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

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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...
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 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 (2.25 mg in 0.1 ml) and amikacin (0.4 mg in 0.1 ml). On day 8, laterobarbital 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.

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′-TNANACATGCAAAGTCGAKCG-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 422T (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 882 (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 μg 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, laterobarbital 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 cefazidime, cepimef, 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 μg ml⁻¹) lower than the aqueous humour concentration of cefuroxime 1 h after drug instillation (756 μg 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.


