

Eubacterial PCR for Bacterial Detection and Identification in 100 Acute Postcataract Surgery Endophthalmitis

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PURPOSE. To evaluate eubacterial PCR compared with conventional cultures for detection and identification of bacterial agents in ocular samples from patients with acute postcataract endophthalmitis.

METHODS. Broad-range eubacterial PCR amplification was used, followed by direct DNA sequencing in ocular samples (aqueous humor, vitreous samples from tap or vitrectomy) from 100 consecutive patients presenting with acute postcataract endophthalmitis. Bacterial cultures were performed on the same ocular samples by using traditional methods (brain-heart infusion broth).

RESULTS. At the time of admission, the detection rate was not significantly different between cultures and PCR (38.2% for cultures versus 34.6% for PCR in aqueous humor samples; 54% versus 57% in vitreous from a vitreous tap). In contrast, in the vitreous obtained from vitrectomy, after intravitreal injection of antibiotics, PCR detected bacteria in 70% of the cases, compared with 9% in cultures. By combining PCR and cultures, bacterial identification was obtained in 47% of aqueous humor samples at admission, in 68% of vitreous samples from a vitreous tap at admission, and in 72% of vitreous samples from pars plana vitrectomy. Gram-positive bacteria predominated (94.3%). The concordance between cultures and PCR was 100%. The contamination rate was 2%.

CONCLUSIONS. Cultures and eubacterial PCR are complementary techniques for bacterial identification in eyes with acute postcataract endophthalmitis. PCR technique was needed for identification of the involved microbial pathogen in 25% of all the cases. Eubacterial PCR is more effective than cultures in detecting bacteria in vitreous samples from patients with previous intravitreal administration of antibiotics. (*Invest Ophthalmol Vis Sci.* 2008;49:1971-1978) DOI:10.1167/iov.07-1377

Bacterial endophthalmitis, with an estimated incidence between 0.07% and 0.3%,^{1,2} is among the most severe complications of cataract surgery,^{1,3-6} one of the most prevalent surgical procedures in the aged population (2.5 million per year in Europe).⁷ Today microbiologic diagnosis of endophthalmitis remains routinely based on isolation of the involved microorganisms in culture, successful in approximately 22% to 30% of cases in aqueous humor (AH)^{1,3,4,8} and in 40% to 69% in vitreous.^{3,9-13}

In previous pilot studies of acute and delayed-onset postoperative endophthalmitis, we¹⁴ and others¹⁵⁻²⁰ obtained promising results by using amplification of eubacterial ribosomal DNA (16S rDNA) from ocular samples. However, there are no data on a large series of patients after cataract surgery, in a real-life environment (i.e., schedule sampling according to the treatment and patient progression). Therefore, the French Institutional Endophthalmitis Study (FRIENDS) group designed a prospective and multicenter study to evaluate further the contribution of direct amplification and sequencing of bacterial 16S rDNA from ocular samples collected in 100 consecutive patients with acute postcataract endophthalmitis, before treatment and after intravitreal injections of antibiotics.

METHODS

Patients

One hundred patients (100 eyes) with acute (<6 weeks after cataract surgery) endophthalmitis were consecutively included in this prospective study (March 2004–December 2005) in four French University Hospitals. The study adhered to the Declaration of Helsinki guidelines for research involving human subjects and was approved by the Institutional Review Board (CPP Lyon B). Informed consent was obtained from the subjects after explanation of the study. The therapy strategy (mainly the requirement and timing of pars plana vitrectomy [PPV]) was left to the discretion of each surgeon.

The diagnosis of acute endophthalmitis was made on the basis of clinical features, including decreased vision, periocular pain, and anterior and posterior segment inflammation (all patients had vitreous infiltration diagnosed by biomicroscopy or ophthalmic ultrasound).

On admission, an immediate tap of AH (in three centers) and/or vitreous (in one center) was performed followed by intravitreal

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injection of vancomycin (1 mg) and ceftazidime (2.25 mg). The patients were also treated with a broad-spectrum intravenous antibiotic regimen (ciprofloxacin and piperacillin or fosfomycin) for 5 days and topical drugs (corticosteroids, tropicamide). For ethical reasons, a second AH ($n = 53$) or vitreous sample ($n = 57$) was collected from the patients only if a second intravitreal injection and/or a PPV was needed (in cases of visual acuity less than counting fingers, retinal detachment, or anatomic and/or functional aggravation; Fig. 1). PPV was always performed after a minimum of one intravitreal injection of antibiotics.

All eyes were sampled after two instillations of 5% aqueous povidone iodine solution in the conjunctival sac (1 minute) and after one rinse with balanced-salt solution. AH (150–200 μL) and/or vitreous samples (200–300 μL) were collected just before the intravitreal injection of antibiotics in a sterile syringe. Fourteen patients had AH samples and a vitreous tap at admission. Undiluted vitreous samples (500 μL) were also collected during PPV. Ocular specimens were separated into two samples at the time of sampling under aseptic conditions and one half was directly injected in brain-heart infusion broth (BHI; 10 mL, pH 7.4 ± 0.2 ; ADM 88440; AES Laboratories, Combourg, France), then rapidly sent to the microbiology laboratory (delay <1 hour) for conventional cultures. The other half was stored in a DNA-free microcentrifuge tube for PCR and transferred at 4°C to a single hospital center (delay <48 hours). In some cases (Fig. 1), a second ocular sample (aqueous humor, vitreous tap, or PPV) and a third ocular sample (PPV) may have been obtained when intravitreal injection of antibiotics was needed or when a PPV was performed. These samples were analyzed with the same microbiologic techniques.

Control Patients

Two populations of control patients were included: (1) patients undergoing a planned ocular surgery without ocular inflammation ($n = 50$) and (2) patients with diagnosis of noninfectious uveitis (intraocular lymphoma, $n = 5$) or nonbacterial uveitis (toxoplasmosis, $n = 5$). The control samples (AH, $n = 35$ and vitreous, $n = 25$) were obtained with the same sterile conditions and analyzed with the same techniques as the infectious specimens. Microbiologic tests were performed by investigators blind to the origin of the sample.

Bacterial Cultures

BHI broths were subcultured on enriched media, including Columbia blood agar and chocolate agar supplemented with polyvitex (bioMérieux, Lyon, France), when bacterial growth was detected or systematically after 2 weeks of culture incubation if no growth was detected. For positive cultures, a Gram stain was performed, and bacterial identification and antibiotic susceptibility testing were performed according to the recommended methods.²¹

Eubacterial PCR Technique

Bacterial DNA extraction, 16S rDNA PCR amplification, and DNA sequence analysis were performed as previously described.^{22,23} Eubacterial PCR detected 500 colony-forming units (cfu) of *Staphylococcus epidermidis*.²³ Amplification of the human β -globulin gene served as an internal positive extraction and amplification control.²² The 16S rDNA sequences obtained were compared with those available in the GenBank, EMBL, and DDBJ databases with the program BIBI (Bio Informatic Bacterial Identification; <http://pbil.univ-lyon1.fr/bibi/query.php>). Identification to the species level was defined as a 16S rDNA sequence similarity of 99% or greater with that of the GenBank prototype strain sequence. Identification to the genus level was defined as a 16S rDNA sequence similarity of 97% or greater with that of the GenBank prototype strain sequence. A failure to identify was defined as a 16S rDNA sequence similarity less than 97% with sequences deposited in GenBank at the time of the analysis.²⁴

To differentiate genetically closely related species of *Streptococcus*, a portion of the superoxide dismutase gene (*sodA*) was amplified using SOD-UP and SOD-DOWN primers.²⁵ The amplified *sodA* sequence was then compared to that in the data bases.

Statistical Analysis

Statistical analysis was performed with commercial software (SPSS, ver. 12.0; SPSS, Chicago, IL). For the comparison of culture and PCR results for ocular samples, the nonparametric McNemar test was used. Mean comparisons were made with the nonparametric Mann-Whitney test. Significance was accepted at $P < 0.05$.

RESULTS

Forty-nine (49%) male and 51 female patients were included, with a mean age of 69.2 ± 14.7 (SD) years. The median delay of acute endophthalmitis after surgery was 5 days (range, 2–35).

Initial Ocular Samples before Antibiotic Injection

Aqueous Humor. AH samples were collected from 76 eyes just before the first intravitreal injection (Fig. 1a; Table 1). Overall, PCR was positive in 26 (34.6%) of 75 of the analyzed samples, and cultures were positive in 26 (38.2%) of 68, which led to a global bacteria identification in 36 (47.3%) of 76 cases. For the 67 eyes that benefited from both PCR and cultures, the distribution of positive and negative samples was not significantly different according to the microbiologic technique used ($P = 0.18$). Of the 41 samples negative with cultures, four had positive PCR (9.7%).

Vitreous. In 38 patients, vitreous samples were obtained by using a vitreous tap just before injection of antibiotics (Fig. 1B; Table 2). Overall, PCR was positive in 21 (56.7%) of 37 samples, cultures were positive in 19 (54%) of 35, which led to a global bacteria identification in 26 (68.4%) of 38 cases. Of the 34 eyes that had both PCR and cultures, the rate of positivity between the two techniques was not significantly different ($P = 0.99$). Of the 15 samples that were negative with cultures, 6 (40%) had positive PCR results.

AH Samples Obtained after Intravitreal and Systemic Administration of Antibiotics

In the subgroup of 76 patients who underwent an initial AH tap, a second AH sampling was performed in 53 eyes after one intravitreal injection of antibiotics (Fig. 1A, 2). At admission, 17 (32%) of 53 in this group had initially positive PCR and 18 (38.3%) of 47 had positive cultures. After one intravitreal injection, cultures were positive in 6 (13.6%) of 44 cases and PCR in 11 (23.4%) of 47. Paired comparisons at the time of the second sampling showed no significant difference between cultures and PCR ($P = 0.68$). When PCR and cultures were combined, the bacteria were identified in 14 (26.4%) of 53 cases.

The effect of one intravitreal injection of antibiotics was evaluated in these 53 patients. When comparisons were made individually (Fig. 2)—that is, for each ocular sample—the positivity rate of PCR decreased from 32% to 23.4% ($P = 0.001$), whereas the positivity rate of cultures decreased from 38.3% to 13.6% ($P = 0.036$). The second AH tap gave an additional microbiologic diagnosis in only 4 of the 53 (7.5%) patients, including three positive PCRs and one positive culture.

Vitreous Samples Obtained from PPV after Intravitreal Antibiotic Therapy

In the overall series, 57 patients underwent a PPV with a mean delay from diagnosis of 4.5 ± 4 days, after an average of 1.75 ± 0.5 intravitreal antibiotic injections (Table 3). The PCR and cultures of the vitreous samples were positive in 40 (70.1%) and 5 (8.8%) of the 57 cases ($P < 0.001$), respectively. When PCR and culture results were considered together, the infectious agent was identified in 41 (72%) of 57. In negative cultures, PCR was positive in 35 (73%) of 48 cases.

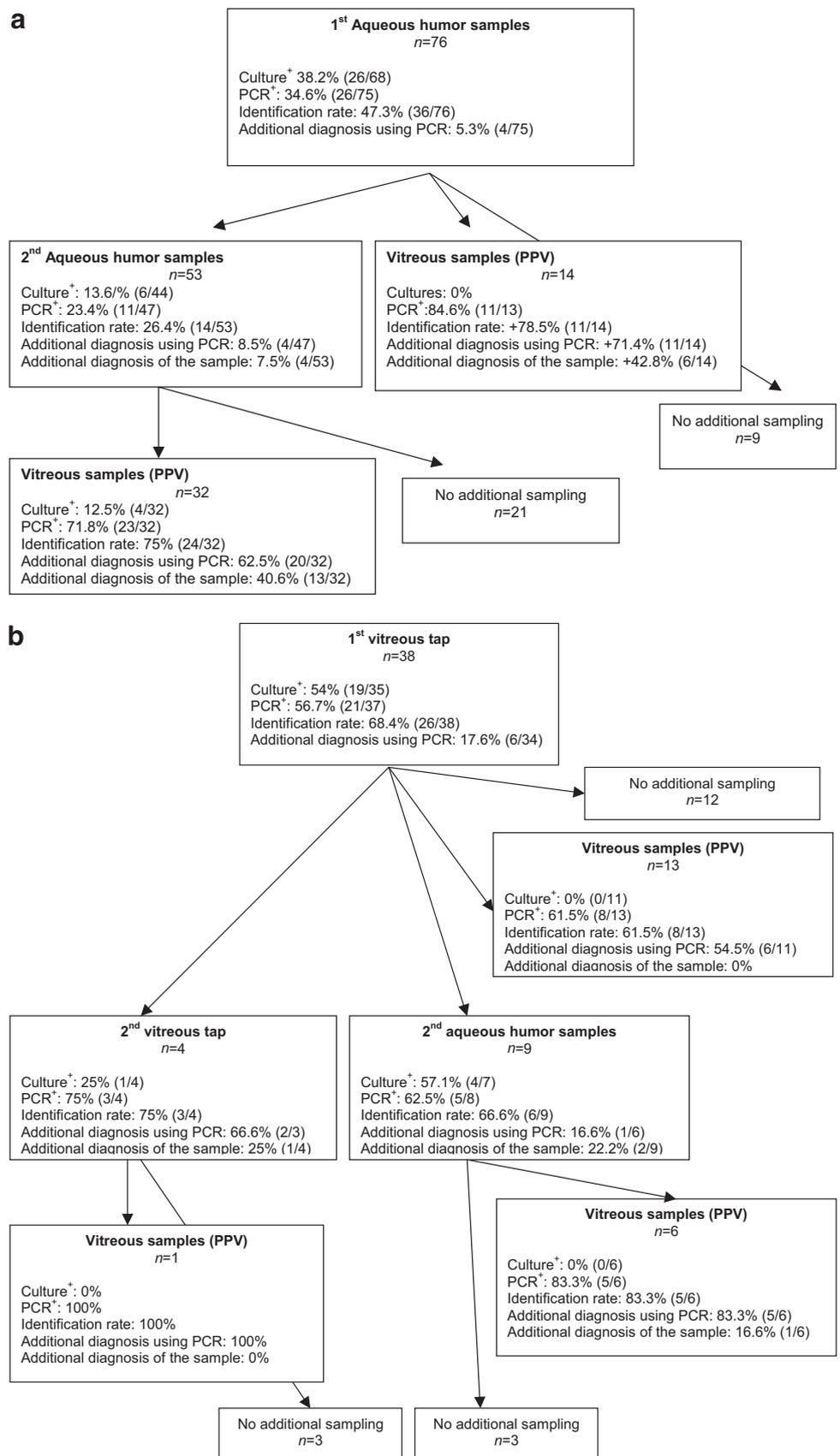


FIGURE 1. Analysis flow chart of aqueous humor samples (a) and vitreous tap (b). The calculated percentages of positivity are based on the number of positive samples/the number of samples analyzed using the microbiologic technique (and not necessarily on the total number of eyes, since only one technique was performed for a few eyes). The number of additional diagnoses using PCR is calculated as the number of cases diagnosed using only PCR/total number of samples tested using cultures + PCR. The number of additional diagnoses of the sample is the percentage of cases of the series non-diagnosed by the previous sample and diagnosed with the new sample. A patient may have several ocular samples (an average of 2.2)—that is, 22 patients with one ocular sample, 49 with two samples, and 33 with three samples. Additional ocular samples could have been performed when intravitreal injection of antibiotics was needed or when a PPV was performed. Fourteen patients had both AH sample and vitreous tap at admission (no. 119, 121, 122, 124, 125, 133, 134, 135, 136, 151, 152, 160, 163, and 166). This explains why 76 AH samples (a) and 38 vitreous taps (b) were performed at admission.

The sensitivities of cultures and PCRs of vitreous specimens collected after intravitreal injection of antibiotics were evaluated in a subgroup of 19 patients (Fig. 3) who had a vitreous tap at admission. Before treatment, 14 (73.7%) of 18 had

initially positive PCR and 10 (58.8%) of 17 had positive culture. After one (n = 13) or two (n = 6) intravitreal injections of antibiotics, PCR was positive in 13 (81.2%) of 16 eyes tested, and cultures were positive in only 1 case. The positivity rate of

TABLE 1. Results of PCR and Cultures of Aqueous Humor Samples from 76 Patients with Acute Postcataract Surgery Endophthalmitis, before Intravitreal Antibiotic Injection

AH 1 Samples	16S rDNA PCR ⁻	16S rDNA PCR ⁺	16S rDNA PCR Not Performed
Culture ⁻	<i>n</i> = 37 No final identification (32, 33, 38, 43, 45, 46, 49, 52, 59, 61, 62, 71, 109, 111, 112, 113, 117, 125, 126, 145, 153, 169) Bacteria identified in a different ocular sample*: <i>Staphylococcus epidermidis</i> (28, 120, 123) <i>S. epidermidis/capitis</i> (50) <i>S. epidermidis/caprae/capitis/saccharolyticus</i> (66, 166) <i>S. warneri/pasteuri/auricularis/piscifermentans/lugdunensis</i> (48) <i>S. aureus/sp. anaerobius/baemolyticus</i> (47) <i>Streptococcus mitis/oralis/sanguinis</i> (127) <i>Strep. mitis/pneumoniae/oralis/sanguinis</i> (74) <i>Strep. parasanguinis</i> (59) <i>Strep. oralis</i> (116) <i>Strep. mutans</i> (128) <i>Granulicatella elegans/adiacens/balaenopterae</i> (64, 124)	<i>n</i> = 4 <i>S. epidermidis</i> (177) <i>S. epidermidis/capitis</i> (114, 115, 149)	<i>n</i> = 1 Bacteria identified in a different ocular sample*: <i>S. epidermidis/caprae/capitis/saccharolyticus</i> (69)
Culture ⁺	<i>n</i> = 10 <i>S. epidermidis</i> (209, 54, 57) <i>S. lugdunensis</i> (130) <i>S. hominis</i> (35, 63) <i>S. aureus</i> (122, 141, 144) <i>Agrobacterium/Bradyrhizobium japonicum/Rhizobium galegae</i> (152)	<i>n</i> = 16 <i>S. epidermidis</i> (31, 37, 56, 73, 119, 135) <i>S. lugdunensis</i> (163) <i>S. aureus</i> (34) <i>Strep. sanguinis</i> (55) <i>Strep. cristatus/sinensis/oligofermentans</i> (160) <i>Strep. oralis</i> (108, 133) <i>Enterococcus faecalis</i> (68) <i>Strep. dysgalactiae</i> (121) <i>Proteus vulgaris</i> (134)	
Culture not performed	<i>n</i> = 2 No final identification (101) Bacteria identified in a different ocular sample*: <i>Abiotrophia defectiva</i> (53)	<i>n</i> = 6 <i>S. epidermidis</i> (75, 136, 140) <i>S. epidermidis/capitis</i> (51, 58) <i>S. aureus</i> (132)	

At the initial sampling, cultures were not performed in eight eyes and PCR was not performed in one additional eye, because the AH sample volume was insufficient to perform both techniques. Parentheses contain patient identification numbers.

* A different ocular sample was used after the first sample and the first intravitreal injection of antibiotics—that is, at the time of the second intravitreal injection of antibiotics or at the time of PPV.

PCR did not differ ($P = 0.3$) before and after injection of antibiotics, whereas the positivity rate of cultures decreased ($P = 0.07$). Most patients (9/10 cases, 90%) with a positive vitreous tap culture at admission had a negative PPV sample culture after antibiotic administration. The positivity of PCR on PPV vitreous samples did not differ significantly according to the number of intravitreal injections before PPV (1.5 ± 0.7 in the group with PCR⁻ versus 1.6 ± 0.6 in the group of PCR⁺; $P = 0.7$) or the delay until PPV was performed (4.4 ± 2.2 vs. 4 ± 1.8 days, $P = 0.5$).

Testing of the PPV vitreous samples resulted in an additional microbiologic diagnosis in 42.8% of the patients compared with microbiologic results of AH samples collected before treatment (Fig. 1A), but in none of the patients with vitreous tap analysis on admission (Fig. 1B).

PCR Amplification of Part of the *sodA* Gene

The *sodA* gene was amplified when eubacterial PCR allowed amplification of 16SrDNA belonging to the *Streptococcus* genus, but without specifying the species involved. The amplification of the *sodA* gene identified *Strep. oralis* in one of the eight cases studied (no. 133). In the other cases when *sodA* PCR or sequencing failed, cultures allowed isolation of *Strep. sanguinis* in two cases and *Strep. oralis* in two other cases.

Overall Results

Overall, when the results of AH and vitreous samples were pooled, the microbiologic diagnosis was obtained in 70% of the cases. Bacteria were Gram-positive in 94.3% of the cases (Table 4). PCR was necessary (i.e., positive PCR with negative cultures) for the microbiologic diagnosis on initial AH in 4 patients (4/41, 9.7%, and 6 additional patients with no cultures), on initial vitreous tap in 6 patients (6/15, 40%, and 1 additional patient with no culture), and on PPV vitreous in 35 patients (35/48, 72.9%, and 1 additional patient with no culture). If we considered all the ocular samples from each patient, PCR was necessary for microbiologic diagnosis in 25% of the patients, all of whose cultures were negative (PCR⁺, cultures⁻). Furthermore, if we considered only the results of PCR of all ocular samples, PCR was sufficient for microbiologic diagnosis in 61% patients (PCR⁺ with cultures⁻ or ⁺).

The correlation between culture and PCR results showed a high concordance (100%). However, in two cases, 16S rDNA sequencing allowed more reliable bacterial identification than did phenotypic methods applied to positive cultures. In case 160, *Strep. sanguinis* was identified from culture, whereas 16S rDNA analysis revealed the *Strep. oligofermentans/cristatus/sinensis* group. In the second case (no. 121), a β -hemolytic

TABLE 2. Results of PCR and Cultures of Vitreous Samples from 38 Patients with Acute Postcataract Surgery Endophthalmitis, before Intravitreal Antibiotic Injection

Vitreous Tap	16S rDNA PCR ⁻	16S rDNA PCR ⁺	16S rDNA PCR Not Performed
Culture ⁻	n = 9 No final identification (125, 161, 164, 174, 187, 189, 191, 197) Bacteria identified on a different ocular sample*: <i>Staphylococcus lugdunensis</i> (130)	n = 6 <i>S. epidermidis/caprae/capitis/saccharolyticus</i> (166, 186) <i>S. epidermidis/capitis/saccharolyticus</i> (171) <i>Granulicatella elegans/adiacens/balaenopterae</i> (124) <i>Strep. dysgalactiae</i> (121) <i>Haemophilus influenzae</i> (36)	n = 1 Bacteria identified in a different ocular sample*: <i>S. lugdunensis</i> (163)
Culture ⁺	n = 5 <i>S. epidermidis</i> (119, 190) <i>S. aureus</i> (122, 173) <i>Streptococcus pneumoniae</i> (182)	n = 14 <i>S. epidermidis</i> (135, 136, 198) <i>S. epidermidis, caprae, capitis, saccharolyticus</i> (172) <i>S. epidermidis, capitis</i> (175) <i>S. lugdunensis</i> (154, 180, 194) <i>S. sanguinis</i> (165) <i>Strep. cristatus/sinensis/oligofermentans</i> (160) <i>Gemella haemolysans</i> + <i>Strep. oralis</i> (162) <i>Strep. oralis</i> (133,† 151) <i>Rhizobium radiobacter</i> (168) <i>Agrobacterium/bradyrhizobium japonicum/Rhizobium galegae</i> (152)	
Culture not performed	n = 2 Bacteria identified on a different ocular sample*: <i>S. epidermidis</i> (134) <i>S. aureus</i> (132)	n = 1 <i>Strep. oralis/parasanguinis</i> (148)	

Because of a small amount of vitreous, PCR was not performed in one sample and cultures were not performed in three samples. Parentheses contain patient identification numbers.

* The different ocular sample was performed after the first sample—that is, at the time of the second intravitreal injection of antibiotics or at the time of PPV.

† 133 identified as a *Strep. oralis* by *sodA* PCR.

Streptococcus could not be identified at the species level with phenotypic methods, whereas *Strep. dysgalactiae* was identified using 16S rDNA sequencing. A coinfection was found in one patient (case 162) with identification of *Strep. oralis* together with *Gemella hemolysans*.

No control samples were positive by culture or PCR. The rate of contamination was estimated as 0% for the control specimens and 2% for samples from endophthalmitis patients. We considered as a possible contaminant an *S. epidermidis* strain detected by PCR in the second AH sample collected from patient 134 and a *Strep. gordonii* strain grown from the first AH sample collected in patient 64.

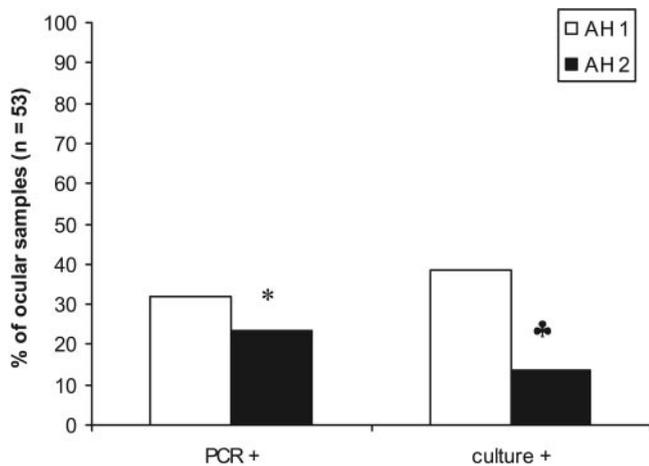


FIGURE 2. Positivity of PCR and cultures of AH before and after antibiotic treatment (n = 53). On the second AH sample, nine samples were not analyzed for cultures and six for PCR, because of the limited volume of sample. The positivity rate of PCR and cultures decreased significantly after one intravitreal injection of antibiotics. *P = 0.001. ♣P = 0.036.

DISCUSSION

Detection of microorganisms from postoperative endophthalmitis is of primary importance to allow confirmation of the infective nature of the observed postoperative inflammation, and to conduct a more rational and appropriate therapy. This prospective multicenter study evaluated eubacterial PCR in a large series (n = 100) postcataract acute endophthalmitis, and the results showed cultures and PCR to be complementary techniques providing a microbiologic diagnosis in 70% of the cases. This study also highlights the high superiority of eubacterial PCR over cultures for assay of PPV vitreous samples in patients with previous intraocular administration of antibiotics.

Although this study was not designed to compare AH and vitreous samples, the fact that one center systematically performed vitreous tap at admission, limiting the bias of the sampling technique, gave us the opportunity to compare both specimens. The results strongly suggest that initial vitreous tap was more effective than AH samples in detecting bacteria in cultures, as previously reported^{3,9-13} or by PCR. The low

TABLE 3. Results of PCR and Cultures of Vitreous Humor Samples Obtained during Pars Plana Vitrectomy from 57 Patients with Endophthalmitis

Vitreous Samples (PPV)	16S rDNA PCR ⁻	16S rDNA PCR ⁺	16S rDNA PCR Not Performed
Culture ⁻	<i>n</i> = 13 No final identification (49, 62, 112, 117, 125, 145, 164, 189) Bacteria identified on a different ocular sample†: <i>Staphylococcus epidermidis</i> (56) <i>S. epidermidis/capitis</i> (58) <i>S. lugdunensis</i> (154) <i>S. aureus</i> (141) <i>Streptococcus parasanguinis</i> (60)	<i>n</i> = 35 <i>S. epidermidis</i> (28, 57, 73, 75, 120, 135, 136, 140, 186) <i>S. epidermidis/caprae/capitis/saccharolyticus</i> (66, 69, 166) <i>S. epidermidis/capitis</i> (50, 51, 114) <i>S. lugdunensis</i> (130, 194) <i>S. warneri/pasteuri/auricularis/piscifermentans/lugdunensis</i> (48) <i>S. aureus</i> (122) <i>S. aureus/haemolyticus</i> (47) <i>Strep. sanguinis</i> (55, 165) <i>Strep. ristatus/sinensis/oligofermentans</i> (160) <i>Granulicatella elegans/adiacens/balaenopterae</i> (64, 124) <i>Strep. oralis/parasanguinis</i> (148) <i>Strep. oralis</i> (108, 116) <i>Strep. mitis/oralis/sanguinis</i> (74, 127)* <i>Strep. mutans</i> (128) <i>Agrobacterium/Bradyrhizobium japonicum/rhizobium galagae</i> (152) <i>Abiotrophia defectiva</i> (53) <i>Enterococcus faecalis</i> (68) <i>Proteus vulgaris</i> (134)	<i>n</i> = 3 No final identification (169) Bacteria identified in a different ocular sample†: <i>S. epidermidis</i> or <i>capitis</i> (175) <i>S. epidermidis/capitis/saccharolyticus</i> (171)
Culture ⁺	<i>n</i> = 1 <i>S. aureus</i> (144)	<i>n</i> = 4 <i>S. epidermidis</i> (123, 209) <i>S. aureus</i> (34) <i>Strep. oralis</i> (151)	
Culture not performed		<i>n</i> = 1 <i>Haemophilus influenzae</i> (36)	

Some of vitrectomized patients benefitted from AH sample and vitreous tap at admission and are listed in the PPV groups in Figure 1 and Figure 2. Parentheses contain patient identification numbers.

* 74, 127 were not identified as *Strep. pneumoniae* after PCR *soda*.

† The different ocular sample was performed before PPV—that is, at the time of the first and/or of the second intravitreal injection of antibiotics

sensitivity of cultures and PCR in AH samples was also supported by the fact that in some patients with negative AH cultures and PCR, detection of microorganisms was further obtained in vitreous samples obtained during PPV.

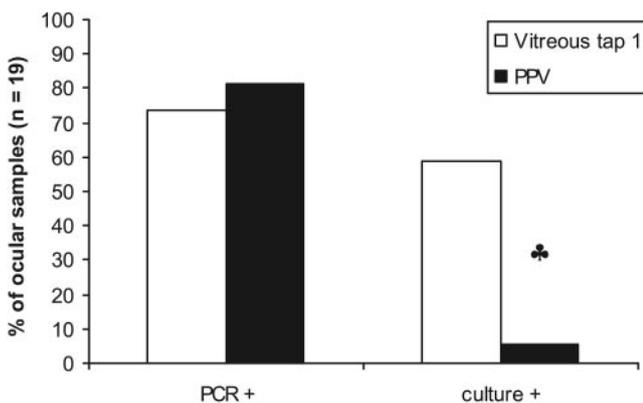


FIGURE 3. Positivity of PCR and cultures in vitreous samples before (vitreous tap) and after antibiotic treatment (vitreous from pars plana vitrectomy, PPV). After antibiotic treatment, three samples were not analyzable by PCR. The positivity rate of cultures decreased after injection of antibiotics, whereas the positivity rate of PCR did not differ. ♣*P* = 0.07.

Our data show that the complementarity of the two techniques was more evident in vitreous samples: Eubacterial PCR detected bacteria in 10% of culture-negative cases in the first AH sample, in 40% in untreated tap vitreous samples, and in 73% of treated PPV vitreous samples. Furthermore, when all ocular samples were analyzed, PCR was sufficient for diagnosis in 61% of the 100 patients. In PPV vitreous samples, previous administration of antibiotics may inhibit bacterial growth but PCR may still detect bacterial DNA of living or killed bacteria.

On the other hand, cultures may have been contributive in PCR⁻ cases (15% for the initial ocular samples), without specificity of the bacterial spectrum. The negativity of PCR tests in culture-positive samples may be related to a lack of sensitivity (amplification of DNA, small inoculum). It is also possible that the DNA from some bacterial strains was not efficiently amplified by a given set of primers.²⁶

Our results confirm the high superiority of PCR compared with culture of vitreous samples after intraocular antibiotic administration.¹⁴ In this context only can PCR replace cultures, the number of viable colonies being probably too low for an effective growth in culture medium. However, even if the rate of positive cultures was sharply decreased by treatment, a single injection of intravitreal antimicrobial agents may be insufficient to eradicate the bacteria,²⁷ since in our series 13%

TABLE 4. Identification of Bacteria Involved in 100 Eyes with Acute Endophthalmitis after Cataract Surgery

	Bacteria	Number	
Gram positive	Coagulase negative <i>Staphylococcus</i> (28, 31, 35, 37, 50, 51, 54, 56, 57, 58, 63, 66, 69, 73, 75, 114, 115, 119, 120, 123, 135, 136, 140, 149, 166, 171, 172, 175, 177, 186, 190, 198, 209)	34	
	<i>Staphylococcus aureus/haemolyticus</i> (29, 34, 47, 122, 132, 141, 144, 173)	8	
	<i>S. lugdunensis</i> (130, 154, 163, 180, 194)	5	
	<i>S. warneri pasteurii/auricularis/piscifermentans/lugdunensis</i> (48)	1	
	<i>Streptococcus mutans</i> (128)	1	
	<i>Strep. sanguinis</i> (55, 165)	2	
	<i>Strep. cristatus/sinensis/oligofermentans</i> (160)	1	
	<i>Strep. oralis</i> (108, 116, 133, 151, 162)	5	
	<i>Strep. oralis/parasanguinis</i> (148)	1	
	<i>Strep. parasanguinis</i> (60)	1	
	<i>Strep. mitis/oralis/sanguinis</i> (74, 127)	2	
	<i>Strep. pneumoniae</i> (182)	1	
	<i>Strep. dysgalactiae</i> (121)	1	
	<i>Enterococcus faecalis</i> (68)	1	
	<i>Abiotrophia defectiva</i> (53)	1	
	<i>Granulicatella elegans/adiacens/balaenopterae</i> (64, 124)	2	
	<i>Gemella haemolysans</i> (162)	1	
	Gram negative	<i>Proteus vulgaris</i> (134)	1
		<i>Haemophilus influenzae</i> (36)	1
<i>Agrobacterium/Bradyrhizobium japonicum/Rhizobium galegae</i> (152)		1	
<i>Rhizobium radiobacter</i> (168)		1	
Total		71*	

The group of coagulase-negative *Staphylococcus* (CNS) includes *S. epidermidis*, *S. capitis*, *S. hominis*, *S. saccharolyticus*, and *S. caprae*. Parentheses contain patient identification numbers.

* 71 bacteria were identified in cultures and/or PCR in 70 patients. One patient had a co-infection of two bacteria: *Gemella haemolysans* + *Strep. oralis* (162).

of AH samples and 8% of PPV samples were positive for cultures after injection of antibiotics.

This study made it possible to estimate the additional diagnosis allowed by analyzing several intraocular samples. In case of a negative first AH sample, a second AH sample seems useless. In the same way, a sample from PPV was not contributive when performed after an initial vitreous tap (Fig. 1B). In contrast, vitreous from PPV is of interest after AH sampling, since in approximately 40% of cases a bacterium may be detected (mainly by PCR). One should be aware that this analysis suffers from bias associated with the decision to perform PPV or administer a new injection of antibiotics (the sampling was done at this time), which may limit this conclusion.

There were some discrepancies between the positivity rate of PCR in our study and that reported in the literature. Three main factors may be responsible for the different results obtained by different laboratories: the PCR technique, the nature of the ocular sample, and the population studied. This study was focused on a homogeneous population of patients with acute postcataract endophthalmitis, whereas other studies differ in the number of patients (from 5 to 55), the bacterial spectrum, the delay of postoperative endophthalmitis,^{14,15,18,19,28,29} the type of surgery,¹⁴ and the inclusion of endogenous or posttraumatic endophthalmitis.^{19,29}

When eubacterial PCR allowed detection of *Streptococcus* sp. DNA, identification of bacteria at the species level was not always possible, since some *Streptococcus* species share greater than 99% homology in their 16S rDNA sequences.³⁰ For example, it may be difficult to differentiate the following species: *Strep. mitis*, *Strep. oralis*, *Strep. sanguinis*, and *Strep. pneumoniae*. In this case, amplification and sequencing of another gene (e.g., *sodA*) may allow differentiation of these species. In practice, *sodA* partial gene sequencing should be considered only in cases with negative cultures.

The concordance between eubacterial PCR and conventional cultures was considered 100%. However, the level of identification may be different between the two techniques. In some cases, the identification of subtypes was not possible by

PCR when a 16S rDNA sequence had less than 97% similarity with sequences deposited in GenBank.²⁴ For example, the *S. epidermidis/caprae/capitis/saccharolyticus*, *S. warneri/pasteurii/auricularis/piscifermentans/lugdunensis* or *Strep. mitis/oralis/sanguinis/pneumoniae* sequence may not allow an identification of the bacteria at the species level.

Use of a scrupulous PCR technique is necessary to avoid false-positive results, which includes a meticulous ocular disinfection to avoid contamination with conjunctival ocular flora during sampling. Contamination must also be avoided during the processing of ocular samples at each step of the procedure. In the control samples, the false-positive rate in this study was 0%, lower than that reported in other studies (nearly 5%).^{16,20} Contamination was not considered a limiting factor in this study.

This prospective study gives insight into the microbial flora responsible for acute endophthalmitis after cataract surgery: Gram-positive bacteria, especially coagulase negative *Staphylococcus* (CNS) species, remain the most common causative organisms in this context.^{3,31} Compared with a previous study reported in France (GEEP study)^{1,32} there seems to be a decline in Gram-negative bacteria (5.7% vs. 15%). Moreover, this study, including approximately 15% to 20% of the total number of cases per year in our country, gave a good estimation of the strains involved in acute postoperative endophthalmitis in France.

The limitations of this study are mainly related to the real-life sampling protocol (i.e., the lack of analysis using one technique because of the limited volume of ocular specimens in a few cases and the bias associated with the second ocular sampling: AH or PPV). Clearly, the rate of positivity of some samples after the initial intravitreal injection of antibiotics may depend on the cause of PPV—particularly the severity of the clinical presentation and probably the virulence of the bacteria involved. The limits of this PCR sequencing technique may stem from lower sensitivity when compared with PCR techniques targeting a specific microorganism, the inability to provide identification at the species level for some taxons, and the cost (US \$179). However, this study more accurately re-

flects the usefulness of both techniques if applied to routine practice in patients with acute postcataract endophthalmitis.

In conclusion, this prospective study on a large series of acute postcataract endophthalmitis showed that conventional cultures and eubacterial PCR are complementary. PCR was necessary for the microbiologic diagnosis in 25% of the cases and was sufficient in 61% of the cases. Finally, this study provides data for a modern ocular sample analysis strategy: PCR and cultures on vitreous samples at admission and PCR on pretreated vitreous.

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APPENDIX

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