UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA" BUCHAREST
DOCTORAL SCOOL
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BACTERIAL CONTAMINATION OF THE CONJUNCTIVA IN PATIENTS ABOUT TO UNDERGO CATARACT SURGERY

THESIS ABSTRACT

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List of published scientific papers

Articles published in scientific journals:

1.The ocular surface bacterial contamination and its management in the prophylaxis of post cataract surgery endophthalmitis. A review. **Simina Daniela Soare,** Larisa Ilie, OtiliaCosteliu, Ana Cristina Ghiță, Liliana Mary Voinea, Aurelian Mihai Ghita. Romanian Journal of Ophthalmology, Volume 65, Issue 1, January-March 2021. pp:2-9. ISSN-online DOI:10.22336/rjo.2021.2

2.The conjunctival bacterial contamination in patients undergoing cataract surgery-study. **Daniela Simina Soare (Dogaru),** Larisa Ilie, Ana Cristina Ghita, Aurelian Mihai Ghita. ACTA MEDICA TRANSILVANICA June 27(2):34-37. ISSN-online ISSN 2285-7079

Abbreviations and symbols:

CO2 - Carbon dioxide

Coryne – Corynebacterium

CV – Cardiovascular

DB – Type II Diabetes

E. coli – Escherichia coli

HIV – Human Immunodeficiency Virus

MRSA - Methicillin-resistant Staphylococcus aureus

MRSCN- Methicillin-Resistant Coagulase Negative Staphylococcus aureus

MRSE - Methicillin-Resistant Staphylococcus Epidermidis

MSSA - Methicillin-sensitive Staphylococcus aureus

RENAR - Romanian Accreditation Association

Res – Resistance

SA - / Staph A - Staphylococcus aureus

Staphylococcus c- / Staph c- - Coagulase-negative Staphylococcus

Staph c+ - Coagulase-positive Staphylococcus

Spp. – Species

Strep – Streptococcus

SE- Staphylococcus epidermidis

SP – Staphylococcus pasteuri

SH - Staphylococcus haemolyticus

Introduction

Cataract is the most common cause of blindness worldwide (Resnikoff S,2002, Kovin N,2020, Lee Cameron M,2017). Cataract is responsible for between 47.8% and 51% of all blindness (Study for the VLE, 1990-2010).

The incidence of cataract increases with age. Thus, between the ages of 55-64 the incidence of cataract is 3.9% and over the age of 80 it is 92.6% (Yu-Chi L, 2017). In terms of gender distribution, women are affected more than men, with an average incidence of 26% in women as compared to 22.6% in men (Yu-Chi L, 2017) (Klein BE, 1998).

Currently, the only approved and effective treatment for cataract, regardless of etiology, is the surgical approach (MajidMoshirfar 1, 2022). Romania is ranked 23rd in the European Union for the number of cataract surgeries performed (https://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do). The current particularity of cataract surgery is that, over the past decades, the number of surgeries performed on an outpatient basis has increased. A study performed in the United States showed a significant trend in the use of outpatient surgery centers for cataract surgery from 2001 (43.6%) to 2014 (73.0%). An estimated 53 million outpatient surgeries are performed each year in the US. Years ago, most of these patients required hospitalization (Brian C. Stagg, 2018). The outpatient cataract surgical treatment is more convenient for both the surgeon and the patient. Patients are admitted, have surgery and go home within hours thereafter. Europe has also seen an increase in the number of surgeries performed on an outpatient basis, with Portugal performing less than 10% outpatient cataract surgeries in 2000 but rising to 96% in 2014. In Austria, the number of same-day surgeries increased from 1% in 2000 to 72% in 2014. A significant increase was also recorded in France, Ireland, Italy and Luxembourg. However, in some Central and Eastern European countries, such as Romania, Poland, Croatia and Lithuania, outpatient surgery accounts for less than half of all cataract surgeries (OECD (2016)).

Studies have shown that the impact of cataract on the quality of life has varied by gender, age, year and socioeconomic status and has persisted since 1990, increasing with age, and the impact has been greater in countries with a lower socioeconomic status (Rui Fang, 2019, Lixia L, 2017).

Although the success rate of cataract surgery is 95%, complications may still occur, with an estimated incidence of 1.2%. Complications of cataract surgery include posterior capsular rupture, cystoid macular edema, retinal detachment and endophthalmitis. (Mats L, 2021). The most serious postoperative complication is endophthalmitis. It can cause irreversible vision loss (Kresloff MS 1, 2005).

Of all post eye surgery endophthalmitis cases, 90% occur after cataract surgery (Simakurthy S., 2021). There are 3 categories of risk factors for endophthalmitis:

- pre-operative: active infections of the appendages of the eyeball (blepharitis, conjunctivitis, dacryocystitis) and contamination of the administered topical solutions or of the surgical instruments;

- operative: wound abnormalities, long surgery time, vitreous loss or contaminated intraocular irrigation solutions;

- post-operative: germ infections, of which the most frequent are with the coagulase-negative Staphylococcus species (Simakurthy S., 2021).

Most post-operative endophthalmitis cases are triggered by bacteria in the conjunctival fornix (60-80%) (Ciulla TA, 2002).

The most common bacteria colonizing the eye surface are Staphylococcus aureus and coagulase-negative Staphylococci (Shadakshari S. Math, 2019).

Among the germs involved in the onset of post-operative endophthalmitis, the main bacteria responsible for the onset of acute endophthalmitis (less than 6 weeks following eye surgery) is Staphylococcus epidermidis (Ciulla TA, 2002). With chronic endophthalmitis (more than 6 weeks after cataract surgery), the most common trigger bacteria are Propionibacterium acnes, followed by coagulase-negative Staphylococcus, then fungal infections (Aspergillus, Candida and others) (Maalouf F, 2012).

Studies show that out of the microbial causes of endophthalmitis, 85.1% are caused by gram-positive bacteria, 10.3% by gram-negative bacteria, and 4.6% of all infections are caused by fungi (Gentile RC, 2014).

The resistance profile of bacteria may be different depending on the geographical area in which the study is conducted. As a result, literature data and treatment plans in professional guidelines may not be effective in sterilising the eye surface. Adapting the treatment to the specifics of a given center is important for the correct and timely determination of the

appropriate antibiotic treatment, so as to achieve a complete and speedy recovery of the patient and, at the same time, to prevent the development of new bacterial antibiotic resistance.

In the future we intend to follow up this PhD thesis with a study in which the sterilization of conjunctival secretions is performed without the use of antibiotic-based formulations and the treatment is carried out by using different sterilization techniques for conjunctival secretions such as povidone-iodine, chlorhexidine or ozonated liposomal oil formulations.

II. Personal contribution

Chapter 5

Analysis of the patients included in the study

5.1. Work hypothesis and main goals

As a result of the overuse of topical antibiotics in ophthalmic practice and of the emergence of new generations of compounds of the same class, this PhD thesis aims to draw conclusions concerning the frequency of the types of bacteria that contaminate the eye surface in patients undergoing outpatient cataract surgery and the antibiotic resistance profile of the bacteria. All this is necessary in order to achieve the most targeted pre-operative treatment and to prevent the risk of post-operative complications.

The objectives of this PhD thesis were the following:

- determination of the frequency of germs present in the conjunctiva in patients from the geographical area and clinic where they presented for outpatient cataract surgery;
- distribution of these germs by gender, age and comorbidities;
- study of germ antibiotic susceptibility;
- comparison of the data obtained with the relevant literature.

5.2. General research methodology

5.2.1. Patient selection and group study

We studied 516 patients who presented to the Ocularcare clinic in Bucharest for outpatient cataract surgery between 2013-2016. The patients were chosen regardless of

gender, age, background (rural or urban) or comorbidities. They were from Bucharest and the surrounding areas.

We conducted a careful patient history (age, comorbidities and their treatments, occurrence of other eye conditions such as glaucoma, dry eye syndrome, presence of topical ocular medication) and a complete ophthalmological examination including visual acuity assessment, biomicroscopic examination, intraocular pressure and fundus examination. All patients had cataracts in various stages of development.

The patients included in the study were asymptomatic. Their eye secretions were collected before surgery as a protocol to prevent intra- and post-operative infections.

All patients included in the study had conjunctival secretion collected from the eye that was going to undergo cataract surgery one week before the operation.

All labs where the conjunctival secretion was cultured were RENAR (Romanian Accreditation Association) accredited.

The collection of conjunctival secretions was performed in different laboratories. The disadvantage in this situation was that more people were involved in the collection and analysis of the cultures and the antibiotic susceptibility, which may interfere with the final results. On the other hand, this situation reflects the current medical practice in which the physicians treat patients whose conjunctival secretions have been collected and processed and whose antibiotic susceptibility has been determined in different laboratories. The conjunctival secretion was collected from the conjunctival sac with a moistened sterile swab. Two swabs were used: one swab, for the microscopic examination (Gram stain to determine the morphology of the germs, by dividing them into Gram-positive or Gram-negative bacteria and Giemsa stain to assess the inflammatory reaction) and the second one for the insemination on appropriate culture media. All cultures from the conjunctival secretions of patients included in the study were inoculated onto blood-agar and agar-chocolate culture media and incubated in 5% CO2 atmosphere for 24 hours at 35-37 degrees Celsius.

The bacteria were identified according to the conventional methods used for each class of microorganisms.

The result of the conjunctival secretion test was sent on average 5 days after collection. In case of positive results, an antibiogram was performed and we applied the appropriate treatment according to the therapeutic schemes from the scientific literature. Following a

seven-day post-treatment interval, we repeated the conjunctival secretion test. The group of patients positive for the second conjunctival secretion test was treated again according to the antibiogram. A week after completion of the treatment, the third conjunctival secretion test was collected. Thus, we obtained the third group of positive patients, who were treated according to the antibiogram. We compared the germ types present in the three conjunctival secretions analyzed, the susceptibility of the bacteria to antibiotics and the impact of repeated treatments on the antibiotic resistance of the bacteria.

In order to identify at-risk patient groups, all study participants completed a comorbidity questionnaire.

The following groups of patients were established: groups of patients with comorbidities (patients with diabetes, patients with a history of viral hepatitis, patients with cardiovascular disease, oncology patients, patients with other infections, patients with chronic topical eye medication, patients with dry eye syndrome) and groups of patients without comorbidities (control group).

We studied the risk of bacterial contamination of the conjunctiva by gender, age group and comorbidity types. The data were compared with those in the relevant literature. In the study we also looked at the susceptibility of bacteria to antibiotics.

The data were analyzed using IBM SPSS Statistics 2. We also used general descriptive statistics for the studied variables (graphical representation). The data were extracted using the Infoword computer system and then centralised in Microsoft Excel. The graphs were created in Microsoft Excel and the tables, in Microsoft Word.

The statistical tests used were: t-student for independent samples, the chi-squared test and the One Way Anova test. A level of $p<0.05$ was accepted as being statistically significant.

5.2.1.1. Distribution of patients by age group

The ages of the patients in the total group ranged from 33 to 98 years, with an average age of approximately 69 years. The ages of the patients vary around the average age by +/- 10 years (Table I.1, I.2).

I.1 Distribution of patients by age

	No. patients	Minimum	Maximum	Average	Standard deviation
Age	516	33	98	68.74	10.351

I.2. Age ranges

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	up to and including 50 years	35	6.8	6.8	6.8
	51-55 years	17	3.3	3.3	10.1
	56-60 years	26	5.0	5.0	15.1
	61-65 years	66	12.8	12.8	27.9
	66-70 years	112	21.7	21.7	49.6
	71-75 years	110	21.3	21.3	70.9
	over 75	150	29.1	29.1	100.0
	Total	516	100.0	100.0	

5.2.1.2. Distribution of patients by gender

More than half of the patients in the group (63%) are women and 37% are men (Table I.3).

I.3 Distribution of patients by gender

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	Male	191	37.0	37.0	37.0
	Female	325	63.0	63.0	100.0
	Total	516	100.0	100.0	

5.2.1.3. Distribution of patients by comorbidities

More than half of the patients (51.6%) have comorbidities and the remaining 48.4% have no comorbidities.

The patients are assigned to several groups by the comorbidity type, with some patients in more than one group at the same time. Below are the groups and the percentage of total patients in each group: CV disease group (26%), topical treatment group (22.7%), other diseases group (16.3%) , DB group (8.9%), dry eye group (6%), viral hepatitis group (2.7%), infections group (2.5%), oncology group (1.6%) and control group (48.4.%);

5.2.2. Analysis of conjunctival secretion in patients of the initial study group

The initial conjunctival secretion analysis, performed before surgery, showed that 15.3% of the patients in the group had a positive secretion and 84.7% had a negative conjunctival secretion (Table I.4.).

I.4. Analysis of the conjunctival secretion in patients from the initial group

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	negative	437	84.7	84.7	84.7
	Positive	79	15.3	15.3	100.0
	Total	516	100.0	100.0	

5.2.2.1. Distribution of patients from the initial group in relation to first secretion and gender

20.9% of the men and 12% of the women showed positive secretion upon initial examination of the conjunctival secretion.

I.5. Distribution of patients from the initial group in relation to first secretion and gender

			Gender		Total
			male	female	
Secretion	negative		151	286	437
		secretion	34.6%	65.4%	100.0%
		gender	79.1%	88.0%	84.7%
	positive		40	39	79
		secretion	50.6%	49.4%	100.0%
		gender	20.9%	12.0%	15.3%
Total			191	325	516
		secretion	37.0%	63.0%	100.0%
		gender	100.0%	100.0%	100.0%

According to the chi-squared test, there are substantial differences between men and women with respect to the presence of secretion, i.e. the incidence of secretion in men is higher than in women (the asymptotic significance associated with the chi-square test statistic is less than 0.05, implying the rejection of the null hypothesis of no difference at a 5% significance level).

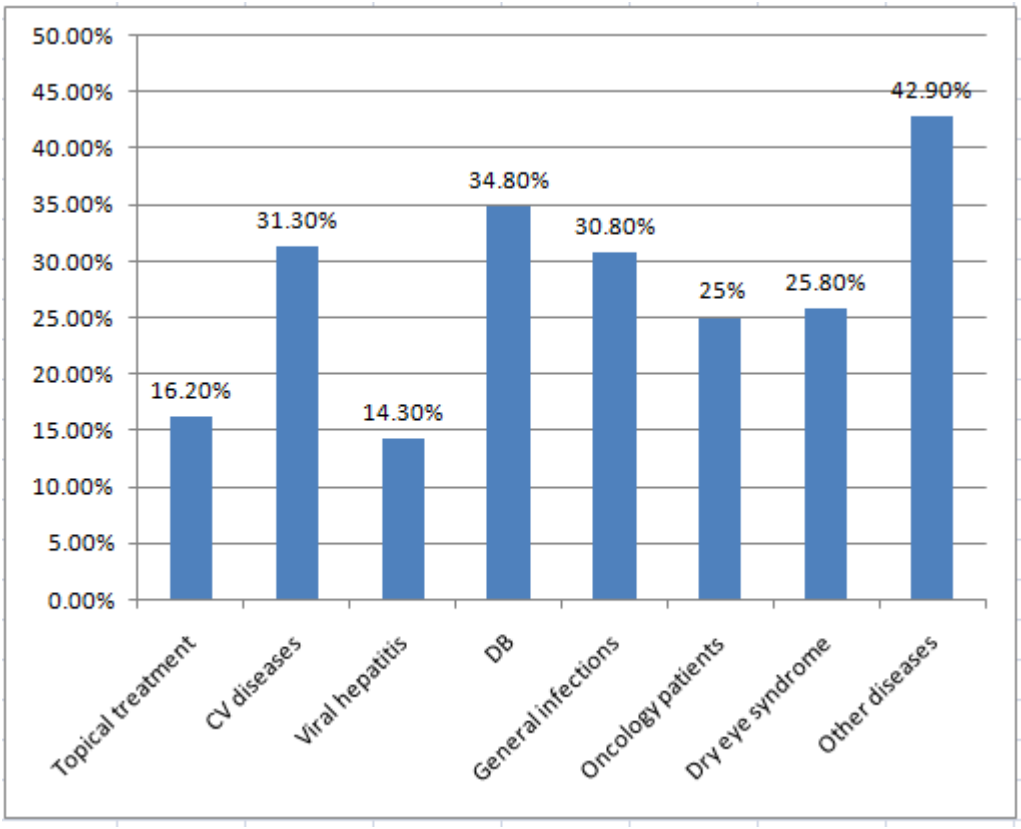
5.2.2.2. Distribution of patients from the initial group by first secretion and age

29.1% of the patients with a positive secretion are over 75 years old, followed by the patients aged 61-65 years (20.3%) and by those in the age range 66-70 years (19.0%) (Table I.6.).

I.6. Distribution of patients from the initial group by first secretion and age

			Age group							Total
			up to and including 50 years	51-55 years	56-60 years	61-65 years	66-70 years	71-75 years	over 75	
Secretion	negative		31	15	21	50	97	96	127	437
		Secretion	7.1%	3.4%	4.8%	11.4%	22.2%	22.0%	29.1%	100.0 %
		Age group	88.6%	88.2%	80.8%	75.8%	86.6%	87.3%	84.7%	84.7%
	positive		4	2	5	16	15	14	23	79
		secretion	5.1%	2.5%	6.3%	20.3%	19.0%	17.7%	29.1%	100.0 %
		Age group	11.4%	11.8%	19.2%	24.2%	13.4%	12.7%	15.3%	15.3%
Total			35	17	26	66	112	110	150	516
		Secretion	6.8%	3.3%	5.0%	12.8%	21.7%	21.3%	29.1%	100.0 %
		Age group	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

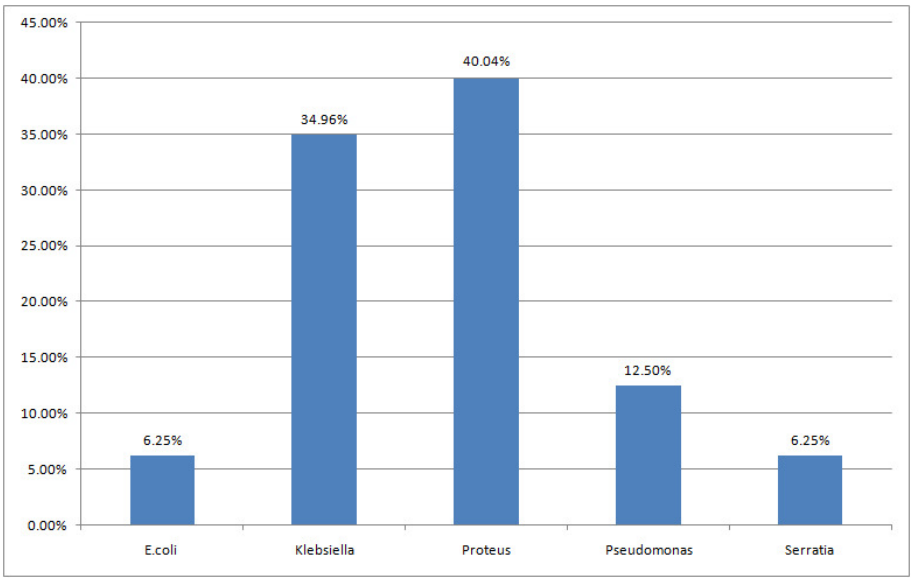
5.2.2.3. Distribution of patients from the initial group by first secretion and comorbidity type



1.1. Initial conjunctival secretion by comorbidity type

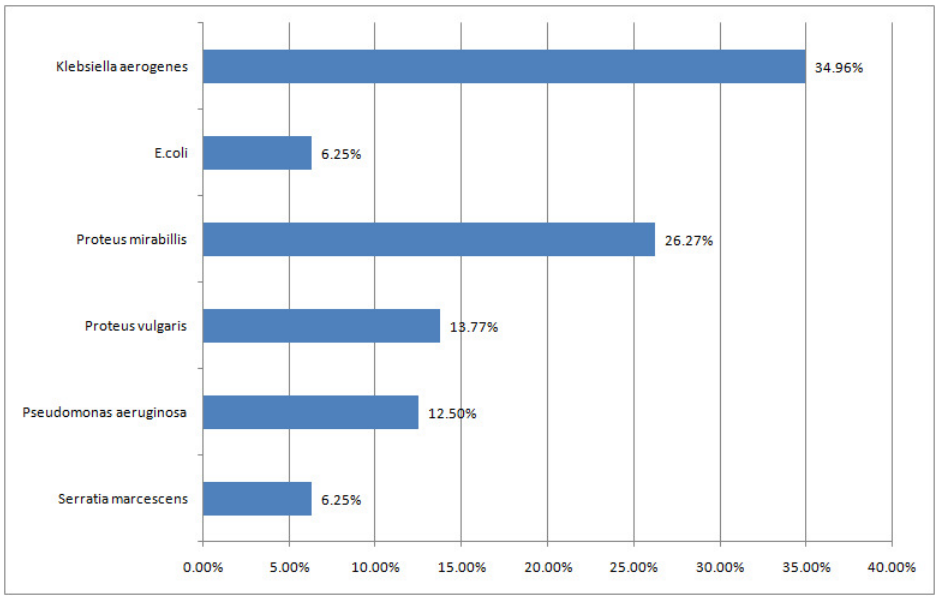
Out of the initial patient group, 42.9% of those with positive secretions belonged to the group with other diseases, 34.80% to the group with DB, 31.30% to the group with CV diseases, 30.80% to the group with general infections, 25.80% to the group with dry eye syndrome, 25% to the group of oncology patients, 16.20% to the group with topical treatment, and 14.3% to the group of patients with viral hepatitis (Graph 1.1.).

5.2.2.4. Analysis of conjunctival secretion by types of bacteria and Gram staining



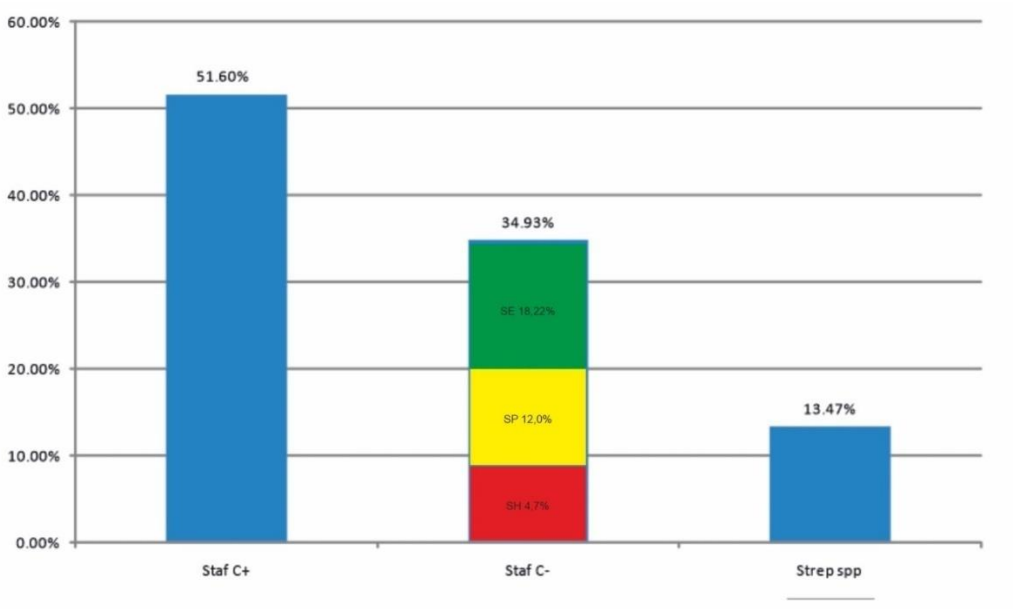
1.2. Distribution of Gram-negative bacteria categories

Out of the Gram-negative bacterial categories, the most frequent was Proteus (40.04%), followed by Klebsiella (34.96%) (Graph 1.2.).



1.3. Distribution of Gram-negative bacteria types

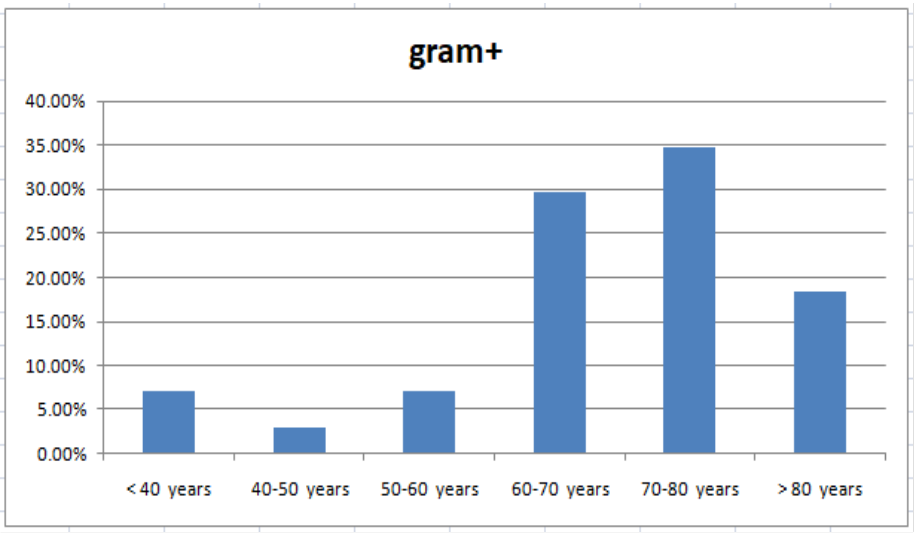
Out of the Gram-negative bacteria types, the most frequent was Klebsiella aerogenes (34.96%), followed by Proteus mirabilis (26.27%) (Graph 1.3.).



1.4. Distribution of Gram-positive bacteria types

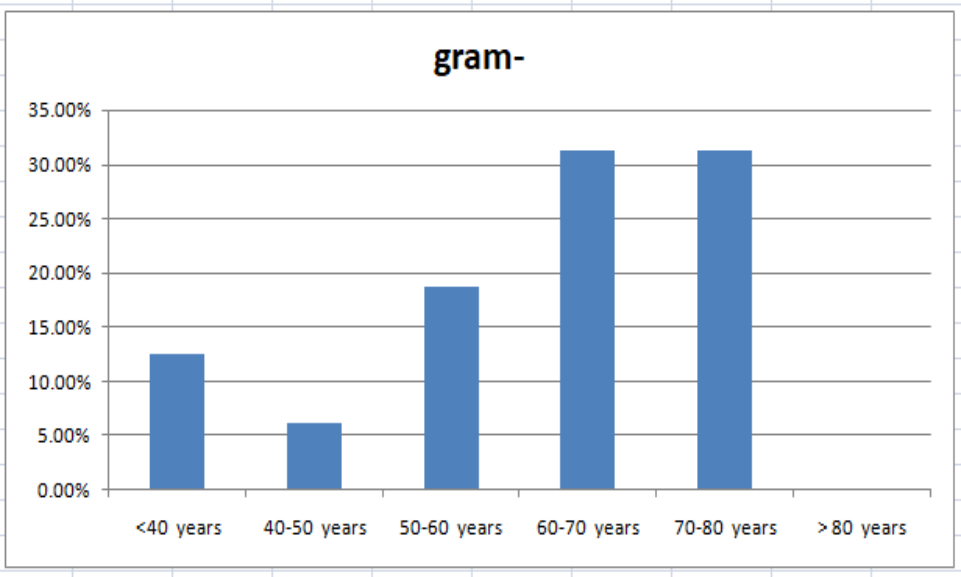
Out of the Gram+ bacteria, the most common was Staph spp. (86,53%), followed by Strep spp. (13,47%) (Graph 1.4.).

5.2.2.4.1. Distribution of bacteria by Gram stain and age group



1.5. Distribution of Gram+ bacteria by age group

34.69% of the patients with Gram+ bacteria are in the age group 70-80 years and the fewest (3.06%), in the age group 40-50 years (Graph 1.5.).



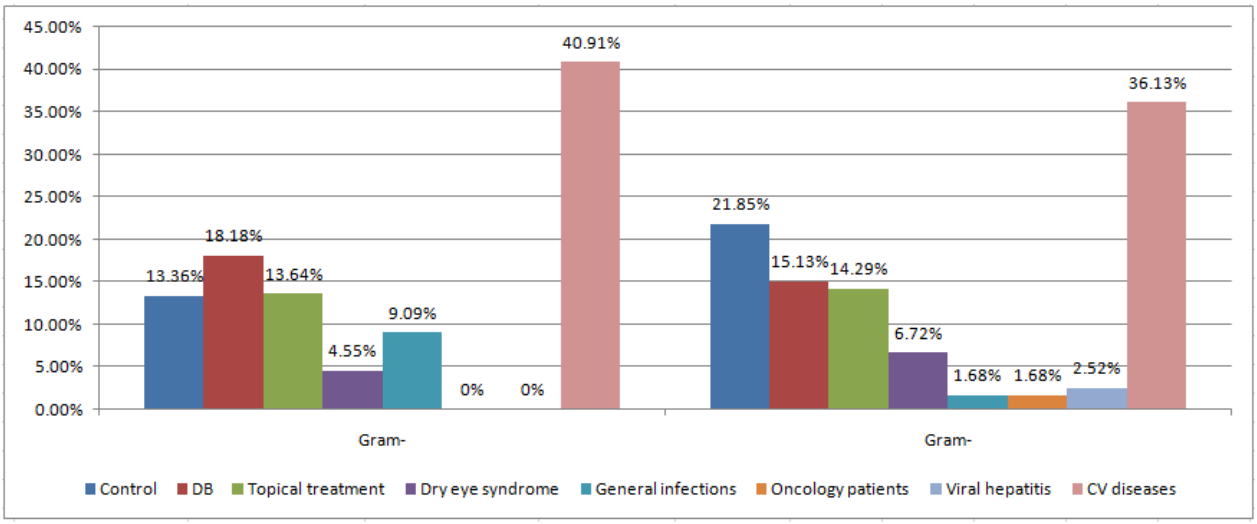
1.6. Distribution of Gram- bacteria by age group

An equal percentage of patients (31.25%) with Gram-positive bacteria are in the age groups 60-70 years and 70-80 years. No patient over the age of 80 had Gram - bacteria (Graph. 1.6.).

5.2.2.4.2. Distribution of bacteria by Gram stain and comorbidity types (Table I.7.).

I.7. Distribution of Gram+ and Gram- germs in each comorbidity group

Comorbidity	Gram +	Gram –
Control group	26,30%	73,70%
Type II Diabetes	19,30%	80,70%
CV diseases	45,60%	54,40%
Viral hepatitis	2,60%	97,40%
Oncological diseases	1,80%	98,20%
Infections	3,50%	96,50%
Topical treatment	17,50%	82,50%
Dry eyes	7,90%	92,10%



1.7. Distribution of Gram+ and Gram- germs in each comorbidity group

Distribution of Gram+ and Gram- germs in every comorbidity group

40.91% of the Gram- bacterial patients had CV disease, 18.18% had DB, 13.64% underwent topical treatment and 13.63% belonged to the control group, 9.09% had infections and 4.55% had dry eyes.

36.13% of patients with Gram+ bacteria had CV disease, 21.85% belonged to the control group 15.13% had DB , 14.29% were in the topical treatment group, 1.68% belonged to the infection and oncology groups, 6.72% had dry eyes, and 2.52% of the total number of Gram+ patients were in the viral hepatitis group (Graph 1.7.).

Chapter 6

Study of patients who tested positive on the first conjunctival secretion test (second patient group)

Out of the initial group of patients (516), 15.3% had positive secretions. They formed the second group of patients (78).

6.1. Correlation between bacteria types and comorbidity types

Older patients are more frequent candidates for cataract surgery (Raczynska D, 2016). In these patients the rate of bacterial contamination of the conjunctiva is higher due to more comorbidities and decreased local and general immunity.

II.1. The distribution of bacteria in patients with comorbidities and in the control group

	Staphylococcus	Streptococcus	Proteus	Klebsiella	Pseudomonas	Serratia	E. Coli
DB	68,6%	12,6%	18,8%	0	0	0	0
CV diseases	70,8%	14,6%	12,2%	2,4%	0	0	0
Viral hepatitis	50%	50%	0	0	0	0	0
Oncological diseases	50%	50%	0	0	0	0	0
Infections	25%	25%	25%	0	0	0	25%
Dry eyes	62,5%	25%	0	0	0	12,5%	0
Chronic topical treatment	73,5%	10,6%	10,6%	0	0	5,3%	0
Control	66,6%	11,1%	5,6%	11,1%	5,6%	0	0

The analysis of the bacteria present in patients who were positive in the first conjunctival secretion test (group 2 patients) showed that patients in the control group (without comorbidities) had a higher percentage of coagulase-negative Staphylococcus (11.1%) compared to the coagulase-positive group (5.6%). Streptococcus was accounted for 11.1% and the Gram-negative bacteria present in the control group were Klebsiella (11.1%), Proteus and Pseudomonas (5.6% each).

In patients with comorbidities, Staphylococcus infection was the most common, while Streptococcus and Gram-negative bacteria were less common (Table II.1.).

Out of all Staphylococcus strains, Staphylococcus aureus (25.8%) was more common than Staphylococcus epidermidis (2.4%), and out of all Staphylococcus aureus strains, methicillin-sensitive Staphylococcus was present only in the group of patients with cardiovascular disease (2.4%). Methicillin-resistant Staphylococcus was found in the group of patients with dry eyes (12.5%) and in the group of patients with chronic topical treatment (5.3%).

6.2. Distribution of patients by gender

II.2. Distribution of patients with positive conjunctival secretion by gender

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	male	39	50.0	50.0	50.0
	female	39	50.0	50.0	100.0
	Total	78	100.0	100.0	

Half of the patients in the second group were women and half were men (Table II.2).

6.3. Distribution of patients by age group

Most of the patients in the second group (28.2%) were aged 75 years and older, 20.5% were between 60 and 64 years, 19.2% were between 65 and 69 years and only 2.6% belonged to the 50-54 age group.

6.4.Distribution of the patients by bacterial susceptibility to topical antibiotics

The bacteria in the first conjunctival secretion test had the highest susceptibility to Ciprofloxacin (67.95%), Gentamicin (62.8%), Levofloxacin (42.3%) and Tobramycin (41%), followed by Ofloxacin (20.5%), Netilmicin (16.7%), Moxifloxacin (15.4%), Azithromycin (9%), fusidic acid (7.7%) and Kanamycin (2.6%);

6.4.1. Correlation between antibiotic susceptibility of the bacteria in the first conjunctival secretion test and gender

A statistically significant relationship between bacterial susceptibility to the antibiotic and the gender of the patients was observed with only some antibiotics. Thus, with Ciprofloxacin, it has been determined that men CARRY bacteria with a higher susceptibility to this antibiotic as compared to women. With Netilmicin, women showed bacteria with a higher susceptibility as compared to men (Table II.3).

II.3. Correlation between antibiotic susceptibility of the bacteria in the first conjunctival secretion test and gender

Antibiotic	Susceptibility in women	Susceptibility in men
Ciprofloxacin	56,4%	76,9%
Netilmicin	25,6%	7,7%

6.4.2. Correlation between antibiotic susceptibility of the bacteria in the first conjunctival secretion test and age group

We studied the possibility of a correlation between the susceptibility of bacteria to antibiotics and age group but found that the antibiotics for which there was a link between age and antibiotic susceptibility did not have a corresponding topical treatment (Cefoxitin, Cotrimoxazole, Ampicillin, Vancomycin, Cefazolin, Cefadroxin).

6.5.Distribution of the patients by bacterial susceptibility to topical antibiotics

We studied the resistance of bacteria from the first conjunctival secretion test to topical antibiotics and found that they had the highest resistance to Gentamicin (14.1%), followed by Ciprofloxacin and Azithromycin (7.7%), then Tobramycin (5.1%), Moxifloxacin and Kanamycin (3.8%). For Levofloxacin, Netilmicin and Ofloxacin, the resistance was low (1.3%).

6.5.1. Correlation between antibiotic resistance of the bacteria in the first conjunctival secretion test and gender

7.5% of the women presented with Kanamycin-resistant bacteria. All Kanamycin resistant patients are female. The statistically significant differences between men and women with respect to Kanamycin resistance are confirmed by the chi-square test for a 10% significance level (Table II.4.).

II.4. Correlation between bacterial resistance to antibiotics and gender

			Kanamycin res.		Total
			0	1	
gender	male	gender	38	0	38
			100.0%	0.0%	100.0%
		Kanamycin res.	50.7%	0.0%	48.7%
	female	gender	37	3	40
			92.5%	7.5%	100.0%
		Kanamycin res.	49.3%	100.0%	51.3%
Total		gender	75	3	78
			96.2%	3.8%	100.0%
		Kanamycin res.	100.0%	100.0%	100.0%

6.5.2. Correlation between antibiotic resistance of the bacteria in the first conjunctival secretion test and age group

Out of all the topical antibiotics studied, the chi-square test confirms statistically significant differences between patients by age group at the 10% significance level for Ciprofloxacin and Kanamycin (Table II.5.).

II.5. Antibiotic resistance of bacteria by age group

Age group	**Ciprofloxacin**	**Kanamycin**
<50 years	0%	0%
50-54 years	50%	50%
55-59 years	40%	20%
60-64 years	0%	0%
65-69 years	0%	6,7%
70-74 years	6,7%	0%
>75 years	9,5%	0%

Chapter 7

Study of patients who tested positive on the second conjunctival secretion test (third patient group)

For the 78 patients who tested positive at the first conjunctival secretion test (15.3% of the total number of patients included in the study), the treatment was performed according to the antibiogram. Following treatment, a second conjunctival secretion test was performed and a total of 17 patients were still positive and 62 tested negative. The analysis of the conjunctival secretion in the positive patients (the third group of patients) showed that the most common bacteria was SA (58.8%), followed by Staphylococcus c- (11.8%). The remaining bacteria were Enterococcus spp, Serratiamarcescens, Staph alb hem c-, MRSA+, Klebsiellaspp (5.9%) (Table III.1.).

III.1. Bacteria at the second secretion test (group 3)

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	SA	10	58.8	58.8	58.8
	Enterococcus spp	1	5.9	5.9	64.7
	Staphylococcus c-	2	11.8	11.8	76.5
	Serratia marcescens	1	5.9	5.9	82.4
	Staph hem alb c-	1	5.9	5.9	88.2
	MRSA+	1	5.9	5.9	94.1
	Klebsiellaspp	1	5.9	5.9	100.0
	Total	17	100.0	100.0	

7.1. Distribution of the patients by bacterial susceptibility to antibiotics

In the second secretion test, patients showed bacteria sensitive to the following topical antibiotics: Gentamicin (23.5%); Moxifloxacin (11.8%); Kanamycin (11.8%); Tobramycin (11.8%); Ciprofloxacin (11.8%); Tetracycline (5.9%);

7.1.1. Distribution of the patients by bacterial susceptibility to antibiotics and age group

All patients aged 50-54 years and half of the patients in the age group 55-59 years are sensitive to Ciprofloxacin. For a 5% significance level, the chi-squared test indicates a statistically significant linear relationship between age group and Ciprofloxacin susceptibility. There is just one patient with Tetracycline sensitivity, who is in the age group 50-54 years. There is a statistically significant relationship between age group and Tetracycline susceptibility at a 1% significance level.

33.3% of the patients aged 65-69 years and half of the patients aged 75 years and over are sensitive to Chloramphenicol. The chi-squared test indicates a linear relationship between age group and susceptibility to this antibiotic at a 10% significance level.

7.1.2. Distribution of the patients by bacterial susceptibility to antibiotics and comorbidity types

Only antibiotics that have a corresponding droplet administration and that showed statistically significant antibiotic susceptibility by comorbidity type according to the chi-squared test at a 10% significance level shall be presented.

In the second secretion test, all patients in the control group were found to have Ciprofloxacin- and Gentamycin-susceptible bacteria; all patients in the oncology group were found to have Gentamycin-susceptible bacteria and all patients in the other disease group were found to have Kanamycin-susceptible bacteria.

7.2. Distribution of the patients by bacterial resistance to antibiotics

In the second secretion test, the group of positive patients presented with bacteria resistant to the following antibiotics: Gentamicin (23.5%), Ciprofloxacin (23.5%), Tetracycline (17.6%), Levofloxacin (11.8%), Tobramycin (11.8%), Moxifloxacin (5.9%), Netilmicin (5.9%), Azithromycin (5.9%), Kanamycin (5.9%).

7.2.1. Distribution of the patients by bacterial resistance to antibiotics and age group

The bacteria in the second conjunctival secretion test showed resistance to Ciprofloxacin in 33.3% of the patients aged 65 to 69 years and 50% of the patients aged 75 years and older and to Gentamicin in 66.7% of the patients aged 75 years and older.

7.2.2. Distribution of the patients by bacterial resistance to antibiotics and comorbidity type

All patients in the oncology group were resistant to Ciprofloxacin.

Ciprofloxacin was the only topical antibiotic to which patients showed a statistically significant resistance.

Chapter 8

Study of patients who tested positive on the third conjunctival secretion test (fourth patient group)

For the 17 patients who were positive in the second secretion test, treatment was repeated according to the antibiogram and then the third conjunctival secretion test was performed. Out of these, 8 patients stayed positive (41.2%) and 9 patients stayed negative (58.8%) (Table IV.1.).

IV.1. Study of patients who tested positive on the third conjunctival secretion test

Negativing		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	no	8	41.2	47.1	47.1
	yes	9	58.8	52.9	100.0
	Total	17	100.0	100.0	

Out of the 8 positive patients (group no. 4), only one patient (5.9%) retained susceptibility to the same bacteria and 7 of them (35.3%) became susceptible to a different bacteria than the original one. The most frequently collected bacteria from the conjunctiva was also SA (11.8% of the patients), but these are only two out of the total number of 8 positive patients (Table IV.2.).

IV.2. Bacteria at the third secretion test

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	SA	2	11.8	25.0	25.0
	St c-	1	5.9	12.5	37.5
	SA MSSA	1	5.9	12.5	50.0
	St spp	1	5.9	12.5	62.5
	St c- MRSE	1	5.9	12.5	75.0
	SA MRSA	1	5.9	12.5	87.5
	St c-MRSCN	1	5.9	12.5	100.0
	Total	8	47.1	100.0	
w/o bacteria		9	52.9		
Total		17	100.0		

Out of the patients who became susceptible to other bacteria (35.3%), 5 were women (29.40%) and two were men (5.9%) (Table IV.3.).

IV.3. Types of bacteria in the conjunctival secretion tests 2 and 3 and their distribution by gender

Bacteria_secretion2	Bacteria_secretion3	Gender
SA	SA	Female
Enterococcus spp	SA MSSA	Female
SA	St spp	Female
SA	Staph - MRSE	Male
SA	-	Male
Staphylococcus c-	SA	Female
SA	-	Female
SA	-	Female
Serratia_marcescens	-	Female
SA	-	Male
SA	-	Male
SA	stc-	Male
SA	-	Female
St hem alb c-	-	Male
SA MRSA+	-	Female
Klebsiellaspp	SA MRSA	Female
Staphylococcus c-	Staphylococcus – MRSCN	Female

8.1. Distribution of the patients by bacteria type and comorbidity type

All chi-squared tests showed that there was no statistically significant relationship between the type of bacteria in third secretion test positive patients and the comorbidity type at a 1% significance level.

In the third secretion test, the control group included only one patient with MRSA. In the infection group and the viral hepatitis group no patient was positive. Only one patient (MSSA) belonged to the oncology group. In the group of patients with CV disease, 40% of the patients

had a SA infection and 20% of the patients in the group presented with MSSA, St. Spp and MRSCN.

One patient belonged to the DB group, and was infected with SA, two were part of the topical treatment group of which one patient had a ST spp infection and one, MRSE. Only one patient was part of the dry-eyes group, and they were infected with St spp.

Chapter 9

General conclusions and personal contribution

9.1. General conclusions

As a result of the analysis of the study group, we have drawn the following conclusions:

1. The patients in the present study ranged from 33 to 98 years, most of them (29.1%) being over 75 years old. Out of these, 63% were women and 37% were men;

2. The initial conjunctival secretion test showed that 15.3% of the patients included in the study had a positive conjunctival secretion and 84.7% had a negative secretion;

3. The analysis of the first conjunctival secretion test by age group showed the highest frequency in the age group over 75 years (29.1%), followed by the age group 61-65 years (20.3%);

4. With the first conjunctival secretion test, the bacteria most frequently identified in patients without comorbidities (control group) were coagulase-negative Staphylococcus, Streptococcus and Klebsiella (11.1%), while coagulase-positive Staphylococcus, Pseudomonas and Proteus accounted for 5.6% of all germs;

5. The most common bacteria identified in the first conjunctival secretion test in patients with comorbidities was Staphylococcus (66.33%), of which Staphylococcus aureus (25.8%) and Staphylococcus epidermidis (2.4%) were the most common. Out of the Staphylococcus aureus strains, methicillin-sensitive Staphylococcus was present only in the group of patients with cardiovascular disease (2.4%), and methicillin-resistant Staphylococcus was found in the group of patients with dry eyes (12.5%), as well as in the group with chronic topical treatment (5.3%). Streptococcus and some Gram-negative bacteria such as: Pseudomonas , Corynebacterium, Serratia and Escherichia Coli were less common;

6. In our study, out of all Gram-positive bacteria, the highest percentage was that of Staphylococcus spp. (86,53%), it being divided into coagulase-positive Staphylococcus (51.6%) and coagulase-negative Staphylococcus (34.93%), followed by Streptococcus spp. (13,47%);

7. Out of the Gram-negative bacteria, the most common was Proteus (40.04%), (Proteus mirabillis 26.27% and Proteus vulgaris 13.77%), followed by Klebsiella (34.96%), Pseudomonas aeruginosa (12.5%), Serratia marcescens (6.25%) and Escherichia coli (6.25%);

8. The analysis of the correlation between the types of comorbidities and the occurrence of positive conjunctival secretion showed that there was a statistically significant relationship in the groups of patients with diabetes, cardiovascular disease, other associated diseases or dry eye syndrome and that there was no statistically significant relationship in patients with associated hepatitis, oncological disease, infections or chronic topical treatment (Glaucoma);

9. In our study, the frequency of coagulase-positive Staphylococcus was higher in patients with diabetes (40%) than in the control group (20%), and coagulase-negative Staphylococcus was more present in the conjunctival secretion of patients from the control group (25%) than from the group of patients with diabetes (12.5%);

10.Gram-positive bacteria were most common in patients without comorbidities (except patients with cardiovascular disease, where these bacteria were more common than in the control group), and Gram-negative bacteria were more common in patients with comorbidities (except patients with cardiovascular disease, where these bacteria were less common than in the control group);

11. The distribution of Gram-negative bacteria by age group showed that the contamination rate was low in the 40-50 age group (6.25%). It increased in the 50-60 age group (18.75%) and then continued to grow, being the same in the 60-70 and 70-80 age groups (31.25%). Over the age of 80, no patient was contaminated with Gram-negative bacteria;

12. The contamination of the conjunctiva with Gram-positive bacteria increased with each decade of age, from 3.06% in the age range 40-50 years to 34.69% in the decade 70-80 years. All patients over 80 years were Gram-positive;

13. The bacteria in the first conjunctival secretion test had the highest susceptibility to Ciprofloxacin (67.95%), Gentamicin (62.8%), Levofloxacin (42.3%) and Tobramycin (41%), followed by Ofloxacin (20.5%), Netilmicin (16.7%), Moxifloxacin (15.4%), Azithromycin (9%), fusidic acid (7.7%) and Kanamycin (2.6%);

14. In the first conjunctival secretion test, the correlation between bacterial susceptibility to antibiotics and gender showed that men had bacteria with a higher susceptibility to

Ciprofloxacin than women, while women had bacteria with a higher susceptibility to Netilmicin than men;

15. The bacterial resistance to antibiotics in the first conjunctival secretion test showed the following: Gentamicin (14.1%), Ciprofloxacin and Azithromycin (7.7%), Tobramycin (5.1%), Moxifloxacin and Kanamycin (3.8%), Levofloxacin, Netilmicin and Ofloxacin (1.3%);

16. The analysis of the first conjunctival secretion test showed that women had a higher resistance to Kanamycin than men (all patients with Kanamycin resistance were women);

17. In the first conjunctival secretion test, Kanamycin resistance was statistically significant in patients in the age groups 50-54 years (50%), 55-59 years (20%) and 65-69 years (6.7%).

18. In the initial conjunctival secretion test, the Ciprofloxacin resistance was highest in patients in the age group 50-54 years (50%) and 55-59 years (40%), followed by the age groups over 75 years (9.5%) and 70-74 years (6.7%). The patients aged 60 to 69 years had no Ciprofloxacin-resistant bacteria. Out of the topical antibiotics, a statistically significant relationship between bacterial resistance to antibiotics and age group was established only for Ciprofloxacin and Kanamycin;

19. The bacteria in the second conjunctival secretion test were sensitive to the following topical antibiotics: Gentamicin (23.5%); Moxifloxacin (11.8%); Kanamycin (11.8%); Tobramycin (11.8%); Ciprofloxacin (11.8%); Tetracycline (5.9%);

20. The distribution of the patients who had a positive second secretion test in terms of bacterial susceptibility to antibiotics and by age group showed a statistically significant correlation to some of the topical antibiotics. Therefore, all patients aged 50-54 years and half of the patients in the age group 55-59 years were susceptible to Ciprofloxacin; there was only one patient susceptible to Tetracycline, who was in the age group 50-54 years; 33.3% of the patients aged 65-69 years and half of the patients aged 75 years and over were susceptible to Chloramphenicol;

21. In the second secretion test, all patients in the control group presented with bacteria susceptible to Ciprofloxacin and Gentamycin; all patients in the oncology group presented with bacteria susceptible to Gentamycin and all patients in the other disease groups presented with bacteria susceptible to Kanamycin;

22. The bacteria in the second conjunctival secretion test showed an increased resistance to Gentamycin and Ciprofloxacin (23.5%), followed by Levofloxacin and Tobramycin (11.8%) and a lower resistance to Kanamycin, Azithromycin, Netilmicin and Moxifloxacin (5.9%);

23. The distribution of patients who were positive in the second secretion test in terms of bacterial resistance to antibiotics and by age group showed that one third of the patients in the age group 65- 69 years and half of those over 75 years were resistant to Ciprofloxacin and two thirds of the patients over 75 years were resistant to Gentamicin;

24. The bacterial resistance in the second conjunctival secretion test as compared to the first secretion test increased for all types of topical antibiotics used, with the greatest increase for Ciprofloxacin (23.5% versus 7.7%) and Levofloxacin (11.8% versus 1.3%);

25. The topical use of Chloramphenicol, of aminoglycosides (Netilmicin) and of fluoroquinolones (Moxifloxacin and Levofloxacin) was a reasonable treatment option;

26. Out of the 17 patients who tested positive in the second conjunctival secretion test, 8 patients stayed positive (41.17%) and 9 patients became negative (58.8%) after the treatment;

27. Out of the 8 patients (41.2%) who were positive in the third conjunctival secretion test, 7 were infected with a different bacteria than in the first secretion test (35.3%) and only one patient was still infected with the same bacteria as in the initial secretion test (5.9%). Out of the patients who became susceptible to other bacteria (35.3%), 5 were women (29.4%) and 2 were men (5.9%).

28. In the third conjunctival secretion test, there was no statistically significant relationship between the bacteria strain and the comorbidity type;

29. Our study highlighted the importance of repeating the conjunctival secretion test following treatment, as patients may become infected with a different type of bacteria;

30. Adequate treatment of the eye surface prior to cataract surgery is effective as a prophylactic measure, lowering the risk of severe post-operative complications;

31. A follow-up protocol should be established for patients undergoing cataract surgery mainly on an outpatient basis, including monitoring of comorbidities, examination of conjunctival secretion, treatment of antibiogram positive patients and repeat testing of conjunctival secretions in treated patients;

32. We recommend avoiding the routine use of pre-operative antibiotic therapy in all patients, given the increasing germ resistance, and instead using antiseptic solutions to provide a sterile pre- and intra-operative eye surface.

9.2. Personal contributions

The only effective treatment for cataract, regardless of its etiology, is the surgical approach. Some decades ago, most patients required hospitalisation for cataract surgery, but now the worldwide trend is for an increase in outpatient surgery. In view of this important trend of using outpatient surgery centers for cataract surgery, in this thesis we aimed to develop a protocol for monitoring patients undergoing outpatient surgery in order to avoid the occurrence of serious post-operative complications.

Considering the heterogeneity of bacteria on the eye surface and their variability from one country to another, but also within different parts of the same country, we considered that it was important to know the local particularities of the patients in the geographical area where the study was conducted. This will help us to achieve swift therapeutic success for the patient, and to have a targeted approach to the bacteria on the eye surface, thus combating the growth of acquired antibiotic resistance. We carried out this study over a period of 4 years, on patients with different ages and comorbidities, who presented in an ophthalmological centre in Bucharest for outpatient cataract surgery. We determined the frequency of the types of pathogenic bacteria present on the eye surface, their distribution by age, gender and comorbidities, their susceptibility and resistance to antibiotics.

Given the occurrence of new forms of antibiotic resistance, we plan to study the possibility of sterilizing the conjunctival secretions using alternative methods such as antiseptic solutions like povidone-iodine, chlorhexidine or pre-operative home treatment with ozonized liposomal oil solution, in order to minimize the use of antibiotic treatment.

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