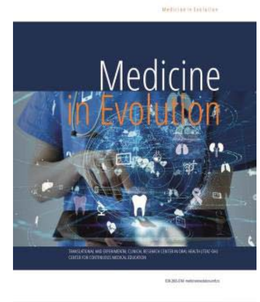
# Pilot study on antibiotic resistance of conjuctival bacteria - RAGS study



**Popa-Cherecheanu M.[1,2], Ionescu D.[3,4], Grigore R.[1,5], Munteanu G.S.[1,7], Simion-Antonie C.B.[1], Bejenaru P.L.[1],Berteşteanu Ş.V.[1,5], Ionescu T.P.[3], Deleanu D.G.[6], Popa-Cherecheanu A.[1,6]**

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest*
[2]*Agrippa Ionescu Clinical Emergency Hospital, Bucharest*
[3]*Titu Maiorescu University, Bucharest*
[4]*Victor Gomoiu Clinical Hospital for Children, Bucharest*
[5]*Colţea Clinical Hospital, Bucharest*
[6]*Emergency University Hospital, Bucharest*
[7]*Central Military Emergency University Hospital "Dr. Carol Davila"*

*Correspondence to:*
*Name: Deleanu Dan George*
*Address: 169 Splaiul Independenţei, Bucharest*
*Phone: +40 727 082 380*
*E-mail address: dangeorge_deleanu@yahoo.com*

## Abstract

The main purpose of this study is to compare the resistance profile of bacteria isolated from conjunctival secretions in our center with that of bacteria isolated in the ARMOR 2013 study. A total of 1591 samples were analyzed, successively collected for 5 years. Of these, 53.5% had a positive result, 37.6% were negative, and 8.9% were contaminated with saprophytic flora, the latter not being included in the statistics. The resistance of *Staphylococcus aureus MRSA / MSSA* to clindamycin, chloramphenicol and ciprofloxacin obtained in the RAGS study is higher than that recorded in the ARMOR study. The resistance of *coagulase-negative staphylococci* (*MR CoNS / MS CoNS*) to tobramycin obtained in our study is higher than that recorded in the ARMOR study, the same being true for chloramphenicol. The resistance of *Pseudomonas aeruginosa* to tobramycin obtained in the RAGS study is also higher than that recorded in the ARMOR study.

**Keywords:** antibiotic resistance, conjunctival secretion, ocular antibiotics

## INTRODUCTION

The bacterial resistance profile to antibiotics may vary depending on the geographical location (Olson et al. 2010), so the antibiotic therapy schemes in the guidelines are not always fully applicable in all regions and there may be variations from one country to another. or even between centers. Therefore, it is important to know the local particularities so that the treatment can be adapted accordingly, both to achieve the best possible therapeutic success for the patient, but also to combat possible new resistance to antibiotics.

### *Aim and objectives*

The main purpose of this study is to compare the resistance profile of bacteria isolated from conjunctival secretions in our center with that of bacteria isolated in other studies, conducted mainly in the United States (Hsu et al. 2015). The study with which we set out to compare our results is ARMOR 2013.

The ARMOR study (Antibiotic Resistance Monitoring in Ocular Microorganisms) is a program developed to monitor ocular pathogens in the United States. The initial results of the ARMOR study based on isolates collected from 34 institutions during 2009 and were published in 2011 (ARMOR 2009), and data between 2009-2013 (ARMOR 2013) were published in 2017. The ARMOR study extends the data collected in a wide range of studies (TRUST study) 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 (Haas et al. 2009, Asbell et al. 2008, Dar et al. 2016).

## MATERIAL AND METHODS

The study is descriptive, observational, transversal, conducted within the Department of Ophthalmology of the University Emergency Hospital Bucharest. The data were extracted using the informatic system Infoword Hospital and then centralized in Microsoft Excel, the statistical analysis being performed in SPSS and the statistical computer https://www.socscistatistics.com/tests/ for the chi-square calculation.

The study included all samples of conjunctival secretions collected between 2014 and 2018. Based on the pathological product harvested at the conjunctival level, bacterial cultures were performed and the susceptibility to different antibiotics was tested, focusing on the resistance of certain categories of germs and on certain antibiotics, analyzed in the ARMOR 2013 study as well.

We named this pilot study **RAGS** (**R**esistance to **A**ntibiotics of **G**erms in conjunctival **S**ecretions).

## RESULTS AND DISCUSSIONS

A total number of 1591 consecutive samples from different patients, collected between 2014-2018 were analyzed. Of the 1591 bacterial cultures, 851 of them (53.5%) had a positive result, 599 (37.6%) were negative, while 141 (8.9%) were contaminated with saprophytic flora. Contaminated samples were not included in the subsequent statistical analyzes.

Only bacteria isolated from positive cultures were analyze (see Table 1).

Table 1. Distribution of bacteria isolated from conjunctival secretions

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | *Bacillus spp.* | 1 | .1 | .1 | .4 |
| | *Enterobacter spp.* | 8 | .9 | .9 | 1.3 |

| | | | | |
|---|---|---|---|---|
| *Enterococcus faecalis* | 1 | .1 | .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 | .1 | .1 | 99.9 |
| *Streptococcus spp.* | 1 | .1 | .1 | 100.0 |
| Total | 851 | 100.0 | 100.0 | |

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

*Staphylococci* were subsequently divided into *Staphylococcus aureus (methicillin-sensitive MSSA / methicillin-resistant MRSA*) and *coagulase-negative staphylococci* (as well as *methicillin-sensitive MS CoNS / methicillin-resistant MR CoNS*).

The bacteria marked in bold in Table 1 are of special interest, as they are part of the set of bacteria analyzed in the ARMOR study and we aim to compare the results of RAGS study with the results of ARMOR study.

We will analyze the resistance of the studied bacteria (R=resistant) to different antibiotics and we will compare the differences between the results of RAGS and ARMOR studies (statistical significance).

Table 2. Resistance of methicillin-sensitive Staphylococcus aureus to various antibiotics in RAGS and ARMOR studies

| *MSSA* | Susceptibility | Ciprofloxacin | Tobramicin | Clindamicin | Cloramfenicol |
|---|---|---|---|---|---|
| RAGS | R | 20.1% | 10% | 29.6% | 18.4% |
| ARMOR | R | 13.3% | 4% | 6.5% | 0.2% |
| *p*-value | | .008045 | .000203 | <0.000001 | <0.000001 |

Table 2 illustrates the resistance of *MSSA* to various antibiotics. There are still large differences in resistance rates compared to those reported in the ARMOR study; for all antibiotics (ciprofloxacin, tobramycin, clindamycin, chloramphenicol) the difference is statistically significant and very high compared for last two.

Regarding *MRSA* resistance to the same antibiotics, in the RAGS study the resistance to clindamycin and chloramphenicol was statistically significant higher than in the ARMOR study. In the ARMOR study the resistance to chloramphenicol was almost absent (0.7%). Interestingly, the ARMOR study reports a higher percentage of ciprofloxacin resistance (76.1%) compared to 59.9% in the RAGS study (Table 3).

Table 3 Resistance of methicillin-resistant Staphylococcus aureus to various antibiotics in RAGS and ARMOR studies

| *MRSA* | Susceptibility | Ciprofloxacin | Tobramicin | Clindamicin | Cloramfenicol |
|---|---|---|---|---|---|
| RAGS | R | 59.9% | 49.7% | 69.2% | 40.1% |
| ARMOR | R | 76.1% | 40.6% | 30.8% | 0.7% |
| *p*-value | | .000034 | | .000048 | <0.00001 |

Table 4. Resistance of methicillin-sensitive coagulase-negative staphylococci to various antibiotics in RAGS and ARMOR studies

| *MS CoNS* | Susceptibility | Ciprofloxacin | Tobramicin | Clindamicin | Cloramfenicol |
|---|---|---|---|---|---|
| RAGS | R | 13.3% | 26.3% | 6.9% | 11.1% |
| ARMOR | R | 14.4% | 2% | 7.2% | 0.5% |
| *p*-value | | | < 0.00001 | | < 0.00001 |

The resistance of *MS CoNS* to tobramycin and chloramphenicol is very high in the RAGS study compared to the ARMOR study (26.3% compared to 2% respectively 11.1% compared to 0.5%), the differences being statistically significant. It is hypothesized that staphylococcal resistance to methicillin also causes a higher expression of other resistance to antibiotics.

In table 5 we compare *MR CoNS* resistance to the same antibiotics, highlighting much higher levels of *MR CoNS* resistance to chloramphenicol and tobramycin ($p < 0.00001$) in the RAGS study.

Tobramycin resistance of *Pseudomonas aeruginosa* was statistically significantly higher in the RAGS study compared to resistance reported in the ARMOR study (Table 6).

Table 5. Resistance of methicillin-resistant coagulase-negative staphylococci to various antibiotics in RAGS and ARMOR studies

| *MR CoNS* | Susceptibility | Ciprofloxacin | Tobramicin | Clindamicin | Cloramfenicol |
|---|---|---|---|---|---|
| RAGS | R | 54.8% | 68.3% | 32.1% | 36.4% |
| ARMOR | R | 54.6% | 14.4% | 31.4% | 1.2% |
| *p*-value | | | < 0.00001 | | < 0.00001 |

Table 6. Pseudomonas aeruginosa resistance to various antibiotics in RAGS and ARMOR studies

| ***Pseudomonas aeruginosa*** | Susceptibility | Ciprofloxacin | Tobramicin |
|---|---|---|---|
| RAGS | R | 4.7% | 39.5% |
| ARMOR | R | 5.1% | 3.1% |
| *p*-value | | | < 0.00001 |

## CONCLUSIONS

*Staphylococcus aureus (MRSA / MSSA)* resistance to clindamycin, chloramphenicol and ciprofloxacin obtained in the RAGS study is higher than in the ARMOR study. MSSA was susceptible to tobranicyn, but not *MRSA.*

The resistance of *coagulase-negative staphylococci (MR CoNS / MS CoNS)* to tobramycin obtained in our study is higher than that recorded in the ARMOR study, the same being true for chloramphenicol as well.

The resistance of *Pseudomonas aeruginosa* to tobramycin obtained in the RAGS study is also higher than that recorded in the ARMOR study.

These results raise issues related to the therapeutic arsenal actually available for the treatment of superficial and deep eye infections because these two antibiotics (tobramicyn and chloramfenicol) are the most prescribed eye drops, but it also seems to have developed. the highest resistance.

The study has, of course, some limitations related to antibiotic testing of samples obtained from conjunctival secretions, which are not tested in a standardized way on the same set of antibiotics, but most probably with those available in each moment.

However, the results of the study are strong enough regarding increased antibiotic resistance in the context of the overuse of antibiotics in ophthalmic practice.

*Acknowledgment*

All authors have contributed equally and would like to thank their colleagues for the considerable work and support.

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