„CAROL DAVILA”

UNIVERSITY OF MEDICINE AND PHARMACY

BUCHAREST

DOCTORAL STUDIES DEPARMENT

MEDICINE

DOCTORAL THESIS

PhD coordinator:

PROF. UNIV. DR. LILIANA MARY VOINEA

PhD student:

POPA-CHERECHEANU MATEI-ȘERBAN

2022

„CAROL DAVILA”
UNIVERSITY OF MEDICINE AND PHARMACY
BUCHAREST
DOCTORAL STUDIES DEPARMENT
MEDICINE

ANTIBIOTIC RESISTANCE PROFILE OF GERMS IDENTIFIED IN CONJUNCTIVAL SAMPLES

PhD coordinator:
PROF. UNIV. DR. LILIANA MARY VOINEA

PhD student:
POPA-CHERECHEANU MATEI-ȘERBAN

2022

„CAROL DAVILA”
UNIVERSITY OF MEDICINE AND PHARMACY
BUCHAREST
DOCTORAL STUDIES DEPARMENT
MEDICINE

ANTIBIOTIC RESISTANCE PROFILE OF GERMS IDENTIFIED IN CONJUNCTIVAL SAMPLES

ABSTRACT

PhD coordinator:
PROF. UNIV. DR. LILIANA MARY VOINEA

PhD student:
POPA-CHERECHEANU MATEI-ȘERBAN

2022

Motto:

"The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism."

"Persoana necugetată care se joacă cu tratamentul cu penicilină este responsabilă din punct de vedere moral pentru moartea pacientului cu infecție cu germen rezistent la penicilină."

Sir Alexander Fleming, Nobel Prize, 1945

TABLE OF CONTENT

INTRODUCTION

Infections produced by multidrug-resistant microorganisms are associated with increased mortality compared to those caused by sensitive bacteria, also having an important economic impact, estimated at more than 20 billion dollars annually in the United States alone. The Centers for Disease Control and Prevention (CDC) estimates that at least 23,000 people die each year in the United States from an infection with a resistant microorganism (CDC, 2013; CDC, 2016). Moreover, according to a recent report, antibiotic resistance is estimated to cause 300 million premature deaths by 2050, with a loss of approximately $100 trillion to the global economy (CDC, 2013; Demirjian, 2015). The situation is made worse by the lack of a robust arsenal of new antibiotics, resulting in almost untreated infections, leaving clinicians without viable alternatives to treat their patients.

In response to the threat of increasing antibiotic resistance globally, the World Health Organization, the United States Food and Drug Administration, and other major organizations have developed surveillance programs that collect data from the US and the rest of the world (Walsh, 1996).

Two such initiatives are of particular interest in the field of ophthalmology – Ocular Tracking Resistance in the U.S. Today (TRUST) and Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) (Davies, 1994; Schentag, 1998; Levy, 1992).

TRUST is a US multicenter surveillance program started in 1996 in which isolates from more than 200 laboratories are sent to an independent central laboratory for in vitro susceptibility testing. A substudy was initiated in 2005 (Ocular TRUST 1) aiming to prospectively collect data each year as well as retrospectively analyze samples from previous years. The TRUST study specifically analyzes three microorganisms: Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae. S. aureus is subsequently divided into methicillin-susceptible (MSSA) or methicillin-resistant (MRSA) (Paulsen, 1996).

The ARMOR study is a similar surveillance program designed specifically for surveillance of ocular pathogens in the United States. Initial results of the ARMOR study based on isolates collected from 34 institutions during 2009 were published in 2011 (ARMOR 2009), and data from 2009-2013 (ARMOR 2013) were published in 2017. The ARMOR study expands the collected data by TRUST studies by additional analysis of Pseudomonas aeruginosa and coagulase-negative staphylococci (CoNS). The ARMOR 2013 study analyzed a total of 3237 isolates, representing the largest study of its kind to date.

Table I highlights the levels of resistance found in the TRUST and ARMOR studies.

Germ		Penicilin	Azitromicin	Gatifloxacin	Moxifloxacin	Levofloxacin	Ofloxacin	Tobramicin	Ciprofloxacin
Streptococcus penumoniae	TRUST retrospectiv	34.1%	33.4%	0.3%	0.1%	0.1%		95.1%	9.7%
	TRUST prospectiv	18.3%	22.4%	0%	0%	0%		98%	10.2%
	ARMOR	31.8%	34.8%	0.4%	0.3%	0%	0.4%		
MS *Staphylococcus aureus* (MSSA)	TRUST prospectiv	90.2%	45.7%	18.9%	18.9%	18.9%		7.3%	20.1%
	ARMOR		42.8%	13.5%	12%	13.7%	14.1%	4.1%	14.2%
MR *Staphylococcus aureus* (MRSA)	TRUST prospectiv	100%	93.9%	84.8%	84.8%	84.9%		63.6%	84.8%
	ARMOR		93.3%	75.1%	74%	75.9%	76.4%	44.3%	77.3%
MS stafilococi coagulazo-negativi	ARMOR		44.7%	13.9%	13.6%	13.9%	14.2%	6.4%	15%
MR stafilococi coagulazo-negativi	ARMOR		78.3%	55.7%	51.2%	56.8%	56.9%	23.1%	
H. influenzae	TRUST retrospectiv	100%	0.3%	0.3%	0.3%	0.3%		0%	
P. aeruginosa	ARMOR					6.9%	10.1%	3.1%	

Table I. Percentage of resistant bacteria in the TRUST and ARMOR studies *(Grzybowski, 2017)*

WORKING HYPOTHESIS AND GENERAL OBJECTIVES

The profile of bacterial resistance to antibiotics can vary depending on the geographical location, so the antibiotic therapy schemes in the specialist guidelines are not always fully applicable in all regions of the globe, in any country or in any diagnostic and treatment center, and there may be variations from one country to another or even between centers belonging to the same narrow geographical region. That is why it is important to know the local peculiarities so that the treatment can be adapted accordingly, in order to achieve therapeutic success, a degree of healing of the patient as high as possible and as close as possible to restitutio ad integrum, but also to combat the development of new bacterial resistance to antibiotics by applying inappropriate selection pressures on bacteria.

As part of this doctoral thesis, I conducted two studies, called RAGS 1 and RAGS 2 (Antibiotic Resistance of Germs from Conjunctival Secretions 1 and 2).

The main purpose of these two studies is to compare the resistance profile of bacteria isolated from conjunctival secretions at the level of two ophthalmology practice centers in Bucharest with that of bacteria isolated in a major study carried out in the United States of America. The study we aimed to compare our results with is ARMOR 2013.

In addition, we aimed to compare the flora detected in the two studies and the resistance/sensitivity of the same germs in two different centers, close in distance, from the same locality, trying to analyze the common elements and the elements that differentiate them.

The RAGS 1 study was carried out in the Ophthalmology Clinic of the Bucharest University Emergency Hospital, with data collected from patients admitted to continuous hospitalization between 2014-2018. As specific to the Ophthalmology Clinic of the Bucharest University Emergency Hospital, emergency pathology predominates, the hospitalized patients being symptomatic patients from the eye point of view, from whom conjunctival secretions were collected in order to establish the etiology of the eye disease and the therapeutic conduct.

The RAGS 2 study was carried out in the Bucharest Ocular Care Ophthalmology Clinic, with patient data being collected in the same time interval, in order to avoid any change in the bacterial flora over time as an error factor. As specific to the Eye Clinic, chronic pathology predominates, the patients from whom conjunctival secretions were collected being mostly asymptomatic or paucisymptomatic patients, and the collection was done as a pre-operative investigation in view of a scheduled surgical intervention.

SPECIAL PART

CHAPTER 5

STUDY I - RAGS 1 STUDY – Antibiotic Resistance of Germs from conjunctival Secretions 1

A total of 1591 samples from different patients, collected between 2014-2018, were analyzed.

In the positive cultures analyzed, the following bacteria were isolated: Bacillus spp, Enterobacter spp, Enterococcus faecalis, Escherichia coli, Klebsiella spp, Proteus spp, Pseudomonas aeruginosa, Serratia spp, Staphylococcus spp, Streptococcus spp.

Staphylococci were further divided into Staphylococcus aureus (methicillin-susceptible MSSA/methicillin-resistant MRSA), respectively coagulase-negative staphylococci (also methicillin-susceptible MS CoNS / methicillin-resistant MR CoNS).

On the bacteria marked in bold in table V.5. we will stop with a special interest, since they are part of the set of bacteria also analyzed in the ARMOR and TRUST studies, with which we propose to compare our results.

Table V.5. Distribution of bacteria isolated from conjunctival secretions

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Bacillus spp	1	0.1	0.1	.4
	Enterobacter spp	8	0.9	0.9	1.3
	Enterococcus faecalis	1	0.1	0.1	1.4
	Escherichia coli	10	1.2	1.2	2.6
	Klebsiella spp	31	3.6	3.6	6.2
	MR CoNS	93	10.9	10.9	17.2
	MRSA	180	21.2	21.2	38.3
	MS CoNS	121	14.2	14.2	52.5
	MSSA	348	40.9	40.9	93.4
	Proteus spp	9	1.1	1.1	94.5
	Pseudomonas aeruginosa	45	5.3	5.3	99.8
	Serratia spp	1	0.1	0.1	99.9
	Streptococcus spp	1	0.1	0.1	100.0
	Total	**851**	100.0	100.0	

In the analyzed cultures, MRSA was isolated 180 times (21.2%), MSSA 348 times (40.9%), MR CoNS 93 times (10.9%), MS CoNS 121 times (14.2%), Pseudomonas aeruginosa 45 times (5.3%).

In table V.6. an analysis of the antibiotics used in the antibiogram is found. It includes variables such as the number and percentage of tests that were performed with that antibiotic, the number of samples not tested for that antibiotic, the number and rate of antibiotic-susceptible/resistant isolates.

Only antibiotics that were tested in more than 5% of the total positive samples were included in the table, those below this threshold not being included.

Antibiotic	Testări cu antibioticul	Netestate	Sensibil	Rezistent	% testări din culturi pozitive	% culturi sensibile din cele testate	% culturi rezistente din cele testate
Tobramicină	764	87	532	222	89.8%	69.6%	29.1%
Rifampicină	735	116	669	64	86.4%	91%	8.7%
Cloramfenicol	726	125	529	196	85.3%	72.9%	27%
Gentamicină	704	147	455	240	82.7%	64.6%	34.1%
Cefoxitin	703	148	448	255	82.6%	63.7%	36.3%
Kanamicină	685	166	310	368	80.5%	45.3%	53.7%
Ciprofloxacin	685	166	483	198	80.5%	70.5%	28.9%
Eritromicină	534	317	177	357	62.7%	33.1%	66.9%
Doxiciclină	464	387	273	190	54.5%	58.8%	40.9%
Tetraciclină	372	479	169	203	43.7%	45.4%	54.6%
Vancomicină	366	485	366	0	43%	100%	0%
Teicoplanină	337	514	335	2	39.6%	99.4%	0.6%
Neomicină	298	553	194	103	35%	65.1%	34.6%
Clindamicină	233	618	168	65	27.4%	72.1%	27.9%
Oxacilină	218	633	129	89	25.6%	59.2%	40.8%
Claritromicină	201	650	92	108	23.6%	45.8%	53.7%
Ofloxacin	144	707	112	30	16.9%	77.8%	20.8%
Linezolid	108	743	108	0	12.7%	100%	0%
Ceftazidim	103	748	62	40	12.1%	60.2%	38.8%
Amoxicilină + Acid clavulanic	92	759	30	62	10.8%	32.6%	67.4%
Cefuroxim	79	772	21	58	9.3%	26.6%	73.4%
Amikacină	73	778	68	5	8.6%	93.2%	6.8%

Table V.6. Analysis of the antibiotics used in the antibiogram

The table is ordered by the testing rate, the most tested antibiotic in the antibiogram being Tobramycin (89.8%). Other antibiotics such as Rifampicin, Chloramphenicol, Gentamicin, Cefoxitin, Kanamycin and Ciprofloxacin were also tested in over 80% of all isolates. At the other end of the spectrum is Amikacin, tested in only 8.6% of antibiograms.
The resistance of the studied bacteria to different antibiotics was analyzed and a comparison was made with the results obtained in the ARMOR study, starting from the null hypothesis that there are differences between antibiotic resistance in the two studied cohorts, and the statistical significance of these differences was evaluated.

5.2.1. Analysis of the results from the RAGS 1 study compared to the ARMOR study:

We performed the analysis between the RAGS 2 study and the ARMOR study, to validate the observation that there are statistical differences in the resistance to the antibiotics of interest of the studied germs.

5.3. DISCUSSIONS

A first aspect that should be discussed is related to the absence of Haemophilus influenzae and Streptococcus pneumoniae in the samples analyzed during the 5 years studied (2014-2018). Both bacteria are found in the ARMOR study, so a comparison of these germs was not possible. A single culture was positive for strep, but the species of which it is a part of was not mentioned in the lab result.

The percentage distribution of positive samples over the 5 years remained relatively constant. With this in mind, the fact that the distribution of MRSA isolates over the 5 years also remained relatively constant allows us to say that there was no increase in the MRSA rate from one year to the next. We note instead that the MSSA rate is decreasing in 2018 (28.3%) compared to the starting point of 2014 (59.6%). The distribution of Pseudomonas aeruginosa isolates also remained constant.

In the RAGS 1 study carried out at the level of the ophthalmology department of the Bucharest University Emergency Hospital, the percentages of resistance to Chloramphenicol are significantly higher than those recorded in the ARMOR study for the entire range of staphylococci analyzed, both coagulase-positive (MRSA/MSSA), as well as coagulase-negative (MR CoNS/MS CoNS).

In the case of coagulase-negative staphylococci (MR CoNS/MS CoNS), our study obtained higher rates of resistance compared to the ARMOR study and in the case of Tobramycin (68.3% vs 14.4% for MR CoNS, respectively 26.3% vs 2% for MS CoNS). A large difference in resistance to Tobramycin was also obtained in the case of Pseudomonas aeruginosa (39.5% vs 3.1%). These findings are important because Tobramycin can be used in combination with Vancomycin in the case of keratitis, precisely because of the good concentrations it reaches at the corneal level.

In Pseudomonas aeruginosa infections, one of the therapeutic alternatives known to be effective is with some third-generation cephalosporins (Ceftazidime). The resistance of P. aeruginosa to Ceftazidime in our study is 47.7%, almost one out of two positive cultures for this bacterium being resistant.

A significant difference in MRSA antibiotic resistance rates between our study and ARMOR concerns Clindamycin (69.2% vs 30.8%). The same significant difference in Clindamycin resistance is also found for MSSA (29.6% vs 6.5%). However, the same cannot be said about MR CoNS and MS CoNS, situations in which the percentages are very similar. Therefore, it seems that Staphylococcus aureus (MRSA/MSSA) has a higher rate of resistance to clindamycin at the level of the center in Bucharest where the study was conducted compared to the rate obtained in the ARMOR study.

5.4. CONCLUSIONS

1. The average age of the patients in the studied group was 61.47 years, the patients were aged between 1 and 93 years.
2. From the point of view of gender distribution of the study group of patients with positive detected conjunctival secretions, they were represented almost equally: 50.23% - men and 49.77% women.
3. Percentage-wise, there was no great variation over time, over the years, of the samples found positive in the cultures from the collected conjunctival secretions, from the total of the analyzed samples, being around 20%. The percentages varied between 17.9% - 2017 and 21.5% - 2018. The significance could be represented by a percentage stability in the types of hospitalized cases that require conjunctival secretion testing in the Ophthalmology Clinic of SUUB.
4. Most frequently positive conjunctival secretions were detected in the age range of 65-75 years.

5. In the analyzed cultures, MRSA was isolated 180 times (21.2%), MSSA 348 times (40.9%), MR CoNS 93 times (10.9%), MS CoNS 121 times (14.2%), Pseudomonas aeruginosa by 45 times (5.3%). All other remaining bacteria were each isolated to less than 5%.

6. The highest percentage of positive samples for MRSA during the analyzed years (2014-2018) was recorded in 2016 (32.8%), and the lowest in 2015 (8.5%), in the rest of the years the percentages varied in around 21%, i.e. 1/5 of the positive detected secretions.

7. Regarding the percentage distribution of MSSA samples during the study period, in 2014 MSSA isolates represented 59.6% - the maximum value - of the total positive cultures, 17.6% in 2015 - the minimum value, 49.4% in 2016, 39.9 % in 2017 and 28.3% in 2018. Their fluctuation over time was clearly higher than MSSA.

8. In 2015, a very large number of MR CoNS and MS CoNS was recorded compared to the other germs identified. We could not provide a pertinent explanation for these isolated elevated values of MR CoNS and MS CoNS.

9. The level of Pseudomonas aeruginosa isolates underwent small variations during the 5 years of the study, the highest number being recorded in 2014 and the lowest in 2015.

10. The resistance of Staphylococcus aureus (MRSA/MSSA) behaves differently: in the RAGS 1 study, MRSA registers total resistance to Oxacillin and Cefoxitin and quasi-total resistance to Erythromycin; MSSA registers in the same RAGS 1 study less high resistances to the studied antibiotics.

11. A similar situation is recorded in the case of resistance of coagulase-negative staphylococci (MR CoNS/MS CoNS): MR CoNS shows total/quasi-total resistance to Oxacillin and Cefoxitin, and significant resistance to Kanamycin, Erythromycin, Gentamicin, Clrithromycin, etc.; in the same time interval MS CoNS does not show such high resistance to the studied antibiotics.

16. Pseudomonas aeruginosa shows total/quasi-total resistance to Amoxicillin+clavulanic acid, Cephalexin, Cefuroxime, Kanamycin and important to Doxycycline and Chloramphenicol. Pseudomonas aeruginosa shows increased resistance to Ceftazidime, an antibiotic known to be active on this bacterium.

17. Regarding the comparative resistance of the organisms of interest MRSA, MSSA, coagulase-negative staphylococci (MR CoNS/MS CoNS) in the RAGS 1 and ARMOR studies to Vancomycin, no isolate was encountered in either our study or ARMOR of Vancomycin-resistant MRSA (VRSA).

18. The resistance of MRSA, MSSA, coagulase-negative staphylococci (MR CoNS/MS CoNS) to Chloramphenicol is almost zero in the ARMOR study and present in an important percentage in the RAGS 1 study, this difference being statistically significant for all the germ categories mentioned.
19. The resistance of coagulase-negative staphylococci (MR CoNS/MS CoNS) to Tobramycin obtained in the RAGS 1 study is statistically significantly higher than that recorded in the ARMOR study. This result raises questions related to the treatment of bacterial eye diseases, especially severe ones.
20. Resistance of coagulase-negative staphylococci (MR CoNS/MS CoNS) to Ciprofloxacin obtained in the RAGS 1 study is statistically insignificant in the case of MR CoNS and statistically significantly higher in the case of MS CoNS compared to that recorded in the ARMOR study.
21. The resistance of Pseudomonas aeruginosa to Tobramycin obtained in our study is higher than that recorded in the ARMOR study, resistance not validated for Ciprofloxacin resistance.

CHAPTER 6

STUDY II - RAGS 2 STUDY – Antibiotic Resistance of Germs from conjunctival Secretions 2

A total number of 1923 samples from different patients, collected between 2014-2018, were analyzed.

On the bacteria marked in bold in table VI.5. we will stop with a special interest, since they are part of the set of bacteria also analyzed in the ARMOR and TRUST studies, with which we propose to compare our results.

Table VI.5. Types of germs identified in positive cultures from conjunctival secretions

Types of germs identified					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Corynebacterium	3	1.0	1.0	1.0
	E. coli	3	1.0	1.0	1.9
	Enterococcus spp	9	2.9	2.9	4.9
	Klebsiella spp	15	4.9	4.9	9.7
	Proteus spp	18	5.8	5.8	15.5
	Pseudomonas aeruginosa	3	1.0	1.0	16.5
	MRSA	9	2.9	2.9	19.4
	Serratia marcescens	3	1.0	1.0	20.4
	MSSA	186	60.2	60.2	80.6
	Stafilococci CoN	54	17.5	17.5	98.1
	Streptococcus spp	6	1.9	1.9	100.0
	Total	309	100.0	100.0	

In table VI.6. an analysis of the antibiotics used in the antibiogram is found. It includes variables such as the number and percentage of tests that were performed with that antibiotic and the number of samples not tested for that antibiotic out of the total positive samples.

Only the antibiotics that were tested in more than 5% of the total positive samples were included in the table, those below this threshold not being included/mentioned in the presentation of the results.

The table is ordered by the testing rate, the most tested antibiotic in the antibiogram being Gentamicin (75.7%). Other antibiotics such as Ciprofloxacin, Trimethoprim-Sulfamethoxazole, Ampicillin+Clavulanic Acid, 2nd generation Cephalosporins, Erythromycin, Penicillin were also tested in more than 50% of the total isolates. At the other end of the spectrum is Kanamycin (among the antibiotics currently used in ophthalmology), tested in only 6.8% of antibiograms.

Analysis of the results from the RAGS 2 study compared to the ARMOR study:

Similar to the comparative statistical analysis between the RAGS 1 study and the ARMOR study, the analysis between the RAGS 2 study and the ARMOR study was performed to validate the observation that there are statistical differences in the resistance to the antibiotics of interest of the studied germs.

6.4. DISCUSSIONS

A first aspect that should be discussed is related to the absence of Haemophilus influenzae and Streptococcus pneumoniae in the samples analyzed during the 5 years studied (2014-2018). Both bacteria are found in the ARMOR study, so we could not make a comparison of these germs. Also, coagulase-negative staphylococci were treated as a single group, there being no subdivision into MR CoNS and MS CoNS, as it appears in the ARMOR study, which determined that the statistical analysis was not done on subgroups, but on the total batch of isolated from coagulase-negative staphylococci.

A notable difference in MRSA antibiotic resistance rates between our study and ARMOR concerns Ciprofloxacin (33.3% vs 76.1%) and Ofloxacin (0% vs 75.9%). The same significant difference in Ciprofloxacin and Ofloxacin resistance is also found for MSSA (4.4% vs 13.3%, respectively 25% vs 13.9). The same can be said about CoNS Staff, situations in which the percentages are reversed (27.3% vs 14.4%). Therefore, it seems that Staphylococcus aureus (MRSA/MSSA) has a lower rate of resistance to Ciprofloxacin in the RAGS 2 study (performed on patients from a private center in Bucharest) compared to the resistance rate reported in the United States of America in the ARMOR study.

Also, at the level of this center in Bucharest, the percentages of resistance to Chloramphenicol are very variable compared to those recorded in the ARMOR study for the entire range of staphylococci analyzed, both coagulase-positive (MRSA/MSSA) and coagulase-negative (Staph CoN) .

In the case of coagulase-positive staphylococci (MRSA/MSSA), our study obtained slightly higher rates of resistance compared to the ARMOR study and in the case of Tobramycin (50% vs 40.6% for MRSA, respectively 4.3% vs 4% for MSSA).

Table VI.6. Analysis of the antibiotics used in the antibiogram

Antibiotic	Tested with antibiotic	% tests of pozitive cultures	Untested	% not tested	Total positive cultures
Gentamicin	234	75.7%	75	24,3%	309
Ciprofloxaxin	216	69.9%	93	30,1%	309
Sulfametoxazol	180	58.3%	129	41,7%	309
Amoxicilin+acid clavulanic	174	56.3%	135	43,7%	309
Cefalosporin II-nd generation	171	55.3%	138	44,7%	309
Eritromicin	165	53.4%	144	46,6%	309
Penicillin	162	52.4%	147	47,6%	309
Tetraciclin	135	43.7%	174	56,3%	309
Levofloxacin	129	41.7%	180	58,3%	309
Oxacilin	126	40.8%	183	59,2%	309
Cotrimoxazol	120	38.8%	189	61,2%	309
Tobramicin	105	34.0%	204	66,0%	309
Chloramfenicol	93	30.1%	216	69,9%	309
Fosfomicin	90	29.1%	219	70.9%	309
Rifampicin	84	27.2%	225	72.8%	309
Claritromicin	78	25.2%	231	74.8%	309
Ampicilin	75	24.3%	234	75.7%	309
Linezolid	75	24.3%	234	75.7%	309
Ofloxacin	69	22.3%	240	77.7%	309
Vancomicin	69	22.3%	240	77.7%	309
Ceftriaxon	66	21.4%	243	78.6%	309
Moxifloxacin	66	21.4%	243	78.6%	309
Amikacin	51	16.5%	258	83.5%	309
Doxiciclin	51	16.5%	258	83.5%	309
Ceftazidim	48	15.5%	261	84.5%	309
Netilmicin	48	15.5%	261	84.5%	309
Meropenem	45	14.6%	264	85.4%	309
Cefaclor	39	12.6%	270	87.4%	309
Cefepime	36	11.7%	273	88.3%	309
Azitromicin	33	10.7%	276	89.3%	309
Acid fusidic	33	10.7%	276	89.3%	309
Imipenem	33	10.7%	276	89.3%	309
Tazobactam	33	10.7%	276	89.3%	309
Cefoperazon	24	7.8%	285	92.2%	309
Kanamicin	21	6.8%	288	93.2%	309
Cefaclor	18	5.8%	291	94.2%	309
Tigeciclin	18	5.8%	291	94.2%	309

6.4. DISCUSSIONS

A first aspect that should be discussed is related to the absence of Haemophilus influenzae and Streptococcus pneumoniae in the samples analyzed during the 5 years studied (2014-2018). Both bacteria are found in the ARMOR study, so we could not make a comparison of these germs. Also, coagulase-negative staphylococci were treated as a single group, there being no subdivision into MR CoNS and MS CoNS, as it appears in the ARMOR study, which determined that the statistical analysis was not done on subgroups, but on the total batch of isolated from coagulase-negative staphylococci.

Six cultures were positive for streptococcus, but the species to which they belong was not mentioned in the laboratory results.

During the 5 years, only 3 isolates of Pseudomonas aeruginosa were identified, which prevented the comparison regarding this germ.

A notable difference in MRSA antibiotic resistance rates between our study and ARMOR concerns Ciprofloxacin (33.3% vs 76.1%) and Ofloxacin (0% vs 75.9%). The same significant difference in Ciprofloxacin and Ofloxacin resistance is also found for MSSA (4.4% vs 13.3%, respectively 25% vs 13.9). The same can be said about CoNS Staff, situations in which the percentages are reversed (27.3% vs 14.4%). Therefore, it seems that Staphylococcus aureus (MRSA/MSSA) has a lower rate of resistance to Ciprofloxacin in the RAGS 2 study (performed on patients from a private center in Bucharest) compared to the resistance rate reported in the United States of America in the ARMOR study.

Also, at the level of this center in Bucharest, the percentages of resistance to Chloramphenicol are very variable compared to those recorded in the ARMOR study for the entire range of staphylococci analyzed, both coagulase-positive (MRSA/MSSA) and coagulase-negative (Staph CoN) .

In the case of coagulase-positive staphylococci (MRSA/MSSA), our study obtained slightly higher rates of resistance compared to the ARMOR study and in the case of Tobramycin (50% vs 40.6% for MRSA, respectively 4.3% vs 4% for MSSA).

6.5. CONCLUSIONS

1. The average age of the patients in the studied group was 67.97 years, the patients were aged between 21 and 98 years.
2. Gender distribution of the total study group: 38.7% - men and 61.3% women

3. From the point of view of gender distribution of the study group of patients with positive detected conjunctival secretions, they were represented almost equally: 153 men and 156 women (50.48% - men and 49.5% women).

4. Most frequently positive conjunctival secretions were detected in the age range of 60-80 years.

5. In the analyzed cultures, MSSA was isolated 186 times (60.2%), MRSA 9 times (2.9%), coagulase-negative staphylococci 54 times (17.5%), Pseudomonas aeruginosa 3 times (0.1%). All other remaining bacteria were each isolated in less than 5% and are not of interest to the current study.

6. Staphylococcus aureus (MRSA/MSSA) resistance behaves differently: both show lower resistance to Ciprofloxacin and higher resistance to Tobramycin compared to ARMOR in the RAGS 2 study. To Ofloxacin MRSA has significantly less resistance than in ARMOR, but for MSSA the difference is not statistically significant. For other higher-generation quinolones no conclusion on resistance/susceptibility can be drawn because they have been insufficiently tested.

13. Chloramphenicol for MRSA resistance has not been tested, while MSSA has significantly higher resistance than reported in ARMOR.

14. Regarding the resistance of coagulase-negative staphylococci (Staph CoNS) we cannot issue a conclusion about resistance to Chloramphenicol, but it is significantly lower to Ofloxacin compared to ARMOR. It shows no difference in resistance to Ciprofloxacin and Tobramycin; the same situation is for Levofloxacin and Moxifloxacin.

15. For Pseudomonas aeruginosa no conclusion on resistance/susceptibility can be drawn because it was insufficiently identified and tested.

16. Resistance of MRSA, MSSA, coagulase-negative staphylococci (Staph CoN) to Chloramphenicol is almost nil in the ARMOR study and is present in an important percentage for MSSA in RAGS 2.

17. The resistance of MRSA and MSSA to Tobramycin obtained in the RAGS 2 study is statistically significantly higher than that recorded in the ARMOR study, while that of coagulase-negative staphylococci (Staph CoN) is lower.

18. The resistance of MRSA, MSSA and coagulase-negative staphylococci (Staph CoN) to Ciprofloxacin obtained in the RAGS 2 study is statistically insignificantly higher than that recorded in the ARMOR study.

19. The resistance of Pseudomonas aeruginosa to Ciprofloxacin obtained in our study compared to that recorded in the ARMOR study was not validated.

CHAPTER 7

STUDY III - RAGS 1 STUDY versus RAGS 2 STUDY

Starting from the hypothesis that there are significant differences not only inter-continental, interstate and inter-regional (at the level of different areas of a country), but even between different areas of a city, we tried to make a statistical comparison between the groups of patients included in the RAGS 1 study and those included in the RAGS 2 study. In essence, this comparison is made between two models of ophthalmological practice (hospital vs. private), two different models of patient addressability (emergency+chronic vs. predominantly chronic), both premises that can be located in the central area of Bucharest, in different sectors, but with complex addressability in Bucharest and the surrounding areas, but not only.

The 851 positive samples from the RAGS 1 study were compared with the 309 positive samples from the RAGS 2 study.

7.6. CONCLUSIONS

1. The mean age of patients in the RAGS 1 group was 61.47 years, while the mean age of patients in the RAGS 2 group was 67.97 years.
2. In the RAGS 1 study, the patients were aged between 1 and 93 years, and in the RAGS 2 study, the patients were aged between 21 and 98 years.
3. In the RAGS 1 study, in terms of gender distribution, they were represented almost equally - 50.48% men and 49.5% women, and in the RAGS 2 study the gender distribution was 50.23% men and 49.77% women .
4. Most frequently positive conjunctival secretions were detected in the age range of 60-80 years in both studies.
5. In RAGS study 1: MRSA was isolated 180 times (21.2%), MSSA 348 times (40.9%), coagulase-negative staphylococci (MR CoNS 93 times and MS CoNS 121 times) 214

times (25.14 %) and Pseudomonas aeruginosa 45 times (5.3%). In the RAGS 2 study: MRSA was isolated 9 times (2.9%), MSSA was isolated 186 times (60.2%), coagulase-negative staphylococci 54 times (17.5%) and Pseudomonas aeruginosa 3 times (0.1%)

11. All other bacteria were each isolated in less than 5% and were not of interest to the current study.

12. The starting null hypothesis H0 was that the overall resistance/susceptibility to antibiotics in the RAGS 1 study is similar to that found in the patient population in the RAGS 2 study. Statistically significant differences were demonstrated between the 2 studied cohorts to Ciprofloxacin, Chloramphenicol, Gentamicin and Tobramycin, possibly higher in RAGS 1 compared to RAGS 2. This hypothesis was confirmed for Kanamycin (p=0.301).

13. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 trials in terms of MRSA resistance to ciprofloxacin, chloramphenicol, gentamicin, kanamycin and tobramycin, suggesting that the behavior of MRSA is the same across territorial surfaces wider belonging to the same narrower geographical area.

14. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 studies with respect to MSSA resistance to gentamicin, kanamycin and tobramycin, but there are to ciproprofloxacin and coramfenicol, suggesting slightly different behaviors of MSSA on wider territorial surfaces belonging to the same area.

15. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 trials in Staf CoNS resistance to gentamicin, kanamycin and tobramycin, but there are to ciproprofloxacin and coramfenicol, suggesting slightly different behaviors of CoNS staff on wider territorial areas belonging to the same area.

16. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 studies with respect to resistance of Pseudomonas aeruginosa to ciproprofloxacin and gentamicin. This hypothesis could not be validated for Chloramphenicol. All this leads to the hypothesis that Pseudomonas aeruginosa does not have very different characters from one subzone to another.

CHAPTER 8

CONCLUSIONS AND PERSONAL CONTRIBUTION

8.1. CONCLUSIONS

The studies were conducted over a period of 5 years (2014-2018), in parallel, and enrolled a total of 3514 patients.

Following the careful analysis of the study groups, we found the following:

1. In the RAGS 1 study we enrolled 1591 patients, of which 851 were positive (53.48%).
2. In the RAGS 2 study we enrolled 1923 patients, of which 309 were positive (16.06%).
3. I would attribute the difference in the percentage of positive patients to the seeding of conjunctival secretions to the fact that more severe cases are presented in the hospital, while in private practice the cases requiring scheduled surgical intervention predominate, the patients being asymptomatic but subject to a preoperative screening.
4. The mean age of patients in the RAGS 1 group was 61.47 years, while the mean age of patients in the RAGS 2 group was 67.97 years.
5. In the RAGS 1 study, the patients were aged between 1 and 93 years, and in the RAGS 2 study, the patients were aged between 21 and 98 years.
6. The average age of the patients in the 2 studies was similar, but the range of patients who presented for the consultation was slightly different, newborns were also addressed to the hospital, and patients from the end of the 9th decade to the private practice.
7. From the point of view of gender distribution, both in the RAGS 1 and in the RAGS 2 study, they were represented 1:1, i.e. 50.48% men vs 49.5% women in RAGS 1 and 50.23% men vs 49.77% women in RAGS 2. We refer only to patients whose conjunctival secretion samples were positive.
8. There are no differences, in the sense of predominately affecting one sex, in the groups of patients detected positive on the seeding of their conjunctival secretion in any of the 2 RAGS studies.
9. Most frequently positive conjunctival secretions were detected in the age range of 60-80 years in both studies.

10. The age group with the most positive conjunctival secretions is the elderly group, where both chronic pathology and acute and chronic/acute chronic infectious pathology are found.
11. In RAGS study 1: MRSA was isolated 180 times (21.2%), MSSA 348 times (40.9%), coagulase-negative staphylococci (MR CoNS 93 times and MS CoNS 121 times) 214 times (25.14 %) and Pseudomonas aeruginosa 45 times (5.3%). In the RAGS 2 study: MRSA was isolated 9 times (2.9%), MSSA was isolated 186 times (60.2%), coagulase-negative staphylococci 54 times (17.5%) and Pseudomonas aeruginosa 3 times (0.1%) . All other bacteria were each isolated in less than 5% and were not of interest to the current study.
12. Both in the RAGS 1 study and in the RAGS 2 study, MSSA prevailed in a percentage of 40.9%, respectively 60.2%, the next germ identified as frequency being Staf CoN: 25.14%, respectively 17.5%. There are large differences in the frequency of identification of MRSA and Pseudomonas aeruginosa.
13. Both in the RAGS 1 study and in the RAGS 2 study, in the age groups 60-70 years and 70-80 years, the most numerous strains identified were MSSA and Staf CoN.
14. Statistically significant differences in overall bacterial resistance/susceptibility to Ciprofloxacin, Chloramphenicol, Gentamicin and Tobramycin were demonstrated between the 2 cohorts studied, possibly greater in RAGS 1 versus RAGS 2.
15. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 trials in terms of MRSA resistance to ciprofloxacin, chloramphenicol, gentamicin, kanamycin and tobramycin, suggesting that the behavior of MRSA is the same across territorial surfaces wider belonging to the same narrower geographical area.
16. Statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 studies in terms of MSSA resistance to ciproprofloxacin and coramfenicol, suggesting slightly different behaviors of MSSA over wider territorial areas belonging to the same area.
17. Statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 studies regarding resistance of Staf CoNS to ciproprofloxacin and coramfenicol, suggesting the same behavior of Staf CoNS over wider territorial areas belonging to the same area as of MSSA.

18. No statistically significant differences were identified between the 2 cohorts of patients in the RAGS 1 and RAGS 2 studies regarding resistance of Pseudomonas aeruginosa to ciproprofloxacin and gentamicin, the difference in resistance to Chloramphenicol remains unvalidated. All this leads to the hypothesis that Pseudomonas aeruginosa does not have very different characters from one subzone to another.

19. As a more general conclusion related to the differences in sensitivity/resistance of germs between neighboring territorial areas, following the analysis of the data from the RAGS 1 and RAGS 2 studies, it appears that the germs that show multiple resistance, most often germs associated with healthcare , have a uniform behavior over wider areas (e.g. MRSA and Pseudomonas aeruginosa), while germs that still show high sensitivity to antibiotics (e.g. MSSA and coagulase-negative staphylococci) have a slightly different behavior in terms of sensitivity /antibiotic resistance.

8.2. PERSONAL CONTRIBUTION

Sir Alexander Fleming, winner of the Nobel Prize for Medicine (1945) for the discovery of penicillin, gave an interview shortly after the ceremony in which he stated: ***"The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism."***

What is the importance of the two studies and what is the significance of the data collected and analyzed?

From what is known up to this moment, they are the only studies in Romania in which data were collected from two different centers of ophthalmology practice, over a long period - 5 years (2014-2018) and which enrolled a total of 3514 patients (1591 in the RAGS 1 study and 1923 in the RAGS 2 study. Fewer studies of the same magnitude have been reported in (e.g. USA and Italy) which are periodically reviewed to establish the resistance trend.

From the analysis of the 2 studies, several important conclusions emerged, which deserve to be further explored in the future:

- the most frequent germ identified in both studies was MSSA, followed by coagulase-negative staphylococci. MRSA and Pseudomonas aeruginosa, which are most often associated with health care, are more resistant germs and were detected in small percentages in both studies.
- from the point of view of resistance/sensitivity to ciproprofloxacin and coramfenicol, MSSA shows slightly different behaviors on wider territorial surfaces belonging to the same area.
- from the point of view of resistance/sensitivity to ciproprofloxacin and coramfenicol, coagulase-negative staphylococci behave uniformly on larger territorial surfaces belonging to the same area.
- no statistically significant differences were identified in MRSA resistance to ciprofloxacin, chloramphenicol, gentamicin, kanamycin and tobramycin, suggesting that MRSA resistance/susceptibility is the same over wider territorial areas belonging to the same narrower geographical area.
- Pseudomonas aeruginosa does not have very different characters from one subzone to another in terms of resistance/susceptibility to ciproprofloxacin, gentamicin and chloramphenicol.
- as a more general conclusion related to the differences in sensitivity/resistance of germs between neighboring territorial areas, following the analysis of the data from the RAGS 1 and RAGS 2 studies, it appears that the germs that present multiple resistance, most often germs associated with medical care, behave uniformly over wider areas (e.g. MRSA and Pseudomonas aeruginosa), while germs that still show high sensitivity to antibiotics (e.g. MSSA and coagulase-negative staphylococci) behave slightly differently in terms of sensitivity/ resistance to antibiotics

This doctoral study also raises a series of problems, frequently encountered and which have had an important impact in ophthalmology practice:
- the first problem identified is related to the lack of uniformity in the evaluation of the collected samples. We do not refer to the lack of standardization at the general level of laboratories, but we also noticed the lack of uniformity in the examination of samples within the same laboratory. From this derives the fact that some germs have been tested only a few times with some antibiotics and consequently the impossibility of introducing some of the laboratory results into the statistical analysis.
- the second problem is directly related to the previous one and tries to put under the microscope, including for economic reasons, the fact that the testing of the collected samples is done with last-generation antibiotics and which would have no justification to be used in an ocular pathology of type of acute or chronic bacterial conjunctivitis. Hence the need for collaboration and a close dialogue with the medical staff in the bacteriology laboratory to

understand the needs and requirements of the ophthalmologist related to the severity of the case from which samples were collected. The antibiogram should be differentiated; for example, if it is an acute conjunctivitis that requires only topical treatment, a keratitis that could benefit from systemic treatment associated with the topical one, or an endogenous endophthalmitis with a hepatic starting point, testing should be done taking into account the severity of the case and of therapeutic options. This would benefit both patient treatment and the economy of laboratory resources.

- the third major problem is the unavailability in the form of topical treatment of the majority of antibiotics to which the germs are identified as sensitive or intermediately sensitive. Most of the time, ophthalmologists have to resort to extemporaneous antibiotic solutions for treatment, with all their drawbacks (tolerability, stability, etc.).
- the fourth problem, not to be neglected, is related to the fact that the antibiotic guidelines, even if they are developed regionally (e.g. the American guide, the European guide, etc.) give directions to fight infections in systemic, oral or intravenous administration, with dosage well established, but the most used way in ophthalmology is the topical one, and in this sense there is no mention or specification in terms of the concentrations of antibiotic solutions.

Fleming himself, the discoverer of penicillin, predicted not only how useful antibacterial drugs would be, but also how dangerous a world without them could be.

Bucharest, 2022

SELECTED REFERENCES

Albrich WC, Harbarth S. P1310 Healthcare workers - Source, vector or victim of MRSA? *Int J Antimicrob Agents*. 2007. 8(5):289-301. doi: 10.1016/S1473-3099(08)70097-5.

Armstrong RA. The microbiology of the eye. *Ophthalmic Physiol Opt*. 2000. 20(6):429-41

Asbell PA, Sanfilippo CM, Pillar CM, DeCory HH, Sahm DF, Morris TW. Antibiotic resistance among ocular pathogens in the United States five-year results from the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) surveillance study. *JAMA Ophthalmol*. 2015. 133(12):1445-54

Asbell PA, Colby KA, Deng S, et al. Ocular TRUST: Nationwide Antimicrobial Susceptibility Patterns in Ocular Isolates. *Am J Ophthalmol*. 2008. 145(6):951-958. doi: 10.1016/j.ajo.2008.01.025. Epub 2008 Mar 28.

Asbell PA, Sanfilippo CM, Sahm DF, DeCory HH. Trends in Antibiotic Resistance Among Ocular Microorganisms in the United States From 2009 to 2018. JAMA Ophthalmol. 2020;138(5):439-450. doi:10.1001/jamaophthalmol.2020.0155

Azari AA, Barney NP. Conjunctivitis: A systematic review of diagnosis and treatment. *JAMA - J Am Med Assoc*. 2013. 310(16):1721-9. doi: 10.1001/jama.2013.280318.

Baim AD, Movahedan A, Farooq AV, Skondra D. The microbiome and ophthalmic disease. Exp Biol Med (Maywood). 2019 Apr;244(6):419-429. doi: 10.1177/1535370218813616. Epub 2018 Nov 21. PMID: 30463439

Bertino JS. Impact of antibiotic resistance in the management of ocular infections: The role of current and future antibiotics. *Clin Ophthalmol*. 2009;3(1):507-521.

Brown CA. Penicillin In Ophthalmology: The bacteriological, experimental, and clinical evidence of its value, including a personal series of 125 clinical cases. *Br J Ophthalmol*. 1946;30(3):146-167.

CARMIAAM – 2018. Consumul de antibiotic, rezistența microbiană și Infecții asociate Asistenței Medicale în România - 2018

Capriotti JA, Pelletier JS, Shah M, Caivano DM, Ritterband DC. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. *Int Ophthalmol*. 2009. 29(2):81-4. doi: 10.1007/s10792-008-9196-4. Epub 2008 Feb 23.

Cassini A, Hogberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted lifeyears caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019 Jan;19(1):56-66.

CDC. Antibiotic resistance threats in the United States, 2013. *Current*. 2013.

Chu DTW, Plattner JJ, Katz L. New directions in antibacterial research. *J Med Chem*. 1996. 39(20):3853-74. doi: 10.1021/jm960294s.

Ciulla TA, Starr MB, Masket S. Bacterial endophthalmitis prophylaxis for cataract surgery: An evidence-based update. *Ophthalmology*. 2002. 109(1):13-24. doi: 10.1016/s0161-6420(01)00899-5.

Czaplewski L, Bax R, Clokie M, et al. Alternatives to antibiotics-a pipeline portfolio review. *Lancet Infect Dis*. 2016. 16(2):239-51. doi: 10.1016/S1473-3099(15)00466-1.Epub 2016 Jan 13.

Dar OA, Hasan R, Schlundt J, et al. Exploring the evidence base for national and regional policy interventions to combat resistance. *Lancet*. 2016. 387(10015):285-95. doi: 10.1016/S0140-6736(15)00520-6.Epub 2015 Nov 18.

Dave SB, Toma HS, Kim SJ. Changes in ocular flora in eyes exposed to ophthalmic antibiotics. *Ophthalmology*. 2013. 120(5):937-41. doi: 10.1016/j.ophtha.2012.11.005. Epub 2013 Feb 13.

Davies J. Inactivation of antibiotics and the dissemination of resistance genes. *Science. 1994;5157(264):375-382*. 1994. DOI: 10.1126/science.8153624

Davies J. Bacteria on the rampage. *Nature*. 1996. doi.org/10.1038/383219a0

De Kaspar HM, Kreidl KO, Singh K, Ta CN. Comparison of preoperative conjunctival bacterial flora in patients undergoing glaucoma or cataract surgery. *J Glaucoma*. 2004. 13(6):507-9. doi: 10.1097/01.ijg.0000137872.19942.cf.PMID: 15534478

Demerec M. Origin of Bacterial Resistance to Antibiotics. *J Bacteriol*. 1948. 56(1):63-74. doi: 10.1128/jb.56.1.63-74.1948.

Demirjian A, Sanchez G V., Finkelstein JA, et al. CDC grand rounds: Getting smart about antibiotics. *Morb Mortal Wkly Rep*. 2015. 64(32):871-3.doi: 10.15585/mmwr.mm6432a3

Dossarps D, Bron AM, Koehrer P, et al. Endophthalmitis after intravitreal injections: Incidence, presentation, management, and visual outcome. *Am J Ophthalmol*. 2015. 160(1):17-25.e1. doi: 10.1016/j.ajo.2015.04.013.Epub 2015 Apr 16.

Durand ML. ESCRS Guidelines for Prevention and Treatment of Endophthalmitis Following Cataract Surgery. *Clin Microbiol Infect*. 2013. 19(3):227-34. doi: 10.1111/1469-0691.12118.

Egrilmez S, Yildirim-Theveny Ş. Treatment-Resistant Bacterial Keratitis: Challenges and Solutions. Clin Ophthalmol. 2020 Jan 29;14:287-297. doi: 10.2147/OPTH.S181997. eCollection 2020.PMID: 32099313

European Centre for Disease Prevention and Control. Surveillance Atlas of Infectious Diseases [Internet]. Stockholm: ECDC; 2018. Available from: http://atlas.ecdc.europa.eu

European Centre for Disease Prevention and Control. Summary of the latest data on antibiotic consumption in the European Union. Stockholm: ECDC; 2019. ESAC-Net surveillance data November 2019

ESAC-Net Interactive Database. http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial-consumption/esac-net-database/Pages/database.aspx

European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2019.

European Centre for Disease Prevention and Control. Exploring opportunities for support in healthcare-associated infections – Romania, 4–7 July 2016. Stockholm: ECDC; 2017.

European Commission. A European One Health Action Plan against Antimicrobial Resistance (AMR). Brussels: EC; 2017. https://ec.europa.eu/health/amr/sites/amr/files/ amr_action_plan_2017_en.pdf

ECDC Mission Report. ECDC country visit to Romania to discuss antimicrobial resistance issues, 6-10 March 2017. Stockholm: ECDC; June 2018.

Foster A. Vision 2020: The cataract challenge. *J Community Eye Heal*. 2000. 13(34):17-9.

Gentile RC, Shukla S, Shah M, et al. Microbiological spectrum and antibiotic sensitivity in endophthalmitis: A 25-year review. *Ophthalmology*. 2014. 121(8):1634-42. doi: 10.1016/j.ophtha.2014.02.001.Epub 2014 Apr 2.

Goldstein MH, Kowalski RP, Gordon YJ. Emerging fluoroquinolone resistance in bacterial keratitis: A 5-year review. *Ophthalmology*. 1999. 106(7):1313-8.

Graham JE, Moore JE, Jiru X, et al. Ocular pathogen or commensal: A PCR-based study of surface bacterial flora in normal and dry eyes. *Investig Ophthalmol Vis Sci*. 2007. 48(12):5616-23. doi: 10.1167/iovs.07-0588.

Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea*. 2008. 27(1):22-7. doi: 10.1097/ICO.0b013e318156caf2.

Grzybowski A, Brona P, Kim SJ. Microbial flora and resistance in ophthalmology: a review.*Graefe's Arch Clin Exp Ophthalmol*. 2017;255(5):851-862. doi: 10.1007/s00417-017-3608-y

Grzybowski A, Kuklo P, Pieczynski J, Beiko G. A review of preoperative manoeuvres for prophylaxis of endophthalmitis in intraocular surgery: Topical application of antibiotics, disinfectants, or both? *Curr Opin Ophthalmol*. 2016. 27(1):9-23. doi: 10.1097/ICU.0000000000000216.

Haas W, Pillar CM, Torres M, Morris TW, Sahm DF. Monitoring antibiotic resistance in ocular microorganisms: Results from the Antibiotic Resistance Monitoring in Ocular micRorganisms (ARMOR) 2009 surveillance study. *Am J Ophthalmol*. 2011. 152(4):567-574.e3. doi: 10.1016/j.ajo.2011.03.010. Epub 2011 Jun 8.

Hamad B. The antibiotics market. *Nat Rev Drug Discov*. 2010. 9(9):675-6. doi: 10.1038/nrd3267.

Han DP, Vine AK, Blodi BA, et al. Microbiologic factors and visual outcome in the endophthalmitis vitrectomy study. *Am J Ophthalmol*. 1996. 122(6):830-46. doi: 10.1016/s0002-9394(14)70380-0.

Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the endophthalmitis vitrectomy study. *Am J Ophthalmol*. 1996. 122(1):1-17. doi: 10.1016/s0002-9394(14)71959-2.

Hanet MS, Jamart J, Chaves AP. Fluoroquinolones or fortified antibiotics for treating bacterial keratitis: Systematic review and meta-analysis of comparative studies. *Can J Ophthalmol*. 2012. 47(6):493-9. doi: 10.1016/j.jcjo.2012.09.001.

Haripriya A, Chang DF, Namburar S, Smita A, Ravindran RD. Efficacy of Intracameral Moxifloxacin Endophthalmitis Prophylaxis at Aravind Eye Hospital. *Ophthalmology*. 2016. 123(2):302-308. doi: 10.1016/j.ophtha.2015.09.037. Epub 2015 Oct 30.

SCIENTIFIC PUBLICATIONS

Books/Book chapters:

1. Alina Popa-Cherecheanu, Ruxandra Pîrvulescu, Raluca Iancu, Sînziana Istrate, **M Popa-Cherecheanu**, DG Deleanu. Rezistența la antibiotice a florei conjunctivale – O ecuație cu mai multe necunoscute și fără soluție? Oftalmologia Clinică – vol. IV, pag. 265-281, 2021. Editura Pim (acreditată CNCSIS - 66/2010). ISBN 978-606-13-6320-9

Articles in scientific journals:

1. Bacterial resistance in ophthalmology – resistance profile, influencing factors and prevention methods. A review. **Matei Popa-Cherecheanu**, Alina Popa-Cherecheanu, Dan George Deleanu, Mihai Ghiță. Modern Medicine. 2021, Vol. 28, No. 2. ISSN-online 2360-2473. doi.org/10.31689/rmm.2021.28.2.127
2. Pilot study on antibiotic resistance of conjuctival bacteria - RAGS study. **Popa-Cherecheanu M**, Ionescu D, Grigore R, Munteanu GS, Simion-Antonie CB, Bejenaru PL, Berteștieanu ȘV, Ionescu TP, Deleanu DG, Popa-Cherecheanu A. Medicine in Evolution. Volume XXVII, Nr. 2, 2021, Timişoara, Romania. ISSN 2065-376X
3. Laboratory results on investigations of laser beams interactions with chlorpromazine to overpass multiple drug resistance acquired by bacteria. RA Pirvulescu, AR Sterian, ML Pascu, **M Popa Cherecheanu**, MO Romanitan. UPB Sci. Bull, series A, vol 83, Issue 2, 2021. https://www.scientificbulletin.upb.ro/rev_docs_arhiva/reze5d_312762.pdf