Correlation between clinical data and antibiotic resistance in coagulase-negative Staphylococcus species isolated from 68 patients with acute post-cataract endophthalmitis

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Abstract

Coagulase-negative staphylococci (CNS) cause the majority of post-cataract endophthalmitis, which can lead to anatomical and/or functional loss of the eye. This study reports the antibiotic susceptibilities of CNS isolates associated with acute post-cataract endophthalmitis cases and correlates antibiotic resistance with severity and outcome of infection in these patients. Clinical data (initial ocular examination, final prognosis, antibiotic treatment) and the antibiotic susceptibilities of the isolated CNS strains were obtained from 68 patients with postsurgical endophthalmitis recruited during a 7-year period by the FRench Institutional ENDophthalmitis Study (FRIENDS) group. The CNS strains displayed 100% susceptibility to vancomycin, 70% to fluoroquinolones, 83% to fosfomycin, 46% to imipenem and 18% to piperacillin. The most effective antibiotic combinations were fosfomycin plus a fluoroquinolone and imipenem plus a fluoroquinolone, which were considered adequate in 80% and 58% of patients, respectively. Methicillin resistance was significantly associated with older age (p 0.001), diabetes mellitus (p 0.004), absence of fundus visibility (p 0.06), and poor visual prognosis (p 0.03). Resistance to fluoroquinolones was significantly associated with absence of fundus visibility (p 0.05) and diabetes mellitus (p 0.02). This large prospective study demonstrates that methicillin resistance and, to a lesser extent, fluoroquinolone resistance in CNS strains causing postoperative endophthalmitis are both prevalent in France and associated with a poorer visual prognosis. These results emphasize the need for an effective surveillance of this antibiotic resistance and the development of new diagnostic tools for rapid detection for early optimization of antibiotic therapy in endophthalmitis patients.

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Introduction

Post-cataract endophthalmitis is a rare, but serious complication, that can lead to anatomical and/or functional loss of the eye. Most of these infections are caused by bacterial species of the skin and by conjunctival and oral commensal flora, especially coagulase-negative Staphylococcus species [1,2]. Early
treatment includes systemic and intravitreal administration of antibiotics, and in more than half of the cases pars plana vitrectomy. Antibiotics are also frequently used for prophylactic purposes, before eye surgery or during surgery [1]. At the present time, the choice of prophylactic or therapeutic antimicrobial agents depends on the microbial epidemiology of post-cataract endophthalmitis, the antibiotic susceptibilities of the microorganisms involved, and finally the pharmacological and pharmacokinetic characteristics of these antibiotics.

Therefore, optimization of both prevention and treatment of endophthalmitis includes better characterization of the spectrum of microorganisms involved and their susceptibilities to antimicrobial agents. A useful approach would be the combination of culture methods and PCR-based techniques [2,3] so as to detect emerging pathogens and/or antimicrobial resistance, particularly resistance to antibiotics currently used in endophthalmitis strategies. The emergence of resistant microorganisms has been reported in endophthalmitis patients in the USA and India [4–7]. Coagulase-negative staphylococci (CNS) are involved in 33–70% of culture-positive post-cataract endophthalmitis cases [3,4,6,8]. We recently reported that 9% of patients with CNS endophthalmitis have a poor ocular prognosis (visual acuity <20/100) [9]. Severe CNS infections may occur because of specific virulence traits of the strains involved, such as higher adhesion or biofilm formation [10] and/or acquired antibiotic resistance [11], decreasing the effectiveness of local and systemic antibiotic therapies. New data are needed to characterize the prevalence of antibiotic resistance among the CNS population responsible for acute endophthalmitis, especially in Europe where recent data are lacking.

Microbiological and clinical data obtained for postsurgical endophthalmitis cases recruited during a 7-year period were previously published by the FrEnch Institutional ENDophthalmitis Study (FRIENDS) group [2,3,9,12]. Among them, 68 patients developed acute post-cataract endophthalmitis caused by CNS species. The main objective of the present study was to evaluate the in vitro susceptibilities of the CNS isolates obtained from intraocular samples collected in these patients. The secondary objectives were to assess whether the results with routine curative and preventive antibiotic treatments were adequate and to tentatively correlate antibiotic susceptibility data with the initial clinical presentation and final visual and anatomical outcome in these patients.

Materials and methods

Patients
This prospective study included 68 post-cataract surgery endophthalmitis cases (68 eyes) infected with a CNS species between March 2004 and December 2011 in three French university hospitals (Grenoble, Lyon, Saint-Etienne) belonging to the FRIENDS group.

The study followed the Declaration of Helsinki guidelines for research involving human participants and was approved by the local Institutional Review Board (IRB# 5921). The data sheet, sampling techniques and microbiological procedures, antibiotics used for intravitreal injections, and follow-up visits were standardized in the three centres [3]. The therapy strategy (mainly the requirement for and timing of a pars plana vitrectomy) was left to the discretion of each surgeon.

Acute endophthalmitis was diagnosed on the basis of clinical features, including decreased vision, periorcular pain, and anterior and posterior segment inflammation. A baseline evaluation form was completed during the initial examination of each patient and included demographic features, past medical history, ocular history, details of cataract surgery and data of the initial ocular examination. The final prognosis was defined at the 6-month visit.

On admission, an immediate tap of aqueous humor (AH) and/or vitreous was performed followed by intravitreal injection of vancomycin (1 mg) and ceftazidime (2.25 mg). Patients were also treated with a broad-spectrum intravenous antibiotic regimen (ciprofloxacin, ofloxacin, levofloxacin, or fosfomycin + piperacillin or imipenem) for 5 days and topical drugs (corticosteroids, tropicamide).

All eyes were sampled after two instillations of 5% aqueous povidone iodine solution in the conjunctival sac. AH (150–200 μL) and/or vitreous samples (200–300 μL) were collected just before the intravitreal injection of antibiotics. Undiluted vitreous samples (500 μL) were also collected during pars plana vitrectomy. Ocular specimens were separated into two samples at the time of sampling under aseptic conditions and one half was directly injected into brain–heart infusion broth (AES Laboratories, Combourg, France, from 2004 to 2007) or into a paediatric blood culture bottle (Becton Dickinson, Pont-de-ClaiX, France, from 2008 to 2011) and then rapidly sent to the microbiology laboratory for conventional cultures. The other half was stored in a DNA-free microcentrifuge tube for PCR and transferred to a single hospital centre.

CNS strains and antibiotic susceptibility testing
Strains of CNS were isolated from intraocular samples collected from endophthalmitis patients at the time of hospitalization. Culture-positive samples included aqueous humor (33.8%) and vitreous (66.2%) obtained by tap or vitrectomy. CNS strains were identified using conventional phenotypic methods and confirmed by PCR amplification and sequencing of a portion of the tuf gene, as previously described [13]. The CNS
species distribution was as follows: Staphylococcus epidermidis (56 strains, 82.4%), Staphylococcus lugdunensis (n = 5), S. warneri (n = 3), Staphylococcus haimin (n = 1), Staphylococcus haemolytics (n = 2) and Staphylococcus saprophyticus (n = 1).

The antibiotic susceptibilities of the CNS strains were evaluated using the Vitek II automated system in the Grenoble and Saint-Etienne hospitals (bioMérieux, Marcy l’Etoile, France) or the Phoenix system in the Lyon hospital (Becton Dickinson, Pont-de-Clair, France). The results were interpreted in all cases according to the French National Anti-riogram Committee Guidelines (CA-SFM 2010; http://www.sfm-microbiologie.org).

The antibiotics tested were penicillin G, oxacillin, kanamycin, tobramycin, gentamicin, erythromycin, clindamycin, pristinamycin, doxycycline, fusidic acid, cotrimoxazole, rifampicin, vancomycin, teicoplanin, linezolid and fluoroquinolones. The group of fluoroquinolones comprised ofloxacin or levofloxacin, depending on the automated system used. Susceptibility to cefuroxime and moxifloxacin were determined using the CA-SFM expertise rules.

**Adequacy of the systemic antibiotic therapies**

Because all patients received intraocular vancomycin, and no acquired resistance to this antibiotic was found in isolated CNS strains, we only evaluated the adequacy of the systemic antibiotic therapy. The systemic treatment was considered adequate if the bacterial strain was susceptible to the two antibiotics administered, and partially adequate if the strain was susceptible to one antibiotic.

**Statistical correlation between clinical and antibiotic susceptibility data**

Statistical analysis was performed using the SPPS 17.0 software (SPSS for Windows, Chicago, IL, USA). Comparisons between groups were done using the non-parametric Wilcoxon test. The relationships between qualitative data were studied using a chi-square test. Significance was accepted for a p-value <0.05.

**Results**

**Clinical data at baseline and at the 6-month follow up**

The population studied had a sex ratio of 0.79 (30 male, 38 female) and a mean age of 76.2 ± 11.4 years. Systemic risk factors for endophthalmitis were diabetes mellitus (18%) and immunosuppression (7%). The left eye was involved in 56% of the cases. There was no statistically significant seasonal predominance of eye infections, with 33% of cases occurring in spring, 12% in summer, 25% in autumn, and 30% in winter. The delay of ocular symptoms after cataract surgery was on average 4.3 ± 2.7 days. The main symptoms were pain (70% of cases) and decreased visual acuity (100%), which was categorized as follows: >20/400 in 12% of the patients, finger counting in 14%, hand motions in 33%, light perception in 40% and no light perception in 1%. Clinical signs at presentation were eyelid oedema (20%), corneal oedema (58%), hypopyon (88%) and absence of fundus visibility (83%). The mean intraocular pressure was 15.5 ± 7.4 mmHg.

None of the patients received an antibiotic prophylaxis at the time of cataract surgery. At the time of endophthalmitis diagnosis, all patients received a systemic antibiotic therapy consisting of one of the four following combinations: piperacillin plus a fluoroquinolone (levofloxacin, ofloxacin or ciprofloxacin) in 66% of the cases; piperacillin plus fosfomycin in 2%; fosfomycin plus a fluoroquinolone (levofloxacin, ciprofloxacin) in 12%; and imipenem plus a fluoroquinolone (levofloxacin) in 20%. All patients also had an intravitreous injection of antibiotics (vancomycin plus ceftazidime). A pars plana vitrectomy was performed in 49 of the 68 eyes (72%), within a mean 3.6 ± 2.2 days after presentation. Steroids were not injected at the time of the first intravitreous injection but were injected at the end of the pars plana vitrectomy in 36 out the 49 patients (73%).

**Antibiotic susceptibility of CNS strains**

Susceptibility rates >90% were found for vancomycin, pristinamycin, rifampicin and linezolid. Lower susceptibility rates (between 70% and 85%) were found in decreasing order for cotrimoxazole, clindamycin, gentamicin, doxycycline, fusidic acid, tobramycin, gentamicin, rifampicin and linezolid. Lower susceptibility rates (66%) were found for vancomycin, pristinamycin, rifampicin and linezolid. Lower susceptibility rates (between 70% and 85%) were found in decreasing order for cotrimoxazole, clindamycin, gentamicin, doxycycline, fusidic acid, tobramycin, gentamicin, rifampicin and linezolid.

Methicillin susceptibility (susceptibility to oxacillin) was found against CNS strains, with 70% susceptibility of CNS. Bacterial susceptibility to fosfomycin, imipenem and piperacillin treatments was 83%, 46% and 18%, respectively. When considering the different combinations of two antibiotics used for systemic antibiotic therapy (n = 68), treatment was considered adequate in 38% of the cases, partially adequate in 35% and inadequate in 26%. The most effective combination therapies were fosfomycin + fluoroquinolone (adequate in 80%) and imipenem + fluoroquinolone (58%).

**Correlation between resistance patterns and clinical data**

The final visual prognosis (at the 6-month visit) was defined as good (≥20/40) or poor (<20/40) in 64.5% and 35.5% of the 68 cases, respectively. Methicillin resistance was significantly
associated with age (81 ± 8 versus 72 ± 13 years, p 0.001), diabetes mellitus (meti-R 83% versus 36% in the non-diabetic population, p 0.004), absence of fundus visibility (meti-R 51% versus 23%, p 0.06) and visual prognosis (55% in the poor VA group versus 29%, p 0.03).

Fluoroquinolone resistance was significantly associated with absence of fundus visibility (22% versus 0%, p 0.05) and diabetes mellitus (36% versus 13%, p 0.02), whereas statistically non-significant differences were found for the association of this resistance and hypopyon (21% versus 0%, p 0.09) or visual prognosis (23% versus 8.3%, p 0.1).

**Discussion**

This is the first European study on the prevalence of antibiotic resistance in CNS strains infecting a large series of patients with acute post-cataract endophthalmitis. All previously published studies were conducted in the USA [4,6,7,14,15] or India [16]. Our case series was highly homogeneous for the microorganisms involved (CNS strains, including 82.4% S. epidermidis), the type of surgery (cataract), and the absence of cefuroxime prophylaxis. The predominance of S. epidermidis strains was expected because microorganisms causing postoperative endophthalmitis mainly belong to the patients’ commensal flora of the conjunctiva [7,17]. No acquired resistance to vancomycin was found, and this antibiotic remains the reference treatment for CNS infections. In contrast, we found a high prevalence of resistance to β-lactams and fluoroquinolones, which are the antibiotic classes most frequently used for prophylaxis and treatment of these nosocomial infections. Most particularly, methicillin-resistance involved 45% of the CNS strains, which is comparable to the 56% and 52% rates previously reported for endophthalmitis [8] and normal conjunctival samples [17–22], respectively (Tables 1 and 2). A recent retrospective study showed a continuous increase of methicillin resistance in endophthalmitis isolates of S. epidermidis from 31% to 55% during a 25-year period.

Fluoroquinolone resistance was found in 30% of CNS strains and 89% of the methicillin-resistant CNS strains. This is in accordance with previous reports highlighting that both types of resistance are often combined [23]. An increase in fluoroquinolone resistance rate in the normal conjunctival flora was reported between 2003 and 2011, and after repeated use of topical antibiotics [18,24–27]. For endophthalmitis patients, Benz et al. [7] reported a resistance rate of 54% for levofloxacin, but also noted a significant increase in the prevalence of this type of resistance between 2001 and 2006.

The present results show that the CNS strains involved were often resistant to the administered β-lactam and fluoroquinolone compound, with 80% resistance rates for penicillin G, 45% for oxacillin (methicillin resistance) and 30% for the fluoroquinolone compounds ofloxacin and levofloxacin. Therefore, for CNS-related endophthalmitis caused by methicillin-susceptible strains of CNS, piperacillin (which is no more effective than penicillin G against CNS) should be replaced with a β-lactam with a lower resistance rate and effective intraocular penetration such as imipenem. For methicillin-resistant strains, non-β-lactam antibiotics such as fosfomycin and fluoroquinolones should be considered. However, these two antibiotics should not be used alone because of the high risk of resistant mutant selection. Apart from in vitro
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**Results of sensitivity are expressed as % of sensitivity.**

CNS, coagulase-negative staphylococci; n, number of bacterial strains; S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis.
almost all the Ferrand, France). We found that 55% of the CNS strains in our intravenous administration of moxifloxacin in patients infected with a methicillin-resistant strain of S. epidermidis. As stated by Montandon et al. [31], the intravitreal injection of a fluoroquinolone antibiotic prophylaxis to prevent postoperative endophthalmitis. This antibiotic prophylaxis is generalized at this time because of its reported efficacy in a few studies, including a randomized controlled study [35] and the recent commercialization of a cefuroxime preparation that can be used for intracameral injection (Aprokam®; Thea, Clermont-Ferrand, France). We found that 55% of the CNS strains in our series were resistant to methicillin, which implies resistance to almost all the β-lactams currently available. One issue is therefore the true efficacy of intracameral injection of cefuroxime in patients infected with a methicillin-resistant strain of CNS. As stated by Montandon et al. [36] the median aqueous humor concentration of cefuroxime 30 s after intracameral injection was 2742 mg/L, decreasing to 756 mg/L 1 h later. Two questions remain to be solved: first, what are the cefuroxime concentrations in aqueous humor during the first 12 h following intracameral injection; and second, are these concentrations sufficient on methicillin-resistant CNS strains? We studied for the first time the relationship between clinical data and methicillin or fluoroquinolone resistance in CNS-related endophthalmitis. Clinical factors associated with methicillin resistance (diabetes, fundus visibility) in this study are well-known prognostic factors [9]. We previously showed that 9% of CNS-related endophthalmitis cases carried a poor visual prognosis, despite the usual low virulence associated with these bacteria [9]. One hypothesis that remains to be demonstrated could be a relationship between methicillin resistance and virulence. In our study, methicillin resistance has never been associated with resistance to vancomycin, suggesting that intravitreal injection of the latter antibiotic remains theoretically effective. Another cause of intravitreal antibiotic failure may be the poorly characterized pharmacokinetic and pharmacodynamic properties of vancomycin and ceftazidime in the eye tissues after intravitreal injection and the variability of antibiotic diffusion in the vitreous secondary to the organization of the inflammatory vitreous. For example, antibiotic exposure (vancomycin or gentamicin) for less than 2 h may not be adequate to eliminate all microorganisms involved in endophthalmitis. These data highlight that the efficacy of intravitreal antibiotics may be related to many factors, including the resistance pattern.

In conclusion, this large prospective study evaluating the antibiotic susceptibility of CNS strains involved in postoperative endophthalmitis cases demonstrates that methicillin resistance and to a lesser extent resistance to fluoroquinolones are of major concern in France. Given the regional specificities of antibiotic resistance, studies are needed in different countries to analyze the balance between current prophylactic and therapeutic strategies and the observed antibiotic resistance. The high rates of resistance to β-lactams and fluoroquinolones found in this CNS series emphasize the need for European microbial surveillance and new diagnostic tools, especially molecular methods [3] for rapid identification of antibiotic resistance in the microorganisms involved. This will allow early optimization of antibiotic therapy in endophthalmitis patients and possibly improvement of endophthalmitis patient prognosis [38–41].

TABLE 2. Review of the main studies of antibiotic sensitivity from bacteria involved in endophthalmitis

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<tr>
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<td>69%</td>
<td>72%</td>
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<tr>
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<td>67.0%</td>
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<td>84%</td>
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<td>Vancomycin</td>
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<tr>
<td>Linezolid</td>
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Abbreviations: CNS, coagulase-negative staphylococci; S. epidermidis, Staphylococcus epidermidis.
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Transparency declaration

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