Case Report

Unusual acute endophthalmitis due to an as yet unclassified *Acinetobacter gyllenbergii*-like isolate

Emilie Pluquet,¹ Dominique Brémond-Gignac,² Solange Milazzo² and Hedi Mammeri¹

Correspondence

Hedi Mammeri
mammeri.hedi@chu-amiens.fr

¹Service de Bactériologie, Centre Hospitalo-Universitaire d'Amiens, Hôpital Nord, Faculté de Médecine d'Amiens, Université de Picardie Jules Verne, 80000 Amiens, France

²Service d'Ophtalmologie, Centre Hospitalo-Universitaire d'Amiens, Centre Saint-Victor, Faculté de Médecine d'Amiens, Université de Picardie Jules Verne, 80000 Amiens, France

Received 27 September 2010
Accepted 29 March 2011

We report a case of postoperative endophthalmitis due to an as yet unclassified *Acinetobacter gyllenbergii*-like isolate. The functional outcome was rapidly negative despite antibacterial therapy. This novel species, which cannot be identified by classical biochemical methods, may represent an underestimated opportunistic pathogen responsible for acute pyogenic infections.

Introduction

The genus *Acinetobacter* presents an important genetic diversity. Genotypic methods, which have been applied to investigate the phylogeny of the genus since 1986 (Dortet *et al.*, 2006; Juni, 2005), have described 23 recognized species (www.bacterio.cict.fr/a/acinetobacter.html) and 11 genomic species (Dijkshoorn *et al.*, 2007) at the time of writing. *Acinetobacter baumannii* has been frequently shown to be the most prevalent species among *Acinetobacter* strains. It has emerged as a major important worldwide nosocomial pathogen responsible for nosocomial infections and hospital outbreaks. In contrast, little is known about the epidemiology and clinical significance of other *Acinetobacter* species (Seifert *et al.*, 1997), which could be mainly attributable to the difficulty in obtaining a reliable identification. Indeed, most of the newly described *Acinetobacter* species cannot be routinely identified by automated identification systems, due to their absence from the databases of commercial biochemical kits. Moreover, *Acinetobacter* species are components of the commensal human skin and mucous membranes (Rosenthal & Tager, 1975; Taplin *et al.*, 1963). They are therefore common contaminants and there is difficulty in determining whether the isolation of *Acinetobacter* species indicates infection or merely contamination.

This study reports a case of acute endophthalmitis due to an *Acinetobacter gyllenbergii*-like isolate. Endophthalmitis is one of the most devastating diagnoses in ophthalmology. It is a serious intraocular inflammatory disorder affecting the vitreous cavity that can result from exogenous or

endogenous spread of infecting organisms into the eye (Kernt & Kampik, 2010). The incidence of postoperative endophthalmitis (POE) has increased in recent decades (0.5 %) (Hanscom, 2004; Kernt & Kampik, 2010). Prognosis is largely determined by the virulence of the offending organism. *Staphylococcus epidermidis* is the most common organism recovered from patients with culture-proven endophthalmitis (60 % of cases). *Staphylococcus aureus*, streptococci and enterococci are each found in 5–10 % of cases, and Gram-negative species are found in 6 % (Hanscom, 2004). A recent epidemiological study showed that Gram-positive bacteria, such as coagulase-negative staphylococci and streptococci, were also predominant in POE reported in France, whereas Gram-negative bacteria represented only 2 % of cases (Chiquet *et al.*, 2007).

Case report

A 77-year-old woman with no significant past medical history except treated hypertension presented with a 6-month history of major decreased vision with metamorphopsia of the right eye. Visual acuity was 3/10 P4. Ocular examination demonstrated bilateral cataract with epiretinal membrane in the right eye. Optical coherence tomography was performed to visualize the epiretinal membrane and a combined surgical procedure was decided upon. No antibiotherapy was administered prior to surgery as it was not recommended in France for patients without risk factors for developing postoperative infection (Bron & Creuzot-Garcher, 2007). After preoperative decontamination by povidone–iodine, phacoemulsification with a 21 D intraocular lens placement completed by epiretinal membrane dissection with an indocyanine green procedure was performed on the affected eye. Topical oxytetracycline and dexamethasone ophthalmic ointment (Thea) was applied at the end of the procedure. Neomycin and dexamethasone

Abbreviation: POE, postoperative endophthalmitis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the sequence of the variable regions of the *rpoB* gene of the *A. gyllenbergii*-like isolate are JF495491 and JF495490, respectively.

ophthalmic ointment (Thea) was applied once daily for two consecutive days. On postoperative day 1, the anterior segment was clear and the intraocular lens was correctly positioned. The macula presented classical moderate intraretinal haemorrhage as usually observed after epiretinal membrane surgery.

On day 3, the patient presented with pain in the eye that had been operated on. On examination, the patient's vision was only light perception. Slit lamp examination demonstrated conjunctival chemosis, hypopyon of 1/5 of the anterior chamber with severe Tyndall and flair phenomenon confirming endophthalmitis of the right eye. The macula was poorly visualized but B-scan ultrasonography was negative for retinal detachment. Immediately after examination, a specimen of aqueous humour was sent for microbiological testing. Intravitreal injection of vancomycin hydrochloride (1 mg in 0.1 ml) and ceftazidime (2.25 mg in 0.1 ml) was performed during the same operating time, followed by reinforced antibiotic eye drops containing vancomycin (50 mg ml⁻¹) and ceftazidime (50 mg ml⁻¹). Intravenous levofloxacin (500 mg per day) and piperacillin (12 g per day) were also administered. On day 5, a second intravitreal injection of vancomycin and ceftazidime was given.

Gram stain of the initial purulent aqueous humour aspirate revealed numerous leukocytes but no bacteria. After 2 days of aerobic incubation, β -haemolytic colonies grew on sheep-blood-supplemented (5%) Columbia agar. Gram staining revealed Gram-negative coccobacilli with a negative oxidase reaction. The API 20 NE identification system (bioMérieux) yielded good identification for the *Acinetobacter* genus but failed to identify the bacterial species (API 20 NE profile was 0010073).

A genetic identification approach, which consisted of amplifying and sequencing the 16S rRNA (*rrs*) gene of the bacterium, was attempted using the two sets of primers, P13B/UNI14 and PB/BAK2, described by Dortet *et al.* (2006) and Goldschmidt *et al.* (2009). These primers were specifically designed to amplify two DNA fragments covering the whole *rrs* gene of *Acinetobacter* species. However, a specific amplicon was obtained only with one pair of primers, P13B and UNI14. Two other universal primers, 68F (5'-TNANACATGCAAGTCGAKCG-3') and 1406R (5'-ACGGGCGGTGTGTRC-3'), previously reported by Gonzalez *et al.* (1997) and Heijs *et al.* (2007), respectively, were therefore used. These primers yielded a 1338 bp PCR product that was subsequently sequenced on both strands. BLAST analysis of the resulting sequence with known 16S rRNA gene sequences identified the isolate as *A. gyllenbergii* with a maximal identity of 99% with the *A. gyllenbergii* type strain, RUH 422^T (GenBank accession no. AJ293694). The comparative sequence analysis of the 16S rRNA genes showed two mismatches and two gaps. Comparative sequence analysis of the *rpoB* gene was used to confirm the species identity. Two sets of primers, Ac696F and Ac1093R, and Ac1055F and Ac1598R, were used to amplify

two variable regions of the *rpoB* gene, as previously described (Nemec *et al.*, 2009; La Scola *et al.*, 2006). PCR products were subsequently sequenced on both strands and BLAST analysis of the resulting sequences was performed using the BLASTN program and the National Center for Biotechnology Information server (Johnson *et al.*, 2008). The partial 366 bp and 510 bp *rpoB* gene sequences presented highest scores of sequence similarity with *A. gyllenbergii* NIPH 822 (96% sequence similarity; GenBank accession no. EU477158). Out of the hitherto known species, this isolate was most similar to *A. gyllenbergii*.

The resistance phenotype was determined using Etest strips (bioMérieux) on Mueller–Hinton agar plates (Bio-Rad) and interpreted according to the Clinical and Laboratory Standards criteria (CLSI, 2010). The *A. gyllenbergii*-like clinical isolate was resistant (MICs in μ g ml⁻¹) to amoxicillin (256), amoxicillin and clavulanic acid (128) and narrow-spectrum cephalosporins such as cefalotin (512), but it remained susceptible to ticarcillin (4), piperacillin (8), ceftazidime (2), cefepime (8), imipenem (0.5), levofloxacin (0.125), ciprofloxacin (0.06), gentamicin (0.5), tobramycin (1), netilmicin (1), amikacin (1) and trimethoprim–sulfamethoxazole (0.5).

The antibiotic treatment was modified according to the results of susceptibility testing. On day 7, the patient underwent an anterior chamber washing combined with a new intravitreal injection of ceftazidime (2.25 mg in 0.1 ml) and amikacin (0.4 mg in 0.1 ml). On day 8, laterobulbar injections of dexamethasone (2 mg) were given every 3 days for 9 days. An improvement of corneal oedema and hypopyon was observed on day 9 and the infection progressively resolved. The ocular inflammation subsequently resolved but the patient only recovered light perception.

Discussion

Acute-onset POE constitutes one of the most severe complications of ocular surgery. Coagulase-negative staphylococci are most commonly isolated, followed by *S. aureus* and streptococci, while Gram-negative bacilli are isolated in only 6% of cases (Hanscom, 2004). There are few published reports of postoperative *Acinetobacter* species endophthalmitis. Peyman *et al.* (1975) reported the first case of '*Acinetobacter anitratus*' (formerly *Herella* sp.) endophthalmitis after incomplete phacoemulsification. Cohen *et al.* (1992) reported growth of *Acinetobacter lwoffii* in intraocular fluid culture after routine pars plana vitrectomy. Gopal *et al.* (2000) described a case of POE caused by sequestered *Acinetobacter calcoaceticus* after cataract surgery with intraocular lens implantation and Chen *et al.* (2008) reported a case of postkeratoplasty endophthalmitis due to *A. baumannii*. It should be noted that the methods used in the former studies were not sufficient for a proper identification of *Acinetobacter* according to the current nomenclature and that the reported species identities are

doubtful. In the present study, a POE case due to an as yet unclassified haemolytic *Acinetobacter* strain is described.

The taxonomy of the genus *Acinetobacter* has undergone extensive revision since 1986 (Juni, 2005), and currently comprises 23 recognized species (www.bacterio.cict.fr/a/acinetobacter.html) and 11 genomic species that were delineated by DNA–DNA hybridization (Dijkshoorn *et al.*, 2007). The description of *A. gyllenbergii* based on characterization of nine strains recovered from blood, urine, sinus, vaginal, tracheal and wound samples was recently reported by Nemec *et al.* (2009). As for the other newly described *Acinetobacter* species, the clinical incidence of *A. gyllenbergii* may be underestimated due to the absence of a reliable biochemical identification (Nemec *et al.*, 2009), as *A. gyllenbergii* is not included in the database of many automated identification systems, such as API 20 NE and API 32 GN (bioMérieux) (http://www.biomerieux.fr/upload/Bases_de_Donnees.pdf). Only the genetic approach can provide precise identification.

The resistance pattern of the *A. gyllenbergii*-like isolate appears to be similar to those of other *Acinetobacter* species. It very likely produces an AmpC β -lactamase that inactivates aminopenicillins and narrow-spectrum cephalosporins (Jacoby, 2009), but remains susceptible to ceftazidime, cefepime, imipenem, aminoglycosides, cotrimoxazole and systemic fluoroquinolones such as levofloxacin and ciprofloxacin.

Postoperative bacterial endophthalmitis is due to penetration of micro-organisms into the anterior chamber during ocular surgeries and procedures (Hollander *et al.*, 2006; Kernt & Kampik, 2010). The surgical wound can serve as portal of entry for *Acinetobacter* species that are normal inhabitants of human skin (Seifert *et al.*, 1997). The absence of postoperative antibacterial prophylaxis may facilitate this colonization. Despite controversies regarding antibiotic prophylaxis, preoperative povidone-iodine was the first procedure with proven benefit (ESCRS Endophthalmitis Study Group, 2007; Hanscom, 2004; Soriano & Nishi, 2005). However, the significant reduction of acute-onset POE by intracameral cefuroxime at the end of surgery has led to a revision of the European Society of Cataract and Refractive Surgery (ESCRS) guidelines (ESCRS Endophthalmitis Study Group, 2007). Interestingly, the *A. gyllenbergii*-like clinical isolate described in this study presented a low level of resistance to cefuroxime with a MIC value ($32 \mu\text{g ml}^{-1}$) lower than the aqueous humour concentration of cefuroxime 1 h after drug instillation ($756 \mu\text{g ml}^{-1}$) (Montan *et al.*, 2002).

This case report highlights the possible implication of a strain related to the newly described *A. gyllenbergii* species in postoperative intraocular infections. These species may represent underestimated pathogens as they cannot be routinely identified by automated identification systems, due to their absence from the databases of commercial biochemical kits.

References

- Bron, A. & Creuzot-Garcher, C. (2007). [Prophylaxis for postcataract endophthalmitis]. *J Fr Ophthalmol* **30**, 1027–1036 (in French).
- Chen, K.-J., Hou, C.-H., Sun, M.-H., Lai, C.-C., Sun, C.-C. & Hsiao, C.-H. (2008). Endophthalmitis caused by *Acinetobacter baumannii*: report of two cases. *J Clin Microbiol* **46**, 1148–1150.
- Chiquet, C., Cornut, P.-L., Vinh, V., Maurin, M. & Romanet, J.-P. (2007). [Acute endophthalmitis: from bacteria to clinical settings]. *J Fr Ophthalmol* **30**, 1060–1066 (in French).
- CLSI (2010). *Performance Standards for Antimicrobial Susceptibility Testing*, 20th Informational Supplement. M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute.
- Cohen, S. M., Benner, J. D., Landers, M. B., III & Morse, L. S. (1992). Intraocular fluid cultures after primary *pars plana* vitrectomy. *Am J Ophthalmol* **114**, 697–699.
- Dijkshoorn, L., Nemec, A. & Seifert, H. (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* **5**, 939–951.
- Dortet, L., Legrand, P., Soussy, C.-J. & Cattoir, V. (2006). Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol* **44**, 4471–4478.
- ESCRS Endophthalmitis Study Group (2007). Prophylaxis of postoperative endophthalmitis following cataract surgery: results of the ESCRS multicenter study and identification of risk factors. *J Cataract Refract Surg* **33**, 978–988.
- Goldschmidt, P., Degorge, S., Benallaoua, D., Basli, E., Batellier, L., Boutboul, S., Allouch, C., Borderie, V., Laroche, L. & Chaumeil, C. (2009). New test for the diagnosis of bacterial endophthalmitis. *Br J Ophthalmol* **93**, 1089–1095.
- Gonzalez, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Sagittula stellata* gen. nov., sp. nov., a lignin-transforming bacterium from a coastal environment. *Int J Syst Bacteriol* **47**, 773–780.
- Gopal, L., Ramaswamy, A. A., Madhavan, H. N., Saswade, M. & Battu, R. R. (2000). Postoperative endophthalmitis caused by sequestered *Acinetobacter calcoaceticus*. *Am J Ophthalmol* **129**, 388–390.
- Hanscom, T. A. (2004). Postoperative endophthalmitis. *Clin Infect Dis* **38**, 542–546.
- Heijs, S. K., Haese, R. R., van der Wielen, P. W. J. J., Forney, L. J. & van Elsas, J. D. (2007). Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microb Ecol* **53**, 384–398.
- Hollander, D. A., Vagefi, M. R., Seiff, S. R. & Stewart, J. M. (2006). Bacterial endophthalmitis after resident-performed cataract surgery. *Am J Ophthalmol* **141**, 949–951.
- Jacoby, G. A. (2009). AmpC β -lactamases. *Clin Microbiol Rev* **22**, 161–182.
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezuk, Y., McGinnis, S. & Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic Acids Res* **36**, W5–W9.
- Juni, E. (2005). Genus *Acinetobacter*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, pp. 425–437. Edited by G. M. Garrity & other editors. New York: Springer.
- Kernt, M. & Kampik, A. (2010). Endophthalmitis: pathogenesis, clinical presentation, management, and perspectives. *Clin Ophthalmol* **4**, 121–135.

- La Scola, B., Gundi, V. A., Khamis, A. & Raoult, D. (2006).** Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* **44**, 827–832.
- Montan, P. G., Wejde, G., Setterquist, H., Rylander, M. & Zetterström, C. (2002).** Prophylactic intracameral cefuroxime. Evaluation of safety and kinetics in cataract surgery. *J Cataract Refract Surg* **28**, 982–987.
- Nemec, A., Musílek, M., Maixnerová, M., De Baere, T., van der Reijden, T. J. K., Vaneechoutte, M. & Dijkshoorn, L. (2009).** *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. *Int J Syst Evol Microbiol* **59**, 118–124.
- Peyman, G. A., Vastine, D. W. & Diamond, J. G. (1975).** Vitrectomy and intraocular gentamicin management of *Herellea* endophthalmitis after incomplete phacoemulsification. *Am J Ophthalmol* **80**, 764–765.
- Rosenthal, S. & Tager, I. B. (1975).** Prevalence of gram-negative rods in the normal pharyngeal flora. *Ann Intern Med* **83**, 355–357.
- Seifert, H., Dijkshoorn, L., Gerner-Smidt, P., Pelzer, N., Tjernberg, I. & Vaneechoutte, M. (1997).** Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. *J Clin Microbiol* **35**, 2819–2825.
- Soriano, E. S. & Nishi, M. (2005).** Endophthalmitis: incidence and prevention. *Curr Opin Ophthalmol* **16**, 65–70.
- Taplin, D., Rebell, G. & Zaias, N. (1963).** The human skin as a source of *Mima-Herellea* infections. *JAMA* **186**, 952–955.