0	100	100	100	–	–	–	–	100	0	–
Complex pyobacte-riophage	S	42	100	0	60	100	0	0	0	–	–	–	–	0	100	–
	R	58	0	100	40	0	100	100	100	–	–	–	–	100	0	–
Polyvalent pyobacte-riophage	S	54	100	0	60	100	0	0	0	–	–	–	–	0	100	–
	R	46	0	100	40	0	100	100	100	–	–	–	–	100	0	–
Streptococcus bacteriophage	S	–	–	–	–	–	–	–	–	–	–	–	–	0	100	–
	R	–	–	–	–	–	–	–	–	–	–	–	–	100	0	–

Table 4. Proportion of sensitive (S) and resistant (R) Gram-negative bacteria to antimicrobial medicaments and bacteriophages, %

Таблица 4. Доля чувствительных (S) и резистентных (R) грамотрицательных бактерий к противомикробным препаратам и бактериофагам, %

Product		*Enterobacter cloacae*	*Moraxella osloensis*	*Acinetobacter pitii*
Furazidine	S	100	–	0
	R	0	–	100
Amikacin	S	100	–	100
	R	0	–	0
Amoxicillin	S	78	–	0
	R	22	–	100
Amoxicillin + clavulanate	S	78	100	100
	R	22	0	0
Ampicillin	S	78	–	0
	R	22	–	100
Doxycycline	S	–	100	–
	R	–	0	–
Levofloxacin	S	100	100	100
	R	0	0	0
Meropenem	S	100	100	100
	R	0	0	0
Cefepime	S	100	100	0
	R	0	0	100
Cefoxitin	S	100	100	0
	R	0	0	100
Ciprofloxacin	S	100	100	100
	R	0	0	0
Ceftriaxone	S	100	100	100
	R	0	0	0
Cefuroxime	S	100	100	0
	R	0	0	100
Imipenem	S	100	100	100
	R	0	0	0
Ceftazidime	S	100	100	100
	R	0	0	0
Vancomycin	S	100	100	0
	R	0	0	100
Topical antiseptic No. 1	S	100	100	100
	R	0	0	0
Topical antiseptic No. 2	S	100	100	100
	R	0	0	0
10% povidone-iodine	S	100	100	100
	R	0	0	0
5% povidone-iodine	S	100	100	100
	R	0	0	0
3% povidone-iodine	S	100	100	100
	R	0	0	0

Table 4 (continued) / Окончание таблицы 4

Product		*Enterobacter cloacae*	*Moraxella osloensis*	*Acinetobacter pitii*
1% povidone-iodine	S	0	0	0
	R	100	100	100
Moxifloxacin	S	100	100	100
	R	0	0	0
Tobramycin	S	100	33	100
	R	0	67	0
Levomycetin	S	100	100	0
	R	0	0	100
Ofloxacin	S	100	100	100
	R	0	0	0
Gentamicin	S	100	33	100
	R	0	67	0
Benzyldimethyl-myristoylamino-propylammonium	S	100	100	100
	R	0	0	0
Picloxydine	S	100	100	100
	R	0	0	0
Intesti-bacteriophage No. 2	S	0	–	–
	R	100	–	–
Intesti-bacteriophage No. 1	S	0	–	–
	R	100	–	–
Complex pyobacteriophage	S	0	–	–
	R	100	–	–
Polyvalent pyobacteriophage	S	0	–	–
	R	100	–	–

and corresponds to the data of another study, where *S. epidermidis* was isolated from the eyelids more often (94.7%) compared with the conjunctiva (54.28%) [3]. However, our study showed a smaller difference in colonization of the tested areas. Microbial colonization was more various on the conjunctiva. Some bacterial species were found only at specific areas; for example, *A. pitii* and *C. mastitidis* were isolated only from the conjunctiva, *K. kristinae*, *M. luteus*, and *S. capitis* were observed only at the free lid margin, and *Propionibacterium* was identified only in the lacrimal duct aspirate. The conjunctiva and eyelid samples showed no growth of *S. pasteuri* and *S. oralis*, respectively; *S. haemolyticus* and *M. osloensis* were not found in the lacrimal duct aspirate. No growth was most common (34.3%) in the lacrimal duct.

The susceptibility to immunobiological products, in particular bacteriophages, was first studied on a large ocular clinical sample. Of all phages, Staphylococcus bacteriophage No. 2 and intesti-bacteriophage No. 2 showed high antimicrobial activity. Interestingly, dilutions of povidone-iodine to 3% demonstrated *in vitro* antimicrobial activity against 100% of isolated microorganisms.

No isolates were resistant to picloxydine, unlike benzyldimethyl-myristoylamino-propylammonium. Picloxydine also demonstrated high activity against the conjunctival microbiome in another study [12].

The study performed in 2008 demonstrated that before moxifloxacin launch in the pharmaceutical market, microorganisms isolated from the conjunctiva of patients with cataract showed low resistance to FQs and aminoglycosides [13]. In a similar study in 2018, a higher proportion of bacteria were resistant to aminoglycosides, such as tobramycin (21.25%) and gentamicin (17.5%), than to ciprofloxacin (13.75%), ofloxacin (10%), levofloxacin (10%), and moxifloxacin (0%) [12]. Our study shows the opposite trend indicating that a percentage of microorganisms resistant to FQs—ciprofloxacin (16.7%), ofloxacin (16.1%), levofloxacin (16.1%), and moxifloxacin (16.1%)—was higher compared with aminoglycosides, such as tobramycin (3.4%) and gentamicin (3.4%).

CONCLUSION

The antimicrobial activity of the tested AMDs and bacteriophages varied depending on the microorganism

type. Given the changes in AMD activity over time, periodical appropriate monitoring is recommended to adjust antimicrobial prophylaxis accordingly. Our study shows that antiseptics, aminoglycoside antibiotics, Staphylococcus bacteriophage No. 2, and intesti-bacteriophage No. 2 have the highest *in vitro* antimicrobial activity against the isolated microorganisms. Therefore, further studies of Staphylococcus bacteriophage No. 2 and intesti-bacteriophage No. 2 are warranted.

ADDITIONAL INFO

Authors' contribution. All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. Personal contribution of each author: T.Yu. Bogdanova — concept, research and analysis, collecting and preparation of samples, data analysis, writing the main part of the text; A.N. Kulikov — concept, data analysis, making final edits; L.A. Kraeva — concept, collecting and preparation of samples, laboratory examination, data analysis.

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Consent for publication. Written consent was obtained from the patients for publication of relevant medical information within the manuscript.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией. Личный вклад каждого автора: Т.Ю. Богданова — концепция и дизайн исследования, поисково-аналитическая работа, сбор и обработка материалов, анализ полученных данных, написание текста; А.Н. Куликов — концепция, анализ полученных данных, внесение окончательной правки; Л.А. Краева — концепция, обработка материалов, лабораторное исследование, анализ полученных данных.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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