Three cases of post-cataract surgery endophthalmitis due to

*Rhizobium (Agrobacterium) radiobacter.*

Viviane Moreau-Gaudry,¹ Christophe Chiquet,¹,² Sandrine Boisset,³,⁴ Jacques Croize,⁵
Yvonne Benito,³ Pierre Loïc Cornut,⁶ Alain Bron,⁷ François Vandenesch,³,⁴ Max
Maurin,²,⁵

For the French Institutional Endophthalmitis Study (FRIENDS) group

¹ Department of Ophthalmology, CHU Grenoble, Grenoble, F-38043, France
² UJF-Grenoble 1, Grenoble, F-38041, France
³ Centre National de référence des Staphylocoques, Laboratoire de Bactériologie, Centre de
Biologie et de Pathologie Est, Hospices Civils de Lyon, Bron, 69677, France
⁴ Université Lyon 1, Lyon, 69008, France
⁵ Department of Microbiology, CHU Grenoble, Grenoble, F-38043, France
⁶ Department of Ophthalmology, Hôpital Edouard Herriot, Université Claude Bernard Lyon
⁷ Department of Ophthalmology, CHU de Dijon, Dijon University, Dijon, France

Running title: *Rhizobium radiobacter* post-operative endophthalmitis.

Key words: *Rhizobium radiobacter*, endophthalmitis, eubacterial polymerase chain reaction,
cataract surgery
ABSTRACT

We present three unrelated post-cataract surgery endophthalmitis cases caused by *Rhizobium radiobacter*, hospitalized in three different hospitals. Early diagnosis was obtained in two cases by bacterial DNA detection in vitreous samples. All patients recovered from infection, but pars plana vitrectomy was needed in two patients due to rapid clinical deterioration.
CASE REPORTS

Patient 1. An 81-year-old female patient complained of decreased visual acuity (20/63) four weeks after cataract surgery (phacoemulsification) of the right eye (Table 1). Acute post-operative endophthalmitis with severe ocular inflammation was diagnosed. Vitreous (200µL) and aqueous humor (AH; 150-200µL) samples were tested by culture using Brain Heart Infusion broth (AES Laboratories, Combourg, France) and eubacterial PCR, as previously described (6). *Rhizobium radiobacter* was detected by culture in AH and vitreous, and by eubacterial PCR in the vitreous. Species identification was confirmed by PCR amplification and sequencing of the 16SrRNA gene (GenBank accession number: JQ304788) from the vitreous sample and from isolated colonies, whereas phenotypic identification of the isolated strain remained ambiguous. A 440 bp DNA fragment of the 16SrRNA gene was amplified with primer 91E and 13BS as described by Gauduchon *et al.* (11) Amplicon was sequenced the sequence obtained was compared in a phylogenetic analysis with those available in the BIBI Database (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi). Using an agar disk diffusion method (8), the strain was found intermediately susceptibility to third-generation cephalosporins (especially ceftazidim), but susceptible to cefepime, imipenem and fluoroquinolones using interpretative criteria of the CLSI for *Pseudomonas aeruginosa*, as previously suggested (15). The patient received two intravitreal injections of vancomycin (1 mg) and ceftazidime (2 mg), the day of admission and four days later, systemic antibiotics for 7 days (intravenous imipenem 500 mg three times a day and ciprofloxacin 500 mg orally twice a day), sub-conjunctival injections of dexamethasone and topical steroid therapy. One year later, the outcome was favorable.

Patient 2. A 75-year-old male patient presented with acute endophthalmitis of moderate severity (Table1, Figure 1), 9 days after an uncomplicated phacoemulsification. He was followed-up for obstructive sleep apnea and hypertension. Vitreous and AH were sampled and...
the patient received an intravitreal injection of vancomycin plus ceftazidim, a systemic antibiotic therapy (intravenous piperacillin 4 g three times a day and levofloxacin orally 500 mg once a day, 7 days) and topical steroid therapy. The following day, visual acuity was reduced to light perception, a 1 mm hypopion appeared and a pupillary membrane prevented fundus examination. Pars plana vitrectomy (PPV) was performed on day three of hospitalization, and intravitreal injections of antibiotics were repeated the same day and two days later. *R. radiobacter* was detected by culture in AH and vitreous samples collected on hospital admission, and by eubacterial PCR in the PPV sample. Species identification was obtained by 16SrRNA gene sequencing (GenBank accession number: JQ304789), whereas the phenotypic identification of isolated colonies (API 20NE system, BioMérieux, Marcy l’Etoile, France) remained ambiguous between *Sphingomonas paucimobilis* and *R. radiobacter*. The strain was susceptible to piperacillin, third-generation cephalosporins including ceftazidim, imipenem, and fluoroquinolones, but resistant to aminoglycosides. The 6-month follow-up examination revealed optic nerve atrophy and a poor visual acuity (20/125).

**Patient 3.** Sixteen days after an uneventful phacoemulsification, an 84-year-old female patient presented with a severe acute endophthalmitis of the left eye (Table 1). She had a type II diabetes mellitus and has received long-term steroid therapy. The patient received intravitreal injection of vancomycin plus ceftazidim, systemic antibiotics (intravenous piperacillin 4 g three times a day and levofloxacin orally 500 mg once a day) and topical steroid therapy. Because of clinical deterioration, a second intravitreal injection of antibiotics and PPV were performed on the 3rd and 4th days after admission, respectively. Two clinical samples were tested by culture and eubacterial PCR: AH collected at the time of admission, and vitreous collected at the time of PPV. Only culture of the PPV sample yielded *R. radiobacter*, as identified by a phenotypic method (Phoenix ® system, Becton Dickinson, Pont de Claix, France). Eubacterial PCR tests were negative. The strain was susceptible to
piperacillin, third-generation cephalosporins including ceftazidim, cefepime, imipenem and fluoroquinolones, but resistant to tobramycin. Clinical improvement was obtained after PPV, and visual acuity was 20/32 three months after admission of the patient.

With an incidence of 0.07-0.3%, postoperative bacterial endophthalmitis is one of the most feared complications of intraocular surgery as it may result in a severe decrease of visual acuity or even blindness of the involved eye (6). Gram-positive bacteria, especially coagulase negative staphylococci, remain the most common causative organisms whereas gram-negative bacteria account for only 5% of cases (6). We present three unrelated cases of postoperative endophthalmitis due to *Rhizobium radiobacter*, a gram-negative, catalase-positive, oxidase-positive, non fermenting bacterium predominantly found in soil (5). This species’ name has been proposed in 2001 by Young et al. (29) who reunified the genera *Rhizobium*, *Agrobacterium* and *Allorhizobium* into a single genus *Rhizobium*. *R. radiobacter* includes the former synonymic species *Agrobacterium radiobacter* and *Agrobacterium tumefaciens*, which could only be differentiated by the presence of tumorogenic (Ti) plasmids. *R. radiobacter* has been occasionally associated with opportunistic infections in humans, including bacteremia (especially catheter-related bacteremia) (2, 4, 10, 12, 16, 17, 22, 23), pneumonia (18), urinary tract infections (1), peritonitis (13, 27) and endocarditis (26). Most of these infections occurred in hospital settings (5, 15), in patients with immunosuppression (e.g., hematologic malignancies, solid-organ cancers, HIV infection or organ transplantation) (7, 9, 14, 18), and/or bearing a foreign material (e.g., central catheter) (4, 5, 12, 16, 22).

The present case series confirms that *R. radiobacter* is an opportunistic human pathogen occasionally responsible for acute postoperative endophthalmitis (19, 20, 25). The adherence of this bacterium to the intraocular lens during cataract surgery may promote development of
endophthalmitis, as has been reported for *Staphylococcus epidermidis* (21). In contrast, none of the endophthalmitis patients from our series and previous reports (19, 20, 25) had underlying malignancy or were immunocompromized. The onset of symptoms after cataract surgery varied from 4 days (19) to 4 weeks (our case series), and this may partly depend on the bacterial inoculum leading to infection. Most patients suffered from acute endophthalmitis with severe symptoms (19, 25), but a chronic endophthalmitis case was also reported (20). All patients recovered from *R. radiobacter* infection after intravitreal and systemic antibiotic therapy, but the final outcome varied from a total visual recovery to a near complete loss of visual acuity in the affected eye (19, 20, 25, present study). In our case series, a PPV was performed because of rapid clinical deterioration in two cases, and a poor visual outcome was observed in patient # 2 because of optic nerve atrophy.

The mode of contamination in patients with *R. radiobacter* infection remains largely hypothetic. Because *R. radiobacter* is a soil organism (5, 15), endophthalmitis may have resulted from patient’s contact with soil and subsequent conjunctival inoculation with this species either before or after intraocular surgery. Most patients with *R. radiobacter* endophthalmitis reported outdoor activities such as gardening or golfing that may have exposed them to soil bacteria (19, 20, 25). Because *R. radiobacter* is responsible for hospital-acquired infections (5, 15) nosocomial transmission of the pathogen (especially in the operating room) should be considered. However, Lai et al. (15) reported that among 13 patients suffering from *R. radiobacter* infection diagnosed between 1996 and 2002 in a single hospital, the pulsed-field gel electrophoresis profiles of the 13 isolated strains were all different, indicating the absence of nosocomial spread of the bacterium. Our three endophthalmitis cases were geographically and temporally unrelated, and were diagnosed and treated in three different hospitals (Grenoble, Dijon, Lyon) in France. In addition, no series of
endophthalmitis caused by *R. radiobacter* reported in the literature (19, 20, 25) corresponded to an epidemic situation.

Diagnosis of *R. radiobacter* infections primarily relies upon isolation of the pathogen from clinical samples (5). Although isolation of *R. radiobacter* isolates has been considered non-significant in some clinical situations (24, 28), its isolation from intraocular samples in a patient with clinical diagnosis of postoperative endophthalmitis should be considered as evidence of true infection (19). *R. radiobacter* can be isolated on blood-enriched media but also on McConkey agar after 2 to 3 days incubation of cultures. However, difficulties in phenotypic identification of this species may lead to delayed reporting of results to clinicians or even erroneous identifications (20). A rapid and accurate identification of *R. radiobacter* can now be obtained by amplification and sequencing of the 16S rRNA encoding gene (3). This technique can be applied to isolated colonies, but also directly to clinical samples such as vitreous and AH to circumvent the slow growth and fastidious nature of this bacterium. In the present case series, *R. radiobacter* could be isolated from AH and/or vitreous samples in all three patients, but species identification of isolated colonies was obtained by a phenotypic method in case #3 and by 16SrRNA gene sequencing in cases #1 and #2. Eubacterial PCR allowed early detection and identification of *R. radiobacter* in intraocular samples in these two later patients.

*R. radiobacter* strains have been reported to be always susceptible to fluoroquinolones, cefepime (a fourth-generation cephalosporin) and carbapenems (e.g., imipenem and meropenem) (15, 22). Acquired resistances are common for other beta-lactam compounds (especially third-generation cephalosporins such as cefotaxim and ceftazidim), aminoglycosides (gentamicin being most frequently active compound) and other antibiotic
families (15, 22). Our standard therapeutic protocol for endophthalmitis patients includes intraocular injection of vancomycin and ceftazidim. Despite the high concentrations reached in the vitreous, these drugs may not be adapted in case of *R. radiobacter* infection both because vancomycin is not active against this gram-negative bacterium and because of possible acquired resistance to ceftazidime. The intraocular injection of an aminoglycoside (e.g., amikacin) may be more effective. The same holds true for the systemic antibiotic therapy. The combination of a carbapenem and a fluoroquinolone is probably the best choice for strains with acquired resistance mechanisms or when antibiotic susceptibility of the involved strain cannot be assessed. This specific therapeutic strategy would be optimal in case of early detection and identification of *R. radiobacter*. The eubacterial PCR has two advantages over culture: the possibility of rapid detection of *R. radiobacter* directly from clinical samples and reliable species identification. Culture remains however essential for antibiotic susceptibility testing.

In conclusion, *R. radiobacter* is a rare etiological agent of postoperative endophthalmitis. The systematic use of eubacterial PCR for the etiological diagnosis of endophthalmitis allowed rapid detection and accurate identification of this bacterium in two of the three reported cases. Although most patients presented with severe ocular symptoms, the use of local and systemic antibiotics and pars plana vitrectomy in the more severe cases allowed cure of the infection. Patients undergoing cataract surgery should avoid contact with soil in the period surrounding this surgical intervention.
References


bacterial detection and identification in 100 acute postcataract surgery endophthalmitis.


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revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the
inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola*
and *R. vitis*. Int. J. Syst. Evol. Microbiol. 51:89-103
Table 1: Clinical features and microbial identification of endophthalmitis caused by *Agrobacterium radiobacter*.

<table>
<thead>
<tr>
<th></th>
<th>Patient # 1</th>
<th>Patient # 2</th>
<th>Patient # 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms onset after surgery (days)</td>
<td>24</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Visual acuity (VA) on admission</td>
<td>20/63</td>
<td>hand motion</td>
<td>hand motion</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- conjunctival hyperemia</td>
<td>moderate</td>
<td>important</td>
<td>important</td>
</tr>
<tr>
<td>- lid edema</td>
<td>none</td>
<td>present</td>
<td>none</td>
</tr>
<tr>
<td>- Tyndall</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>- Hypopion</td>
<td>1 mm</td>
<td>0.5 mm</td>
<td>1 mm</td>
</tr>
<tr>
<td>- pupillary membrane</td>
<td>none</td>
<td>mild</td>
<td>dense</td>
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<tr>
<td>- intraocular pressure (IOP)</td>
<td>20 mm Hg</td>
<td>9 mm Hg</td>
<td>10 mm Hg</td>
</tr>
<tr>
<td>Posterior chamber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- red reflex</td>
<td>absent</td>
<td>present</td>
<td>present</td>
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<tr>
<td>- visibility of the fundus</td>
<td>altered</td>
<td>good</td>
<td>altered</td>
</tr>
<tr>
<td>- vitritis</td>
<td>important</td>
<td>moderate</td>
<td>important</td>
</tr>
<tr>
<td>- retinal vasculitis</td>
<td>not visualized</td>
<td>yes</td>
<td>not visualized</td>
</tr>
<tr>
<td>Culture positive</td>
<td>AH, VIT</td>
<td>AH, VIT</td>
<td>PPV</td>
</tr>
<tr>
<td>Eubacterial PCR positive</td>
<td>VIT</td>
<td>VIT, PPV</td>
<td>none</td>
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<tr>
<td>Number of intravitreal injections of antibiotics</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Pars plana vitrectomy</td>
<td>no</td>
<td>the 3&lt;sup&gt;rd&lt;/sup&gt; day of hospitalization</td>
<td>the 4&lt;sup&gt;th&lt;/sup&gt; day of hospitalization</td>
</tr>
<tr>
<td>Outcome (visual acuity)</td>
<td>20/25 at 1 year</td>
<td>20/125 at 6 months</td>
<td>20/32 at 3 months</td>
</tr>
</tbody>
</table>
AH and VIT: aqueous humor and vitreous samples collected at the time of hospitalization, before administration of antibiotics.

PPV: vitreous sample collected at the time of pars plana vitrectomy.
Figure legend.

Figure 1. Ocular examination in case # 2.

A- Moderate inflammation of the anterior chamber (pupillary membrane and Tyndall 1+);
B- Moderate vitritis, retinal vasculitis and pre-retinal infiltration.