



## BACTERIAL ETIOLOGY OF ACUTE CONJUNCTIVITIS

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**Abstract.** Acute conjunctivitis is the most frequent ophthalmological affection. The purpose of our study is to determine both the frequency and the drug-resistance of the bacteria involved in acute conjunctivitis' etiology. **Material and methods.** We have isolated 85 bacterial strains coming from conjunctival prelevates, between 2006-2008 in the laboratory of Emergency Clinical Hospital of Craiova. The strains have been isolated and identified using modern bacterial methods. As for the drug-resistance, we used the standardized diffusimetric method, using CLSI/NCCLS standards. **Results.** The most frequent-isolated species were: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, *Peptostreptococcus*. Drug sensitivity was different for each bacterial species. **Conclusions.** Our study proves that in infections' etiology, the most important were Gram-positive bacteria, Gram-negative bacteria coming second. Drug sensitivity proved to be increased, because most strains were wild; the ones with intrahospital origin presented a higher level of drug resistance.

**Keywords:** acute conjunctivitis, drug-resistance

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### Introduction

Acute conjunctivitis is one of the most frequent ophthalmological affections, having a varied etiology [1,3].

Acute conjunctivitis represents an inflammatory affection of the eye conjunctiva, being contagious in most of the cases and sometimes epidemic. Usually the nature of its cause is exogenous: infections (bacteria, viruses, parasites), allergic, traumatic, toxic [3,5].

Conjunctival inflammation may evolve with discreet or intense subjective symptoms, such as irritated eye, foreign-body sensation and burning. The objective symptom is represented by conjunctival hyperemia, with variable intensity, from palpebral conjunctiva congestion, with conjunctival blood-

vessels' dilation, up to the hyperemia of the entire conjunctiva, respecting the perikeratic territory.

Depending on conjunctival secretion, acute conjunctivitis may be: catarrhal, suppurative, pseudomembranous and follicular [3,6].

### Premises and objectives

Starting from the fact that acute conjunctivitis is one of the most frequent eye affections, its cure depending on adequate etiologic treatment, we have proposed to determine isolated prelevates' bacterial frequency and etiology and also isolated strains' sensitivity spectrum.

### Material and methods

From 380 conjunctival prelevates, 85 bacterial strains have been isolated and their sensitivity spectrum was determined. The prelevates were taken from adults, aged between 16 and 55 years.

The methods used in bacteriological diagnosis

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were complex. In the laboratory, the direct exam from conjunctival secretion was done, Gram and Giemsa stains. Afterwards the prelevates were isolated on complex mediums: blood agar in both aerobiosis and anaerobiosis, CO<sub>2</sub> atmosphere or Chapman medium. For *Haemophilus influenzae*, the chocolate agar medium was used.

The identification was done using conventional methods (standardized identification system API bioMerieux). *Chlamydia trachomatis*' identification was done using a quick chromatographic immune method, which determines the chlamydian antigen from the conjunctival secretion in the presence of specific antibodies (*Chlamydia Rapid Test* and Giemsa staining for inclusions' determination).

For drug-resistance testing, standardized diffusimetric A.W.BAUER method was used, approved by CLSI, ex NCCLS USA [2,4]. The medium used for drug resistance testing of the aerobic non-pretentious bacteria was Mueller-Hinton. For *Haemophilus influenzae*, agar HTM (*Haemophilus Test Medium*) was used; for *Streptococcus pyogenes* and *Streptococcus pneumoniae*, Mueller-Hinton agar sheep-blood supplemented medium was used.

For quality control the following reference strains were used: *Staphylococcus aureus* ATCC 25923 for non-pretentious Gram positive bacteria testing and negative control at beta-lactamases testing; *Staphylococcus aureus* 29213 as positive control at beta-lactamases testing; *Escherichia coli* ATCC 25922; *Streptococcus pneumoniae* ATCC 49619; *Haemophilus influenzae* 49247.

The inhibition area interpretation was considered to be sensitive, intermediate and resistant, in the presence of standard number 0,5 National Committee for Clinical Laboratory Standards of McFarland scale with barium sulphate. In drug choosing, we have considered the fact that in acute conjunctivitis, as topical drugs only a few antibiotics are used, which must be used in any pharmacoresistance test according to ABX Guide Johns-Hopkins [4]. The chosen drug sets were different, depending on the bacterial genus and the nutritive necessities, meaning that in pretentious bacteria testing we used other antibiotics than the ones used in non-pretentious bacteria testing (CLSI/NCCLS) [1,2].

## Results

### Acute conjunctivitis' bacterial etiology

From the 380 conjunctival prelevates, only in 85 we determined bacterial strains, the other 295

having different etiologies, especially viral. (see figure 1).

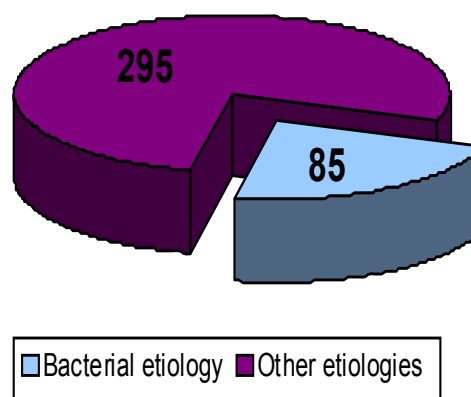


Figure 1. Acute conjunctivitis' etiology

From the 85 isolated strains, the most frequent were *Staphylococcus aureus* (48). The increased number is explained by the fact that *S.aureus*' locations are on the tegument and in the nose, places from where it can contaminate the conjunctiva (continuously exposed to nasal and tegument microorganisms contamination).

*Streptococcus pneumoniae* was isolated from 12 prelevates and *Haemophilus influenzae* from 10 prelevates. *Streptococcus pyogenes* was isolated from 10 prelevates. Figure II.

From the Gram-negative gut bacteria, *Escherichia coli* was isolated from 5 prelevates. Anaerobic bacteria were very poorly involved in acute conjunctivitis' etiology, only 3 strains of *Peptostreptococcus* being isolated. We have also isolated *Chlamydia trachomatis* from 2 prelevates. Decreased incidence of *C. trachomatis* infection in our patients is justified by the fact that the prelevates were taken from adults, the most frequent *C. trachomatis* infections appearing in newborns (genital transmission) [1,8,9]. (see figure 2).

We have not identified nor isolated any strain of *Neisseria gonorrhoeae* because we have not had any prelevates from newborns, where it is frequent [10,11].

### Isolated strains' sensitivity

#### 1. *Staphylococcus aureus* strains' sensitivity

The most frequent ocular infections were determined by *S. aureus*, as other authors also emphasized [3,6,7].

Most of the *S. aureus* isolated strains were methicillin-sensitive (40-83.3%) and only 8 (16.7%) were methicillin-resistant, proving that these strains were wild and not nosocomial. The strains which

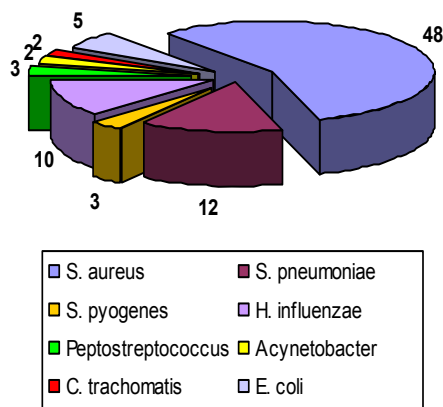


Figure 2. Isolated bacterial strains

were resistant to cefoxitin 30 mcg were reported as methicillin-resistant. Cephalothin was used to represent cephalothin, cefaprim, cefradine, cephalixin, cefaclor and cefadroxil.

All of the methicillin-sensitive *S. aureus* (MSSA) strains were sensitive at all the beta-lactam antibiotics used.

MSSA strains' aminoglycoside sensitivity was increased, 38 (95%) strains were sensitive to gentamicin, kanamycin and tobramycin. Only 2 (5%) of the strains were resistant to aminoglycosides. The gentamicin, kanamycin and tobramycin – aminoglycoside resistance was enzymatic, meaning that

it extends to other aminoglycosides like amikacin and netilmicin, especially altering their bactericide activity and less their bacteriostatic activity, fact that leads to loss of their synergic activity with betalactamines.

MSSA strains' quinolone sensitivity was increased: 36 (90%) were sensitive to norfloxacin, 30 (75%) were sensitive to ofloxacin, 38 (95%) were sensitive to ciprofloxacin and 40 (100%) were sensitive to levofloxacin.

MSSA strains' macrolide sensitivity was tested with erythromycin, this leading to sensitivity to other macrolides (clarithromycin, azithromycin) – 25 strains (62.5%) were sensitive to macrolides.

To tetracyclines, 26 MSSA strains were sensitive (65%).

To linezolid, all MSSA strains were sensitive.

Methicillin-resistant *S. aureus* (MRSA) strains presented an increased resistance not only to betalactamines, but also to aminoglycosides (only one strain was sensitive to aminoglycosides, the other 7 being resistant). The MRSA strains keep their sensitivity to quinolones, except for one strain which was resistant to ofloxacin and intermediary sensitive to norfloxacin and ciprofloxacin. (see table I).

#### 2. *Streptococcus pneumoniae* strains' sensitivity

For penicillin testing, oxacillin 1µg was used, a drug that is more adequate in penicillin-resistance

Antibiotics			MSSA 40 strains	MRSA 8 strains	<i>S. pyogenes</i> 2 strains	<i>S.pneumoniae</i> 12 strains	Peptostr. 3 strains
Classes	Types	Concentration	Sensitive strains	Sensitive strains	Sensitive strains	Sensitive strains	Sensitive strains
Betalactamines	Amoxicillin clavulanic acid	30 mcg	40	1	2	10	3
	Cefoxitin	30 mcg	40	0	2	10	3
	Cefalotin	30 mcg	39	0	2	10	3
	Norfloxacin	10 mcg	36	7	2	8	3
Quinolones	Ofloxacin	5 mcg	30	7	1	8	3
	Ciprofloxacin	5 mcg	29	7	2	9	3
	Levofloxacin	5 mcg	40	8	2	11	3
Aminoglycosides	Gentamicin	10 mcg	38	1	0	9	2
	Tobramycin	10 mcg	38	1	0	9	2
Others	Tetracycline	30 mcg	26	2	2	4	1
	Erythromycin	15 mcg	25	0	1	6	1
	Linezolid	30 mcg	40	5	2	6	1
	Trimethoprim					10	

Table I. Isolated Gram positive bacterial strains' sensitivity

Antibiotics			Haemophilus influenzae	Escherichia coli	Acinetobacter		
Classes	Types	Concentration	Strains	Sensitive strains	Strains	Sensitive strains	
Betalactamines	Ceftriaxone	30 mcg		1		4	0
	Amoxicillin clavulanic acid	30 mcg		1		4	0
	Cefalotin	30 mcg		1		4	0
Quinolones	Norfloxacin	10 mcg		9		4	2
	Ofloxacin	5 mcg		9		5	2
	Ciprofloxacin	5 mcg	10	9	5	4	2
Aminoglycosides	Levofloxacin	5 mcg		10		5	2
	Tobramycin	10 mcg		1		4	2
Others	Gentamicin	10 mcg		1		4	2
	Trimethoprim	10 mcg		1		4	0
	Chloramphenicol	30 mcg		1		4	0

**Table II.** Isolated Gram negative bacterial strains' sensitivity

testing. From the 12 isolated strains, 10 (83.3%) were sensitive to betalactamines and the same strains were sensitive to trimethoprim-sulfamethoxazole.

*S. pneumoniae* strains' sensitivity to aminoglycosides and quinolones proved to be increased, while to tetracyclines, these strains' sensitivity was reduced. (see table I).

#### 3. *Haemophilus influenzae* strains' sensitivity

90% of the *Haemophilus influenzae* isolated strains were sensitive to quinolones, while to betalactamines only one was sensitive (10%), the other 9 being resistant.

The betalactamine resistance was induced by beta-lactamase secretion and was associated with tetracycline, kanamycin, trimethoprim-sulfamethoxazole resistance. (see table II).

#### 4. *Streptococcus pyogenes* strains' sensitivity

*S. pyogenes* isolated strains presented increased sensitivity to betalactamines (100%), while at aminoglycosides both strains were resistant. (see table I).

#### 5. *Escherichia coli* strains' sensitivity

From the 5 *E. coli* isolated strains, 4 (80%) were sensitive to betalactamines and also to other antibiotic classes. Only one strain was beta-lactamase-secretory with extended spectrum, its resistance was associated to aminoglycosides, tetracyclines and trimethoprim. Table II.

#### 6. *Acinetobacter* strains' sensitivity

Isolated *Acinetobacter* strains were resistant to betalactamines and trimethoprim, but sensitive to aminoglycosides and quinolones. (see table II).

#### 7. *Peptostreptococcus* strains' sensitivity

The isolated strains of *Peptostreptococcus*

were sensitive to betalactamines, quinolones and aminoglycosides and only one strain was sensitive to tetracycline, erythromycin and linezolid. (see table I).

Drug resistance for *Chlamydia trachomatis* could not be tested because these species of bacteria do not grow on mediums; adequate treatment is represented by tetracycline or erythromycin, with good results.

## Conclusions

The most frequent bacterial species involved in acute conjunctivitis' etiology are Gram positive bacteria, especially *Staphylococcus*.

This means that the bacteria coming from the oropharynx and/or the tegument can find appropriate conditions in the eye conjunctiva. As for the Gram negative bacteria, probably these microorganisms are vehiculated with foreign bodies that harm the eye only accidentally.

Isolated strains' antibiotic sensitivity proved to be increased, due to the fact that the prelevates came from ambulatory patients.

Hospitalized patients suffer different maneuvers, being this way exposed to nosocomial bacteria, which are much more resistant to antibiotics than the wild strains.

Gram negative bacteria were more resistant to betalactamines, but presented an increased sensitivity to quinolones and a moderate sensitivity to aminoglycosides. They presented an increased resistance to tetracyclines.

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