Molecular Epidemiology in a Cluster of Cases of Postoperative Pseudomonas aeruginosa Endophthalmitis

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Between September and October 1994 we observed three cases of Pseudomonas aeruginosa endophthalmitis in a single ophthalmology center. Endophthalmitis progressed rapidly following surgical intervention, and the three patients completely lost vision in the affected eye. Microbiological surveillance culture specimens were obtained from environmental sites, the operating team, intraocular lenses, irrigation fluids, and surgical equipment. P. aeruginosa was isolated from the internal tubing system of automated cataract surgical equipment. The strains of P. aeruginosa cultured from vitreous and anterior chamber specimens of case patients and from the surgical equipment were analyzed with pulsed-field gel electrophoresis. Genomic DNA typing of these isolates showed an identical banding pattern on ethidium bromide-stained gels. We believe that this is the first reported outbreak of P. aeruginosa endophthalmitis traced to automated surgical equipment. Genomic DNA typing emerged as a practical and reliable option for the epidemiological investigation of the outbreak.

Postoperative endophthalmitis remains the most serious complication of ocular surgery. Although rare, this condition is potentially devastating and often leads to visual loss [1, 2]. The incidence of endophthalmitis after eye surgery is believed to be <0.5%, but it varies in reported series from 0.08% to 1% [3–10]. Staphylococcus epidermidis has emerged as the most common pathogen responsible for endophthalmitis, followed by Staphylococcus aureus and streptococci [9–11]. Gram-negative organisms are implicated in <20% of cases, although their incidence ranges from 6% to 29% in the larger surveys [5, 10–17]. Most of these episodes of gram-negative endophthalmitis are caused by Pseudomonas aeruginosa and Enterobacteriaceae [11].

Post-surgical endophthalmitis is most commonly caused by bacteria derived from the patient’s commensal or colonizing flora [9, 18, 19]. However, postsurgical endophthalmitis may result from exogenous sources such as the operating team, air, contaminated intraocular lenses, irrigation fluids, and surgical equipment [9, 20–22].

Here we report a cluster of cases of P. aeruginosa endophthalmitis that was traced to the use of automated cataract surgical equipment by means of molecular epidemiology.

Patients and Methods

During a 5-week period from 14 September to 18 October 1994, three patients who had undergone extracapsular cataract extraction with intraocular lens implantation developed P. aeruginosa endophthalmitis shortly after surgical intervention. The clinical presentation was characterized by severe local pain, conjunctival hyperemia and chemosis, lid edema, and hypopyon. As soon as the third case developed, we hypothesized an environmental source of infection. The operating theater was closed and an infection control program was implemented.

The staff members were interviewed about the surgical procedures and sterilization methods, and the perioperative and preparation areas were inspected. Vitreous and anterior chamber specimens simultaneously obtained from the three patients were cultured. The previously placed synthetic lenses were removed and cultured at the time of vitrectomy. Samples of various medications, ophthalmic solutions, intraocular lenses, and surgical equipment, as well as selected environmental sites and specimens from the operating team (hand, nose, and throat swabs), were cultured by conventional microbiological methods.

Gram-negative, oxidase-positive bacilli growing on both blood agar and MacConkey agar were identified as P. aeruginosa by standardized methods for detection of gram-negative bacilli (ID 32 GN System; bioMérieux, Marcy l’Étoile, France). To determine the relatedness of the isolates of P. aeruginosa, we used analysis of chromosomal restriction fragment patterns by pulsed-field gel electrophoresis (PFGE), a technique that permits easy characterization of very large fragments of DNA. For this purpose we used field inversion gel electrophoresis (FIGE) [23].

Genome Fingerprinting by FIGE

P. aeruginosa strains isolated were cultured in Luria broth, washed twice with 75 mM of NaCl/25 mM of EDTA (SE buffer; pH, 7.4), and adjusted to 10⁹ cfu/mL in SE buffer. The bacterial suspension was mixed at 45°C with the same volume...
of 2% (wt/vol) low-gelling agarose in SE buffer. The mixture was dispensed into slots and allowed to solidify. The agarose blocks were transferred into 1 mL of EDTA buffer (pH, 9.5) with 1% N-lauroylsarcosine and 0.5 mg of proteinase K (Sigma Chemical, St. Louis) for 15 hours at 56°C. The blocks were then washed twice in Tris-EDTA (TE; pH, 7.4) and equilibrated in an appropriate buffer. DNA digestion was performed overnight at 37°C in 150 μL of restriction enzyme buffer containing 10 U of XbaI (TCTAGA; Sigma Chemical).

The reaction was stopped by the addition of 1 mL of TE buffer at 4°C. FIGE was run at 14°C with a field strength of 5.6 V/cm for 20 hours on 1% agarose gel in 0.5% Tris–boric acid–EDTA (TBE) buffer by means of the CHEF-DRII Electrophoresis System (BioRad Laboratories srl, Segrate, Italy). The forward-to-reverse ratio was chosen to be 3:1. The gel was then stained with ethidium bromide, examined by ultraviolet transillumination, and photographed. To evaluate the relatedness of P. aeruginosa strains, according to the methods of Grothues et al. [23] and Ojeniyi et al. [24], we analyzed the restriction fragment patterns on the gel by side-to-side visual comparison. Moreover, we referred to the criteria proposed by Tenover et al. [25] to interpret the DNA restriction patterns of isolates.

**Results**

Two of the three case patients were male; the patients’ ages were 51, 54, and 79 years. The three patients developed signs and symptoms of endophthalmitis 24 hours (2 patients) and 72 hours (1 patient) after the surgical procedure. The interval between the first and the second recorded case was 13 days, and that from the second to the third case was 20 days. The three patients’ extracapsular cataract extraction with intraocular lens implantation was performed with automated surgical equipment (Optikon Phacovisc P 4000; Optikon Oftalmologia spa, Rome). This instrument, which is specifically designed for use in anterior segment surgery, permits extracapsular cataract extraction to be performed with phacoemulsification or aspiration-irrigation techniques.

No other cases of endophthalmitis were observed among the other 129 patients who had cataract surgery with the same equipment (55 by extracapsular extraction and 74 by phacoemulsification) or among 54 patients who underwent other surgical procedures during the same period. Moreover, a hospital record review did not identify any reported episode of P. aeruginosa endophthalmitis before this cluster.

All patients had a rapid, fulminating course with complete loss of vision despite early intravitreal and systemic antimicrobial therapy. The initial empirical therapy consisted of administration of intravitreal vancomycin and amikacin plus systemic ceftazidime. As soon as the overnight cultures yielded growth of an oxidase-positive, gram-negative rod, of which the colonial morphology was consistent with P. aeruginosa, the intravenous treatment was modified with ceftazidime and amikacin. A secondary vitrectomy, with direct intravitreal injection of amikacin, was repeated in one patient. Two of the three affected eyes were enucleated.

P. aeruginosa was isolated from the vitreous, anterior chamber, and synthetic lens of the three case patients and from 18 specimens collected from the device’s internal tubing system. The organism could not be detected in the other specimens cultured.

All these isolates were susceptible to aminoglycosides, ceftazidime, cefsulodin, ticarcillin, piperacillin, imipenem, pefloxacin, and ciprofloxacin. Genomic DNA typing of the 18 isolates from the internal tubing system of the surgical device and of the three isolates from case patients showed an identical banding pattern on ethidium bromide–stained gels (figure 1). No other strains of P. aeruginosa unrelated to the outbreak were typed.

**Discussion**

Microorganisms causing endophthalmitis are primarily those belonging to the flora associated with the ocular surface, with S. epidermidis being the most frequently isolated [1, 2, 9, 10, 18, 19, 26]. In the larger survey series, gram-negative organisms account for 6%–29% of all cases of bacterial endophthalmitis, with P. aeruginosa being one of the leading pathogens in this etiological group [1, 2, 5, 10–17]. P. aeruginosa endophthalmitis is a well-described clinical syndrome characterized by rapid progression and a poor visual prognosis [1, 12, 27].

Although P. aeruginosa endophthalmitis may be secondary to penetrating ocular trauma, posterior perforation of corneal
ulcers, or septicemia, it occurs most often following ocular surgery [9, 11, 12, 27]. Most of the cases of P. aeruginosa endophthalmitis reported have occurred in a sporadic fashion. However, it is unclear if these sporadic cases are a consequence of intraocular contamination of the conjunctival sac or eyelids by the colonizing flora or result from a breakdown of aseptic technique during ocular surgery [8, 9].

On the other hand, outbreaks of P. aeruginosa postoperative endophthalmitis have been reported [28–30]. In these epidemic cases, the source of infection was traced to the use of contaminated saline solution used for moistening the cornea during operation [28], to the intraoperative use of intrinsically contaminated basal salt solution [29] or indomethacin ophthalmic preparation [30], or to the implantation of contaminated lenses [31].

In this outbreak, the source of contamination was traced to the use of automated surgical equipment. To our knowledge, this is the first report of P. aeruginosa endophthalmitis confirmed by molecular epidemiology. The epidemiological analysis in previous reported outbreaks of P. aeruginosa endophthalmitis was based on antimicrobial susceptibility profiles [29, 31] or phage, pyocin, and serological typing [28].

Chromosomal analysis by PFGE is increasingly being viewed as a new “gold standard” for the epidemiological analysis of hospital infections [32]. Using this reliable epidemiological marker, we have found that the isolates from the three patients with endophthalmitis and those recovered from the automated cataract surgical equipment had an identical banding pattern of genomic DNA. On the basis of this finding, the theater was reopened for operation.

Bacterial contamination of intraocular fluids during extracapsular cataract extraction has been reported to occur in 28%–89% of cases [22, 33, 34]. It has been suggested that fluid from the conjunctival sac, contaminated with bacteria, routinely enters the anterior chamber during extracapsular cataract extraction [22, 33, 35]. However, despite this high frequency of contamination, endophthalmitis develops rarely following surgery, probably as a reflection of the low virulence of the principal contaminants, the small inoculum size, and the bacterial properties of the aqueous humor [22, 30]. Intraoperative communication with the vitreous cavity has been associated with significant increased risk of postoperative endophthalmitis [26, 36]. Moreover, the possibility of intraoperative bacterial contamination from automated cataract surgical equipment has already been observed [21].

Several species of bacteria, including S. epidermidis and Pseudomonas species, were found when the internal tubing, involved in the venting and vacuum mechanism of this automated cataract surgical equipment, was cultured [21]. However, the significance of these data in terms of causation of endophthalmitis is unclear, and we are not aware of any reported cases of endophthalmitis related to contamination of automated equipment. In the present study we have confirmed the possibility of bacterial contamination of the aspiration side of the system, and we have also demonstrated that contamination of the eye from the nonsterile interior part of the machine can occur. We could not determine how and when contamination of the inner workings of the machine occurred, but it is clear that the inability of the equipment to avoid reflux of contaminated material was responsible for the outbreak (although the mode of transmission to the patient was unclear).

We can assume that during the interval of time between the first and the last case of endophthalmitis all the patients undergoing cataract extraction with this equipment were at potential risk for exposure to contamination. However, of 129 patients at risk, only three developed endophthalmitis. On the basis of the devastating nature of the three reported cases and on data from previous reported outbreaks of P. aeruginosa endophthalmitis, we believe that it is improbable that other patients have had intraoperative contamination with the same strain and did not develop endophthalmitis.

Despite the relatively low infection rate, particularly if compared with other reported outbreaks of P. aeruginosa endophthalmitis, the temporal cluster of these infections was readily perceived on a clinical and microbiological basis. However, the possibility exists that an epidemic case may be interpreted as a sporadic complication or as a breakdown of aseptic technique at the time of surgery. Thus, it will be of particular interest for future epidemiological studies to routinely save all the isolates from cases of endophthalmitis. In this setting, as our report highlights, the reliability of DNA fingerprinting holds great promise.

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References