Original article

Bacteriological analysis in the management of conjunctivitis. Comparison of antibiotic resistance between 1982 and 2008

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Abstract

Objective: To demonstrate the need for bacteriological analysis for the rational treatment of infections of the corneal surface, including conjunctivitis.

Methods: The results of 1970 consecutive bacteriological analyses obtained from 2001 to 2008 in patients with ocular hyperemia and discharge were analyzed and compared with a similar study done in 1982 in the same geographical area.

Results: Bacterial growth was obtained in 1044 cases (53%). The most frequent bacteria were Staphylococci spp. (56.6%), followed by Streptococci spp. (21.4%), Haemophilus spp. (12.1%), and other Gram-negative bacteria (9.9%). No antibiotic was effective against all the bacteria isolated. The frequency of resistant bacteria against neomycin, tobramycin, erythromycin and gentamicin was significantly increased with respect to the study of 1982.

Conclusions: Bacteriological analysis of conjunctival specimens is necessary to ensure the choice of an effective antibiotic against bacteria of the ocular surface when the initial treatment fails.

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Análisis bacteriológicos en el tratamiento de las conjuntivitis.
Comparación de la resistencia a antibióticos entre 1982 y 2008

Resumen

Objetivo: Mostrar la necesidad de análisis bacteriológicos para un tratamiento racional de las infecciones de la superficie ocular, incluyendo las conjuntivitis.

Métodos: Los resultados de 1.970 análisis bacteriológicos consecutivos, obtenidos desde 2001 a 2008, en pacientes con hiperemia conjuntival y secreción fueron contados y comparados con un estudio similar realizado en 1982 en la misma área geográfica.

Resultados: Se obtuvo crecimiento bacteriano en 1.044 casos (53%). Las bacterias más frecuentes fueron Staphylococci spp. (56,6%), seguidos de Streptococci spp. (21,4%), Haemophilus spp. (12,1%) y otras bacterias gram-negativas (9,9%). Ningún antibiótico fue eficaz frente a todas las bacterias aisladas. La frecuencia de bacterias resistentes a la neomicina,

Palabras clave: Conjuntivitis Bacterias Antibioticos Resistencia a los antibióticos
tobramicina, eritromicina y gentamicina estaba significativamente aumentada con respecto al estudio de 1982.

Conclusiones: Los análisis bacteriológicos de las muestras obtenidas de la conjuntiva son necesarios para asegurar la elección de un antibiótico efectivo frente a las bacterias de la superficie ocular cuando fracasa el tratamiento inicial.

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**Introduction**

Treatments for conjunctivitis and prophylaxis against infections in ocular surgery or anterior segment traumatic injuries usually begin by choosing a broad spectrum antibiotic. This choice is based on previous studies that have proven the efficiency of the antibiotic with a variety of infectious agents. If bacteriological analysis is carried out, the choice tends to repeat the same initial antibiotic in the absence of a rational basis to choose a different one. This study aims at demonstrating that this approach diminishes the efficiency of antibacterial agents due to the increase of antibiotic-resistant bacteria. In addition, it aims at demonstrating that it is necessary to perform bacteriological analysis with antibiograms in order to choose the most efficient antibacterial agent when the initial treatment fails.

**Subjects, material and methods**

The results of 1970 consecutive bacteriological analyses of private practice patients (Dr. B. Carreras) from 2001 to 2008 were studied.

Sample taking was indicated in cases exhibiting conjunctival hyperemia and secretion and when the involvement of a bacterial infection was suspected or had to be discarded. The patients who were in some type of previous topical treatment interrupted it for at least 48 h before the sample was taken. Prior to the sample taking the conjunctiva was not cleansed or touched with any material and no drops were applied.

The samples were taken from the ocular fundus at the inferior tarsal conjunctiva with commercial devices manufactured specifically for that purpose (Culturette®, Venturi Transystem®). Said devices comprise a stem or plastic with a sterile surgical sponge at one tip, a stopper at the other tip and a tube with rich transport medium. All the elements were under sealed sterile conditions and were opened when the sample was taken. The stems with the sponge were held by the tip of the stopper so that the sponge only made contact with the tarsal conjunctiva prior to be introduced in a transport medium, avoiding contact with anything else except the upper part of the transport medium when being introduced in the tube.

The samples, together with a paper explicitly requesting the inclusion in the antibiogram of a list of available antibiotics in the form of eye drops or ophthalmic cream, were sent to various private clinical analysis laboratories although the majority of the results are from the same lab.

The following culture media were usually utilized: M. Cled and McConkey, cetrimide agar, blood agar, chloramphenicol gentamicin sabouraud agar, actidione with phenol red, incubated at 37 °C. A database was designed (OpenOffice.org Base v3.3.0) to record the results of the bacteriological analysis reports. The following fields were filled in: identification number (automatically assigned), name (text), sex (1 = male, 2 = female), year of birth (number), report month (number from 01 to 12), report year (number), culture (0 = no bacteria growth, 1 = bacteria growth), any type of Gram-negative Bacillus, any type of Gram-positive cocci, any type of staphylococci (Staphylococcus spp.), Staphylococcus aureus, Staphylococci spp. Coagulase-negative (Staphylococcus epidermidis, etc.), Streptococci spp., Neisseria, Escherichia coli, Klebsiella, Enterobacter, Pseudomonas, Hemophilus, Proteus, Serratia (all these with the same values as the culture field, i.e. 0 = no bacteria growth, 1 = bacteria growth), fusidic acid, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, neomycin, norfloxacin, ofloxacin, rifampicin, tetracycline, tobramycin, trimetoprim-sulphametoxazol, vancomycin, clindamycin, cefotaxime, cefazoline (all with the values 0 = not tested, 1 = resistant, 2 = intermediate, 3 = sensitive). Result tables were obtained through the corresponding practices. The resistance percentages were compared among the various bacteria groups and also with the study carried out in 1982 by Al-Hiraki, Carreras and Cantalejo. Statistical contingency tests were performed (Chi square test and Fisher test) to assess significance (Graph-Pad Prism v5.04).

**Results**

Out of the 1970 bacteriological analysis reports, 1386 were of females and 584 of males. The patient ages were comprised between a few months (0 years) and 102 years, with a mean age of 54.4 years, a typical deviation of 22.2 years, and typical error of 0.5 years.

Cultures were positive in 1044 cases (53%): 728 females (69.73% of positive cultures, 52.5% of females) and 316 males (30.27% of positive cultures, 54% of males). The mean age in a positive culture was of 57.8 years, typical deviation 21.5 years and typical error 0.7 years.

The most frequently isolated bacteria in ocular surface exudates were Staphylococci spp. (56.6%), followed by Streptococci spp. (21.4%), Hemophilus (12.1%) and other Gram-negative bacteria (9.9%). Staphylococci spp. Coagulase negative bacteria (Staphylococcus epidermidis, etc.) constituted 41.2% of all the isolated bacteria and Staphylococcus aureus accounted for 13.2%.

The antibiotic available in the form of eye drops or cream which exhibited the higher percentage of resistant bacteria, considering the higher group of isolated bacteria (Fig. 1), was neomycin with 76.7% of resistant bacteria, followed by fusidic acid with 55.2%; tobramycin with 48.7%;
erythromycin with 46.2%; trimetoprim-sulphamethoxazol with 29.4%; ofloxacin with 27.6%; tetracycline with 27.3%; gentamicin with 22%; chloramphenicol with 17.8%; norfloxacin with 17.7%; ciprofloxacin with 13.6% and rifampicin with 5.3% of resistant bacteria.

Excluding *Staphylococcus* spp., fusidic acid took the first place in the percentage of resistant bacteria with 87.6%, followed by neomycin with 76.1%; tobramycin with 43.1%; erythromycin with 35.3%; tetracycline with 31.9%; trimetoprim-sulphamethoxazol with 29.4%; ofloxacin with 28.2%; gentamicin with 18.1%; chloramphenicol with 17.8%; norfloxacin with 15.4%; ciprofloxacin with 12.3% and rifampicin with 10.1%.

Considering only *Staphylococcus* spp. (Fig. 2), the antibiotic which exhibited the higher percentage of resistance was again neomycin with 77.4%, followed by tobramycin with 54.9%; erythromycin with 46.9%; trimetoprim-sulphamethoxazol with 29.3%; ofloxacin with 27%; gentamicin with 25%; tetracycline with 23.9%; norfloxacin with 20.2%; fusidic acid with 18.9%; chloramphenicol with 17.8%; ciprofloxacin with 14.7% and rifampicin with 1.7% of resistant *staphylococci*.

In what concerns *Staphylococcus aureus*, the frequency of resistance against neomycin reached 91.7%; tobramycin 67%; ofloxacin 46.3%; norfloxacin 39.5%; erythromycin 36.5%; trimetoprim-sulphamethoxazol 31.8%; gentamicin 29.9%; ciprofloxacin 25.5%; chloramphenicol 24.8%; fusidic acid 21.2%; tetracycline 19% and rifampicin 5%. Against coagulase negative *Staphylococcus* spp., neomycin continued to exhibit the highest frequency of resistance (74.56%) followed by erythromycin (51.7%); tobramycin (51%); trimetoprim-sulphamethoxazol (28.8%), tetracycline (25.9%), gentamicin (24.2%), ofloxacin (20.5%), fusidic acid (18.3%), chloramphenicol (15.7%), norfloxacin (14.8%), ciprofloxacin (11.7%), and rifampicin (1.7%).

Against *Streptococci* spp. (Fig. 3) the antibiotic exhibiting the highest percentage of resistance was fusidic acid (83.2%), followed by neomycin (72.6%), tobramycin (51.2%), erythromycin (41.7%), ofloxacin (29.7%), trimetoprim-sulphamethoxazol (27.6%), gentamicin (19.7%), chloramphenicol (18%), norfloxacin (17.5%), ciprofloxacin (16.1%) and rifampicin (1.4%).

Against Gram-negative bacteria and bacilli (Fig. 4), fusidic acid (93.1%) remained in the first position as regards frequency of resistance, followed by neomycin (80%), tetracycline (36.1%), tobramycin (32.1%), trimetoprim-sulphamethoxazol (27.3%), ofloxacin (25.9%), rifampicin (18.6%), chloramphenicol (18.2%), gentamicin (15.3%), norfloxacin (12%) and ciprofloxacin (6.9%).

When comparing antibiotics available in the form of eye drops and cream with the present study and the study carried out in Granada in 1982\(^1\) in the same geographical area (Fig. 5), the frequency of resistance of all isolated bacteria against the majority of antibiotics available in the form of eye drops and cream in both studies (neomycin, tobramycin, erythromycin, gentamicin) has increased with statistically significant differences (p < 0.0001 in all cases). However, against chloramphenicol and tetracyclines it has diminished with statistically significant differences (p < 0.0001 in both cases) and has not changed in practical terms for trimetoprim-sulphametoxazol (statistically not significant difference). Considering only
Staphylococci spp., there is a statistically significant increase of resistance to trimetoprim-sulphametoxazol (p = 0.0119), in addition to neomycin (p < 0.0001), tobramycin (p < 0.0001), erythromycin (p < 0.0001) and gentamicin (p < 0.0001), as well as a statistically significant reduction for chloramphenicol (p < 0.0001) and tetracyclines (p < 0.0001). Considering only Gram-negative bacteria, increased resistance against neomycin (p < 0.0001), tobramycin (p = 0.0158) and gentamicin (p = 0.0299) continues to be observed. The reduction of resistance against chloramphenicol and tetracyclines is not statistically significant, although a statistically significant reduction (p = 0.023) is observed in the resistance against trimetoprim-sulphametoxazol.

**Discussion**

The activity of antibiotics against the bacteria isolated in our study is an example of what could be occurring in the general population. In fact, other studies found in the literature refer to the increased resistance of bacteria to antibiotics even though the percentages thereof and the resistance to antibiotics vary from one study to another. This supports the general thesis of this paper about the necessity of carrying out ongoing bacteriological analyses to determine the sensitivity to antibiotics of bacteria in our environment.

The reason for presenting the results of bacteria sensitivity to antibiotics in various groups is to demonstrate the extent at which resistance is due to the characteristics of the antimicrobial spectrum of the antibiotic (as in the case of fusidic acid) or to a general increase in resistance.

The study data evidence the absence of antibacteria among those available in eye drops and creams that covers 100% of bacteria. In addition, we observed a high frequency of resistance to antibiotics which are broadly disseminated in the market. The high percentage of resistance to tobramycin is worthy of note, taking into account that it seems to be the initial antibiotic of choice for many physicians, both ophthalmologists and other specialists.

A comparison with the study made also in Granada in 1982 revealed unequal evolution of antibiotic resistance. In those days (prior to 1982) when cultures and antibiograms were not performed, chloramphenicol and tetracycline eye drops and creams were the antibacterial agents of first choice in the majority of cases. The appearance of other antibiotics in the market may have benefited the antibacterial activity of chloramphenicol and tetracyclines although the antibacterial efficacy of tobramycin, erythromycin and above all neomycin has been greatly reduced. Within the same group and also exhibiting increased resistance, the performance of gentamicin was not as poor as that of tobramycin and neomycin. The fact that it is frequent to administer antibiotics in association with preservatives and corticosteroids may have concealed in many cases their diminished efficiency. To the extent that the results of this study can be applied to the general population, the indication of tobramycin or neomycin as the drug of choice when ocular infections are suspected no longer has a rational basis.

As a result of this study, it could be decided that what should be done is to change the initial antibiotic of choice and use fluoroquinolone for treating infectious conjunctivitis instead of carrying out bacteriological analyses. However, in the long term this approach will lead to diminished efficiency of fluoroquinolone or the antibiotic of choice. Recently, Fintelmann et al. have demonstrated in Staphylococcus aureus cultures that the use of topical fluoroquinolones in a three-month period before the test was associated to a higher frequency of Staphylococcus aureus with resistance to these antibiotics.

Even though a bacteriological analysis in the first visit of a patient with conjunctivitis seems the most adequate approach for treating infectious conjunctivitis, it would be impractical in many cases due to lack of availability besides being too costly in general terms and unnecessary for many experienced
physicians. But if the clinic does not improve significantly after 4–5 days of treatment the initial diagnosis should be reconsidered, topical treatments suspended for at least 48 h and ocular surface samples taken for bacteriological analysis. In addition to improving our therapeutic efficacy, bacteriological analyses will enable an improved selection of initial antibiotic and rotate said choice in order to diminish the increase of resistant bacteria.

**Conflict of interests**

No conflict of interest has been declared by the authors.

**References**