• Clinical Research •

Ocular flora in patients undergoing intravitreal injections: antibiotic resistance patterns and susceptibility to antiseptic picloxydine

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Abstract

● **AIM:** To study antibiotic resistance patterns and susceptibility to eye antiseptic picloxydine of conjunctival flora in patients undergoing intravitreal injections (IVIs).

● **METHODS:** Conjunctival swabs were taken in 4 groups of patients, 20 patients in each group (*n*=80): without IVIs and ophthalmic operations in history (group N1; control group); with the first IVI and antibiotic eye drops Tobrex applied 3d before IVI and 5d after it (group N2); with 20 or more IVIs and repeated courses of antibiotic eye drops (group N3); with the first IVI and antiseptic eye drops Vitabact (picloxydine) applied 3d before IVI and 5d after it (group N4). In groups N2 and N4 swabs were taken at baseline and after the treatment. Efficacy of picloxydine in inhibition of growth of conjunctival isolates susceptible and resistant to antibiotic was studied *in vitro*. Minimal inhibition concentrations (MIC) were determined with microdilution test.

● **RESULTS:** Two of the three patients who had to undergo the IVI procedure showed conjunctiva bacterial contamination. Along with few *Staphylococcus aureus* and Gram-negative isolates susceptible to most antibiotics, the majority (71%-77%) of causative agents were coagulase-negative *Staphylococci* (CoNS), 40%-50% of which were multidrug resistant (MDR). Eye disinfection in the operating room and peri-injection courses of Tobrex or Vitabact resulted in total elimination of isolates found at baseline. However, in 10% and 20% of patients, respectively, recolonization of the conjunctiva with differing strains occurred. In patients with repeated IVI and Tobrex/Maxitrol treatment, the conjunctival flora showed high resistance rates: 90% of CoNS were MDR. In the *in vitro* study, picloxydine showed bactericidal effect against *Staphylococci* isolates both antibiotic resistant and susceptible with MIC≥13.56 μg/mL. Incubation of bacteria for 15min in Vitabact eye drops, commercially available form of picloxydine, 434 μg/mL, showed total loss of colony forming units of all tested isolates including *Pseudomonas aeruginosa*.

● **CONCLUSION:** The confirmed efficacy of eye antiseptic picloxydine against conjunctival bacterial isolates and the presence of its commercial form, 0.05% eye drops, convenient for use by patients before and after injection, make this eye antiseptic promising for prophylaxis of IVI-associated infectious complications.

● **KEYWORDS:** intravitreal injections; conjunctival isolates; antibiotic resistance; picloxydine; Vitabact
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INTRODUCTION

Intravitreal injections (IVIs) are one of the effective, widespread and minimally invasive methods of treatment of various retinal diseases. The effectiveness of such therapy is observed in the treatment of exudative age-related macular degeneration, edema, associated with diabetic retinopathy or retinal vein occlusions. Due to a noticeable increase in the incidence of diabetes mellitus and cardiovascular diseases it is expected that the number of IVIs will steadily continue to rise. Generally, IVI is a safe procedure. However, like any surgical intervention, it carries the risk of potential complications. Infectious complications, associated with IVIs, occur when

a pathogen from the eye surface in the site of the injection penetrates the vitreous cavity. The most dangerous complication is the infectious endophthalmitis with visual impairment threat even in a case of proper and early treatment[1-2].

The importance of antimicrobial treatment accompanying the IVI procedure is obvious. However, to date, there is no single approach to the management of patients regarding the use of antibacterial eye drops before and/or after IVI as a prevention method of inflammatory infectious complications. In 2004 when the practice of IVI was just introduced, ophthalmic antibiotics were widely used for this purpose[3]. But unlike other ocular surgeries, conducted once or twice in a patient's life, where topical antibiotics may be an appropriate prophylactic measure, IVIs are often repeated to the same eye[4]. In such patients, short-term repeated courses of topical antibiotics accompanying IVI may not only reduce the risk of infectious complications but actually enhance it by increasing antibiotic resistance of conjunctival flora. In recent years, this has been confirmed in several studies.

Coagulase-negative staphylococci (CoNS), the most common bacteria on the eye surface[5], demonstrated increased rates of resistance to fluoroquinolones if isolated from eyes repeatedly exposed to one of the following ophthalmic antibiotics ofloxacin/gatifloxacin/moxifloxacin hydrochloride[6]. As shown in the study[7], CoNS isolates from azithromycin-exposed eyes were characterized by increased macrolide resistance. The predominant CoNS strain *Staphylococcus epidermidis* (*S. epidermidis*) developed co-resistance to trimethoprim/sulfamethoxazole, gentamicin and clindamycin in fluoroquinolone-exposed eyes and to trimethoprim/sulfamethoxazole and doxycycline in azithromycin-exposed eyes[7]. Milder *et al*[8] and Dorrepaal *et al*[9] also found the increased antibiotic resistance of conjunctival flora due to repeated use of fluoroquinolone drops. The selection of resistant bacteria does not require so much time. Thus, bacterial colonies with high resistance to gatifloxacin were isolated from the eye of the patient who received only three IVIs with a prophylactic use of this topical antibiotic[9].

Along with the increased resistance, repeated courses of topical eye antibiotics cause changes in the composition of conjunctival flora with the significant increase in the percentage of *S. epidermidis*[10]. The authors note the clinical significance of this fact because *S. epidermidis* is the main causative agent of ocular infections.

Grzybowski *et al*[5] consider IVI to be "a prime example where unnecessary and/or improper use of antibiotics may have serious consequences". An alternative may be the use of antiseptics with efficacy comparable to antibiotics such as povidone-iodine or biguanides. Barkana *et al*[11] proved that there was no significant difference between povidone-iodine, chlorhexidine (cationic biguanide) and ofloxacin in terms of reduction of conjunctival flora, 91.2%, 87.6% and 85.6%, respectively. Merani *et al*[12] showed that aqueous chlorhexidine used as an antiseptic drug before IVI was well tolerated and effective in terms of low rate of endophthalmitis. In conjunctival samples after the treatment with chlorhexidine 0.05%, there was a significant reduction in the total bacterial load (82%) and even greater results were observed for CoNS (90%). In this study by Gili *et al*[13], no povidone-iodine was administered to the patient, eye irrigation was performed only using 0.05% chlorhexidine solution.

When comparing povidone-iodine and chlorhexidine, the former is still considered to be the gold standard for prophylaxis of infectious complications in eye surgery[12]. Along with efficacy, application of brown colored povidone-iodine, in contrast to colorless chlorhexidine, is easier for surgeon due to the visible areas of irrigation. However, povidone-iodine has its deficiencies. There is a cohort of patients with povidone sensitivity, not true immunoglobulin E-mediated allergy[14]. Sensitivity can be expressed in conjunctival hyperemia, irritation (mild to severe) and pain. In these cases, surgeon should consider using another antiseptic drug, for example, chlorhexidine. Thus, Oakley and Vote[15] switched povidone-iodine to 0.1% chlorhexidine solution in patients reporting high levels of pain. As the result, the average pain score decreased from 8 of 10 points to 3 of 10.

Another antiseptic from the group of biguanides is picloxydine dihydrochloride commercially available as Vitabact, 0.05%. Different pharmaceuticals companies worldwide produce eye drops of picloxydine with different brand names: Vitabact (LaboratoiresThéa, France; Ciba Vision, Lithuania; Novartis, Tunisia; Novartis, Excelvision, O.C.A. Vietnam), Medibact (Medipak, Pakistan), Bactavit (Rompharm, Georgia). These approved eye drops could be useful in pre- and post-injection prophylaxis of eye infections. However, we found no data on the use of Vitabact in the management of patients with IVI.

In the study, we confirmed the increased resistance of conjunctival flora in patients with multiple IVIs and antibiotic eye drops courses in anamnesis. We compared efficacy of antiseptic Vitabact and antibiotic Tobrex eye drops in the eye surface decontamination. In the *in vitro* experiments, we proved the bactericidal effect of Vitabact eye drops against both antibiotic susceptible and resistant conjunctival bacterial isolates.

SUBJECTS AND METHODS

Ethical Approval This study was conducted in accordance with the Declaration of Helsinki. All patients were recruited from the Scientific Research Institute of Eye Diseases in Moscow, Russia. The local biomedical ethics committee of the Scientific Research Institute of Eye Diseases approved the

protocol (protocol No.49/4). Informed written consent was obtained from each patient before participation in the study.

This was a prospective case-control study comparing 4 groups of patients, 20 patients in each group (n=80): 1) patients of the control group, comparable in age, without IVI and ophthalmic operations in history (group N1); 2) patients undergoing the first IVI who applied antibiotic eye drops Tobrex based on aminoglycoside tobramycin 3d before IVI and within 5d after it (group N2); 3) patients who received 20 or more IVIs and the concomitant courses of antibiotic eye drops Tobrex, in some courses it was replaced by Maxitrol containing aminoglycoside neomycin, polymyxin B and dexamethasone (group N3); 4) patients undergoing the first IVI who applied antibacterial eye drops Vitabact (picloxydine) 3d before IVI and within 5d after it (group N4).

Exclusion criteria for all groups were the following: age less than 50 years old, use of systemic antibiotics within 3mo, use of ocular hypotensive drops for the management of glaucoma; moreover, use of antibiotic drops and ocular surgery were exclusion criteria for the second, third and fourth groups.

In the standard IVI procedure, the eyelid skin and the area around the eye were treated with a 10% solution of iodopyrone. Next, eyelid speculum was applied. Local anesthetic drops of Alcaine were instilled in the conjunctival sac. The conjunctival cavity was irrigated for 30s with 2.0 mL of 5% povidone-iodine and then with saline solution to wash away povidone residue.

Conjunctival swabs were taken with sterile disposable tampons using a standard procedure (from lateral to medial angle of the eye) in the lower conjunctival fornix to the Amies transport system, which maintains the viability of microorganisms from the time of the material collection to the beginning of the study. Care was taken to minimize the contact with lashes, eyelids and skin. In groups N2 and N4, conjunctival swabs were taken both before (at baseline) and the next day after the end of post-injection treatment with Tobrex or Vitabact.

In positive swabs, the isolated microorganisms were identified and tested for antibiotic susceptibility by BD Phoenix 100 automated identification and susceptibility testing system.

The *in vitro* inhibitory effects of picloxydine in the form of commercially available eye drops Vitabacton the growth of conjunctival isolates was analyzed with the broth microdilution test. The Trypticase Soy Broth (Becton Dickinson, France) containing a series of double-diluted Vitabact in the range 1:2 to 1:32 (corresponded to picloxydine 217.00 to 13.56 μg/mL) or without Vitabact in control samples was used for bacterial growth. Three colonies of each isolate grown for 24h at 37℃ on Columbia agar (Becton Dickinson, France) with 5% sheep blood plates were suspended in phosphate buffer saline (PBS, pH 7.4) with density adjusted to 0.5 McFarland. These stock suspensions were used to inoculate (20 μL) samples of nutrient broth (200 μL) in sterile 96-well plates. The absorbance of bacterial cultures was recorded with Perkin Elmer Wallac 1420 Multilabel Counter (Sweden) at 490 nm at the initial moment, after inoculation, as well as after 24 and 96h growth at 37℃. To confirm the inhibitory effect of picloxydine, probes from the wells were inoculated in Columbia agar plates and cultivated at 37℃ for 24h. The bactericidal effect of Vitabact eye drops was studied with incubation of bacteria, 10^8 colony forming units (CFU)/mL, directly in Vitabact (434 μg/mL picloxydine) for 15min at 25℃ or diluted in phosphate buffer saline (PBS, pH 7.4) 1:16 Vitabact, 27.12 μg/mL picloxydine, for 60min at 25℃. The loss of CFU was controlled by subsequent cultivation on agar plates at 37℃ within 24h. The independent experiments were performed in triplicate.

Also, we assessed the pain score after the procedure of IVI and after the treatment with Tobrexor Vitabact by using the numeric pain rating scale (NPRS).

Statistical analysis was conducted with SPSS *via* contrasting the respective 95% and 99% confidence intervals (based on the estimates of group means and standard deviations). Pearson's Chi-square test was used for testing relationships between categorical variables. McNemar Chi-square test was used on paired nominal data. It was applied to 2×2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal (*e.g.* to determine whether particular microorganisms are found before and after the treatment). To compare mean antibiotic resistance in various isolates at 0, 24 and 96h two-way ANOVA with Tukey post-hoc test was used [separately for the condition without Vitabact and for the condition with Vitabact (1:32 dilution)].

RESULTS

Ocular Flora and Antibiotic Resistance Patterns A total of 120 conjunctival swabs from 80 eyes were collected during the study. Of these 120 swabs, 59 isolates were cultured. *S. epidermidis* composed the body (66.1%, 39/59) of isolates, followed by *Staphylococcus aureus* (*S. aureus*; 11.86%, 7/59), *Staphylococcus hominis* (*S. hominis*; 6.78%, 4/59), *Staphylococcus haemolyticus* (*S. haemolyticus*; 5.08%, 3/59). Also, one isolate (1.69%, 1/59) of each was obtained: *Staphylococcus caprae* (*S. caprae*), *Staphylococcus lugdunensis* (*S. lugdunensis*), and Gram-negative microorganisms–*Enterococcus cloacae*, *Escherichia coli* (*E. coli*), *Pseudomonas auruginosa* (*P. aeruginosa*) and *Pseudomonas luteola* (*P. luteola*).

In the control group N1 (20 eyes), microflora growth was detected in 14 swabs (70%). All the 14 isolates (Table 1) were Gram-positive staphylococci: *S. epidermidis* was found in swabs of 11 patients (78.57%, 11/14), the rest 3 bacteria were *S. caprae*, *S. hominis* and *S. aureus*, each 7.14% (1/14). Thus,

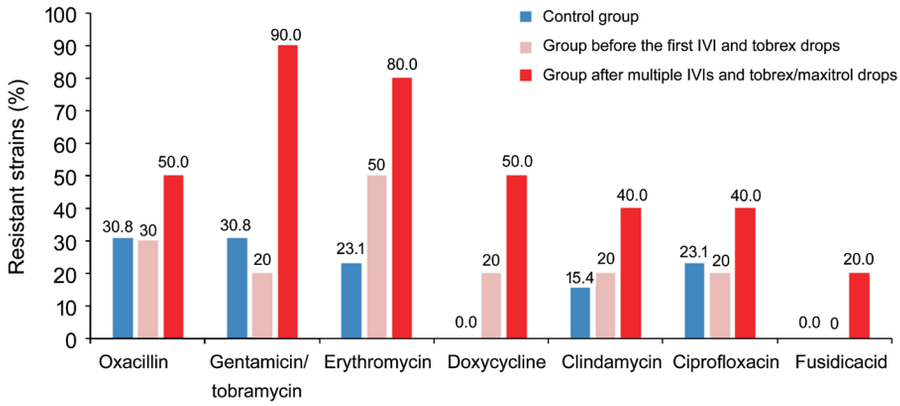

Figure 1 Percentage of antibiotic resistant coagulase-negative *Staphylococcus spp.* (CoNS) isolates in different groups of patients.

Table 1 Bacterial species isolated from conjunctival swabs in different groups of patients *n* (%)

Bacterial species	Control group N1	Group N2		Group N3 after multiple IVIs and antibiotic eye drops treatments
		Before the first IVI and antibiotic eye drops treatment	After the first IVI and antibiotic eye drops treatment	
Gram-positive	14 (100)	13 (92.9)	2 (100)	11 (91.7)
S. epidermidis	11 (78.6)	7 (50.0)	-	8 (66.7)
S. caprae	1 (7.14)	-	-	-
S. haemolyticus	-	1 (7.14)	-	2 (16.7)
S. hominis	1 (7.14)	2 (14.3)	1 (50.0)	-
S. lugdunensis	-	-	1 (50.0)	-
S. aureus	1 (7.14)	3 (21.4)		1 (8.3)
Gram-negative	0	1 (7.14)	0	1 (8.3)
Enterobacter cloacae	-	1 (7.14)	-	-
P. aeruginosa	-	-	-	1 (8.3)

13 out of 14 (92.86%) isolates were CoNS. Among 13 CoNS, 6 isolates were resistant to drugs of 3 to 4 antibiotics classes that is they are multidrug resistant (MDR). Methicillin-resistant staphylococci (MRS) made up 30.77% (4/13) of CoNS isolates (Figure 1). Almost the third of the isolates (30.77%, 4/13) were resistant to gentamicin and tobramycin. Rather high percentage (23.08%, 3/13) of CoNS were resistant to erythromycin or ciprofloxacin. The *S. aureus* isolate was susceptible to all drugs among 21 tested except chloramphenicol.

In the group of 20 patients who had to undergo the first IVI and antibiotic eye drops Tobrex treatment (group N2), the swabs were obtained before (20 eyes) and after (20 eyes) this treatment. Like in the control group, at baseline before the treatment microflora growth was observed in 14 swabs (70%, 14/20). The isolates represented different types of staphylococci, including 10 CoNS (76.92%, 10/13) and 3 *S. aureus* (23.08%, 3/13). Among 10 CoNS, 5 (50%) were MDR and 3 (30%) MRS, 2 (20%) were gentamicin and tobramycin and 2 (20%) ciprofloxacin resistant (Figure 1). This corresponded to the control group in Gram-positive/Gram-negative proportion (Pearson's χ^2=1.04, P=0.31) as well as in bacterial species' structure (Pearson's χ^2=5.22, P=0.63). Actually, all three groups (N1, N2 before the treatment, and N3) were equivalent in Gram-positive/Gram-negative proportion (Pearson's χ^2=1.15, P=0.56) as well as in bacterial species' structure (Pearson's χ^2=12.47, P=0.41. Surprisingly, in this group of patients who had to undergo the first IVI, the percentage of erythromycin resistant CoNS reached 70% (7/10) that was much higher as compared with the control group (Figure 1). As for *S. aureus* isolates, the first one was antibiotic susceptible, the second was resistant to penicillin G and the third to tobramycin and tetracycline. In one case, Gram negative *Enterobacter cloacae* was isolated (Table 1).

In the same group N2 of 20 patients after the first IVI procedure and peri-injection treatment with Tobrex (20 eyes), only two conjunctival swabs were positive. These changes were statistically significant (McNemar χ^2=6.75, P=0.009). In one patient, no growth was observed in swabs taken at the first visit, and after the IVI and Tobrex treatment, antibiotic susceptible *S. hominis* was isolated. In the swabs of the second patient *S. haemolyticus* resistant to erythromycin, chloramphenicol and fosfomycin (including glucose-6-phosphate) was isolated at the first visit, and *S. lugdunensis* resistant to fosfomycin was obtained at the second visit.

In 20 patients (20 eyes) who received 20 IVIs and peri-injection prophylaxis with antibiotic eye drops (group N3), 11 swabs were positive (55%, 11/20), one of them gave the growth of two isolates (*S. epidermidis* and *S. haemolyticus*). Eleven cultures were staphylococci (Table 1), among them ten were CoNS, 80% (8/10) *S. epidermidis* and 20% (2/10) *S. haemoyticus*. The rest one was *S. aureus*. In addition, Gram negative *P. aeruginosa* was detected in one case. As for antibiotic patterns, the conjunctival flora in such patients was characterized by an increase in the number of strains resistant to a wide range of antibiotics. Nine of ten CoNS were MDR (90%). Among these, we found *S. epidermidis* isolate resistant to 11 antibiotic classes. In this group of patients who received repeated courses of aminoglycoside-containing eye drops Tobrex/Maxitrol, 90% (9/10) CoNS and the single isolate of *S. aureus* were gentamicin and tobramycin resistant (Figure 1). This threatening situation encouraged us to try antiseptic picloxydine-containing eye drops Vitabact in peri-injection antimicrobial prophylaxis.

In group N4 of 20 patients who were prescribed antiseptic eye drops Vitabact 3d before the first IVI (20 eyes) and within 5d after it (20 eyes), microflora growth was not detected in 80% of swabs taken next day after the end of the treatment. The swabs of 6 (30%, 6/20) patients were negative at baseline and after the treatment. From 13 positive baseline swabs (65%, 13/20), the majority, namely 12 swabs (92.31%, 12/13) showed *Staphylococci* growth. Ten CoNS isolates were represented by *S. epidermidis* (Table 2). Out of ten, four *S. epidermidis* isolates were MDR (40%, 4/10). Both *S. aureus* isolates were resistant to penicillin G and one of them to ampicillin. In one case the rare *P. luteola* was isolated. The latter was susceptible to all antibiotics tested.

After IVI and prophylaxis with Vitabact the swabs of ten of these patients were negative. In three patients (15%, 3/20), *S. epidermidis* growth was observed both before and after post-injection treatment with Vitabact. However, the cultures isolated before and after the treatment differed in their resistance to certain antibiotics; this fact indicates the elimination of the primary isolated strain as a result of the Vitabact treatment and secondary infection with another strain of *S. epidermidis*. In one patient (5%, 1/20), swab was negative before the treatment, but after the treatment antibiotic-susceptible *E. coli* was isolated, which can also be explained by secondary infection due to eye hygiene breaches.

Conjunctival isolates Growth Inhibition with Picloxydine

Picloxydine (Vitabact) efficacy in inhibition of conjunctival isolates growth was confirmed in the *in vitro* study. In these experiments we analyzed the growth of 44 staphylococci isolates in picloxydine-containing nutrient broth.These isolates included 5 *S. aureus* (1 antibiotic susceptible, 4 resistant to 1-2 antibiotic classes), 33 *S. epidermidis* (2 antibiotic susceptible, 15 resistant to 1-2 antibiotic classes and 16 MDR), 2 *S. haemolyticus* (1 resistant to 2 antibiotic classes and 1 MDR), 3 *S. hominis* (1 susceptible and 2 MDR), 1 *S. caprae* (MDR). Three Gram-negative isolates were also included in the study: *P. aeruginosa*, *P. luteola* and *E. coli.*

Table 2 Bacterial species isolated from conjunctival swabs in group of patients with the first IVI and Vitabact treatment 3d before and 5d after it *n* (%)

Bacterial species	Before the treatment	After the treatment	McNemar χ^2/P
Gram-positive	12 (92.3)	3 (75.0)	7.11/0.008
S. epidermidis	10 (76.9)	3 (75.0)	5.14/0.023
S. aureus	2 (15.4)	-	-
Gram-negative	1 (7.7)	1 (25.0)	-
P. luteola	1 (7.7)	-	-
E. coli	-	1 (25.0)	-

After 24h, we did not detect growth of any staphylococci in series of liquid growth media containing double-diluted Vitabact (from 1:2 to 1:32) that corresponded to decrease in picloxydine concentration from 217.00 to 13.56 μg/mL for every condition (F=0.69; P=0.60). Control suspensions without Vitabact showed equal bacteria growth in each of three conditions, as observed by absorbance increase (F=0.77; P=0.55). In Table 3 we summarized the growth parameters of staphylococci isolates in nutrient broth without addition or in the presence of Vitabact in its lowest concentration (1:32 dilution) tested in our study. In order to identify possible differences in the Vitabact effect on MDR strains, CoNS that made up the most of isolates were grouped according to their antibiotic resistance. *S. aureus* formed one group, as among the few *S. aureus* MDR strains were not isolated. We found no differences in picloxydine inhibitory effect on the growth of isolates, MDR or bacteria resistant to no more than two drugs, as well as CoNS and *S. aureus*.

As Tukey post-hoc test showed, after 96h growth the absorbance changes were insignificant as compared with 24h for growth without Vitabact (P=0.92 for *S. aureus*, P=0.28 for CoNS, P=0.84 for MDR) and for growth with Vitabact (P=0.63 for *S. aureus*, P=0.79 for CoNS, P=0.90 for MDR). Only one isolate, *S. epidermidis* resistant to clindamycin, chloramphenicol and erythromycin, showed the growth of absorbance to the value of about 0.25. Its contribution in the average MDR group absorbance after 96h growth is seen from increased standard deviation (Table 3). The results again did not reveal a difference depending on antibiotic sensitivity and staphylococci species (Vitabact effect was statistically strong in all isolates at 1% level). The difference between 0 and 24h without Vitabactwas statistically significant for *S.aureus* (Tukey post-hoc test, P=0.0001), for CoNS (Tukey

Table 3 Absorbance of *Staphylococci* cultures in nutrient broth growing without addition or with Vitabact (1:32 dilution) that corresponds to 13.56 μg/mL of picloxydine mean±SD

Isolates	Antibiotic resistance	Growth time without Vitabact, h			Growth time with Vitabact, h		
		0	24	96	0	24	96
S. aureus	Susceptible or resistant to 1-2 drugs (n=5)[a]	0.106±0.003	0.857±0.121	0.745±0.160	0.106±0.002	0.116±0.012	0.099±0.008
CoNS	Susceptible or resistant to 1-2 drugs (n=19)[a]	0.106±0.002	0.723±0.142	0.603±0.174	0.108±0.004	0.111±0.011	0.104±0.010
	MDR (n=20)[a]	0.107±0.002	0.712±0.189	0.667±0.223	0.105±0.006	0.119±0.013	0.114±0.034

CoNS: Coagulase-negative staphylococci; MDR: Multidrug resistant; [a]number of isolates.

post-hoc test, P=0.0001), and for MDR (Tukey post-hoc test, P=0.0001).

Probes of each staphylococci culture grown in nutrient broth for 24 or 96h with or without Vitabact dilutions were cultivated further on agar plates within 24h. Those taken from picloxydine-containing liquid media samples showed no growth except one mentioned isolate with the lowest picloxydine concentration tested (13.56 μg/mL). The picloxydine minimal inhibitory concentration (MIC) for staphylococci conjunctival isolates growth was ≥13.56 μg/mL.

Among the Gram-negative bacteria, the most resistant was *P. aeruginosa* isolate. It grew in liquid medium even with 217.00 μg/mL picloxydine content (1:2 deluted Vitabact). For *E.coli* and *P. luteola* the minimal picloxydine concentrations that inhibited the growth of these isolates for 24h were 54.25 and 13.56 μg/mL, respectively. Bactericidal effect during 96h growth in nutrient broth was detected with picloxydine concentrations ≥54.25 μg/mL for *E. coli* and ≥27.12 μg/mL for *P. luteola*.

Incubation of *P. aeruginosa* or *E. coli* (10^8 CFU/mL) directly in Vitabact (434 μg/mL picloxydine) for 15min caused complete loss of CFU as observed by subsequent cultivation on agar plates at 37°C within 24h. Moreover, incubation of these isolates in diluted 1:16 Vitabact, 27.12 μg/mL picloxydine in PBS (pH 7.4), for 60min led to the same. Therefore, lower concentrations of picloxydine were required to achieve a bactericidal effect toward Gram-negative bacteria in the absence of the components of nutrient broth. Staphylococci (4 *S. epidermidis*, 2 antibiotic susceptible and 2 MDR, 2 *S. hominis*, susceptible and MDR, *S. caprae*, *S. haemolyticus* and 2 *S. aureus*) exposed for 15min to Vitabact or 60min to 1:16 diluted Vitabact (27.12 μg/mL picloxydine in PBS) lost CFU, that is complete bactericidal effect was achieved.

With regard to the results of pain levels, the value of pain after the IVI procedure was 7 points (±2). After Tobrex or Vitabact treatment, we observed reduction of pain to 0 points in the both groups within the first 24h of application of eye drops.

DISCUSSION

In our study, at least two of the three patients who had to undergo the IVI procedure showed conjunctiva bacterial contamination: in groups N3 (n=20) and N4 (n=20) the baseline swabs of 14 and 13 patients were positive. Consistent with other studies[5], the majority of bacteria were CoNS (71%-77%) followed by *S. aureus* (15%-21%) and single Gram-negatives. Among CoNS, the most frequently isolated was *S. epidermidis* (70%-100%). *S. epidermidis* is believed to prevent the colonization of conjunctiva by more serious pathogens[16]. CoNS (93%, 87% of these *S. epidermidis*) and *S. aureus* (7%) constituted the ocular flora in the control group (n=20).

Antibiotic resistance rate of ocular flora and especially CoNS in potent ophthalmic patients (groups N3 and N4, preoperatively) requires increased attention when prescribing pre- and post-injection prophylactic antimicrobials. Along with *S. aureus* and Gram-negative isolates susceptible to most antibiotics, 40%-50% of CoNS in our study were MDR. In view of the continuing practice of antibiotic eye drops prophylactic peri-injection treatment in the Russian Federation, we should mention that CoNS resistant to gentamycin/tobramycin were found in 20% and to moxifloxacin in 10% of potent ophthalmic patients.

The patients who received 20 or more IVIs and concomitant prophylactic courses of antibiotic therapy, showed a significant increase in the resistance of the conjunctival flora to a wide range of antibiotics. Percentage of MDR strains reached 75% of all isolates and doubled among CoNS (90%). These patients received courses of antibiotic eye drops Tobrex based on aminoglycoside tobramycin (in some courses replaced by Maxitrol containing aminoglycoside neomycin, polymyxin B and dexamethasone). As the result, we found 4.5 times increase of tobramycin resistant strains (90%) as compared with those isolated preoperatively in groups 3 and 4 (20%). Thus, we strongly suggest testing susceptibilityin patients who are planning to undergo repeated IVI. The results of the test would help the surgeon to avoid prescribing unnecessary and even threatening antibacterial drug and to choose the most appropriate one.

Nowadays asepsis and antisepsis are beneficial rather than use of topical antibiotics as a prevention method of post-injection complications[4]. Aseptic and antiseptic techniques include performing injections in the operating rooms, application of

povidone-iodine and peri-injection prophylactic treatment with antiseptic drops. Ultraclean air and good ventilation are required in operating rooms[2]. In order to reduce the risk of infection, the spread of pathogens from the oral cavity of patients and medical personnel should be minimized[17] by using sterile masks by surgeons and nurses and sterile adhesive eye drapes that isolate patients' nasopharyngeal area and periocular region[18]. It is important to prevent the contact of eyelashes and eyelid margins from the injection site and the needle, through which the medication is injected into the vitreous cavity. This can be achieved by using an eyelid speculum which remove the eyelashes, potential source of infection of the needle tip[17]. The preparation of the ocular surface should include irrigation with a solution of povidone-iodine for at least 30s[19]. Precisely, irrigation of the conjunctiva is needed, not a drop application of the solution, that corresponds to Safar and Dellimore[20] findings. A single application of povidone-iodine demonstrates a bactericidal effect, equivalent to the 3-day course of local antibiotics[21]. Several studies have shown that resistance to povidone-iodine does not develop[22-23] unlike the reported reduced levels of susceptibility to chlorhexidine[24], so we can safely continue using povidone-iodine solution in the operating rooms.

As to pre- and post-injection prophylaxis, in patients, who used the Vitabact antibacterial eye drops 3d before the first IVI and within 5d after it, 80% of swabs taken the day after the end of the treatment were negative. Thus, the effectiveness of a single pre- and post-injection course of Vitabact was close to that of Tobrex (90% of negative swabs). Among positive conjunctival swabs taken the day after the end of post-injection treatment with Tobrex or Vitabact, 2 and 4 isolates were found, respectively. However, these isolates differed in their resistance to certain antibiotics from those found at baseline in the same patients. This means that the isolates found at baseline were eliminated with the povidone-iodine irrigation before the injection and/or eye drops treatment. Most likely, after the end of the eye drops post-injection treatment a rapid, within one day, recolonization of the conjunctiva occurred.

In the *in vitro* study with microdilution test, picloxydine inhibited the growth of 39 CoNS, 5 *S. aureus*, *E. coli* and *P. luteola* isolates regardless of their antibiotic susceptibility. The picloxydine MIC for staphylococci was ≥13.56 μg/mL. Another test with incubation of bacteria 15min in Vitabact eye drops, commercially available form of picloxydine with concentration 434 μg/mL, resulted in total loss of CFU of 10 conjunctival Gram-positive isolates, both antibiotic susceptible or MDR, and Gram-negative *E. coli* and *P. aeruginosa.*

There is a cohort of patients complaining of pain after the IVI. This pain is usually associated with perioperative antisepsis with povidone-iodine, rather than the procedure itself[25]. That's why the absence of discomfort, which we observed in patients who used Vitabact or Tobrex eye drops after the IVI, is an important positive feature. Moreover, patients told that the pain after the IVI decreased with application of these drops even within the first 24h.

In conclusion, the confirmed efficacy of eye antiseptic picloxydine against conjunctival bacterial isolates and the presence of its commercial form, 0.05% eye drops, convenient for use by patients before and after injection, make this eye antiseptic promising for prophylaxis of IVI-associated infectious complications.

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