Nosocomial Outbreak of *Pseudomonas aeruginosa* Endophthalmitis

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CONCISE COMMUNICATION

Nosocomial Outbreak of *Pseudomonas aeruginosa* Endophthalmitis

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We describe an outbreak of nosocomial endophthalmitis due to a common source, which was determined to be trypan blue solution prepared in the hospital's pharmacy service. We assume that viable bacteria probably gained access to the trypan blue stock solution during cooling after autoclaving. The temporal cluster of *Pseudomonas aeruginosa* endophthalmitis was readily perceived on the basis of clinical and microbiological findings, and an exogenous source of contamination was unequivocally identified by means of DNA fingerprinting.

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Pseudomonas aeruginosa is a gram-negative bacillus commonly found in soil and moist environments and capable of surviving and growing in nutrient-poor water. *P. aeruginosa* often colonizes the lungs of patients with cystic fibrosis and has emerged as an important opportunistic pathogen in hospitalized and immunocompromised patients.[1] Small hospital outbreaks of infection with this pathogen are frequent and are usually traceable to a single contaminated source.

The most serious complication of ocular surgery remains postoperative endophthalmitis.[2-4] Endophthalmitis involves inflammation of the intraocular cavities and its adjacent structures and can result in severe complications, such as a loss of visual acuity. The incidence of postoperative endophthalmitis is low, less than 0.5%, although reported rates vary from 0.08 to 1%.[5-7] These complications are caused by patients' own cutaneous flora, such as *Staphylococcus epidermidis, Staphylococcus aureus,* and *Streptococcus* species. Gram-negative bacteria are implicated in fewer than 20% of cases of endophthalmitis,[6] and most of these cases are caused by *Pseudomonas* species and Enterobacteriaceae. However, studies have reported air, intraocular contact lenses, irrigation fluid, and surgery equipment as exogenous sources of postsurgical endophthalmitis.[6,7]

We describe a nosocomial outbreak of *P. aeruginosa* endophthalmitis following cataract extraction that occurred between November 2003 and February 2004 at the ophthalmology service of our hospital, and we report the results of an epidemiological investigation.

METHODS

In January 2004, two cases of postoperative *P. aeruginosa* endophthalmitis after cataract extraction were reported. A nosocomial endophthalmitis outbreak was suspected, which prompted us to carry out an epidemiological and microbiological investigation to identify the source and the route of infection. A retrospective and prospective search for cases was done. A case was defined as endophthalmitis in any patient who had had ophthalmic surgery at the hospital between November 2003 and February 2004; a case was considered confirmed if *P. aeruginosa* was isolated and otherwise suspected. The patient's files were reviewed, and the intraoperative and postoperative outcomes were analyzed. The epidemiological survey was focused on 2 areas: review of the surgical procedure and the sterility of the surgical areas, as well as the patient's exposure to a common source of contamination. The staff members were interviewed about surgical procedures and sterilization methods, and the preoperative and preparation areas were inspected. Vitreous fluid and anterior-chamber fluid specimens obtained from the case patients were cultured. Samples of various medications, ophthalmic solutions (including trypan blue solution), intraocular lenses, surgical equipment, and selected environmental sites were cultured by conventional microbiological methods.

All the isolates were identified on the basis of their appearance on Gram staining, colony morphology, motility, and cytochrome oxidase production. Final identification and susceptibility testing were performed with the semiautomatic MicroScan WalkAway system (Dade Behring). Additionally, all the trypan blue solution samples were inoculated into blood culture bottles and cultured in an automatic culture system for 6 days (Bactec 9240; Roche Diagnostics).

To determine the relatedness of the isolates of *P. aeruginosa*, we used a repetitive-element polymerase chain reaction assay to obtain the DNA fingerprints of the strains.[8,9]

RESULTS

During the epidemic period, 12 (1.3%) of 913 patients who underwent cataract surgery received a diagnosis of endophthalmitis. We identified 6 suspected cases of *P. aeruginosa* endophthalmitis, but we only confirmed 4 cases. The first 2 confirmed cases, in women aged 58 years and 61 years, occurred in January 2004, and the other 2 cases, in a man aged 65 years and a woman aged 68 years, were diagnosed in February 2004.

All 4 patients with confirmed cases developed signs and symptoms of endophthalmitis 24 hours after the surgical procedure. In 1 case, the evolution was good with antibiotic treatment; keratoplasty was required in 1 case, and in 2 cases enucleation was performed.

In all 6 cases, trypan blue solution was used for staining the anterior lens capsule to facilitate the cataract surgery.[10,11] Only 0.5 mL of trypan blue solution was used per procedure, and it was washed off with saline solution during the surgery. The trypan blue solution was mixed in the pharmacy service of our hospital after reconstitution of a commercial trypan

TABLE Results of Cultures of Various Specimens Suspected of Being the Source of Contamination

Specimen cultured	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*	*Alcaligenes xylosoxidans*
Vial 1	+	−	−
Vial 2	+	−	−
Vial 3	+	−	−
Vial 4	+	−	−
Trypan blue stock solution	+	−	−
Autoclave water	+	+	+

blue powder with phosphate buffer solution. This reconstituted solution was sterilized by autoclave and dispensed aseptically in commercial sterile vials. The vials of solution were sent to the operating theater and stored at room temperature. It was intended that the solution be passed through a filter with 0.22-μm pores before use.

Samples were collected from 4 vials of trypan blue solution (1 sample from each different vial) used during cataract surgery that were in the surgical area and from the trypan blue stock solution in the central pharmacy. After 24 hours of incubation, *P. aeruginosa* was isolated from all trypan blue solution samples (Table). Phenotypic identification and antimicrobial susceptibility patterns suggested that the *P. aeruginosa* isolates from clinical samples and the isolates from the trypan blue solution vials had the same biotype and antibiotype. These results prompted us to focus on the trypan blue solution as the common exogenous source of contamination.

We decided to culture all the components of the trypan blue stock solution, before and after the sterilization process, as well as water from the autoclave. All the samples were culture negative for pathogens, except for samples of autoclave water, which yielded *P. aeruginosa, Stenotrophomonas maltophilia,* and *Alcaligenes xylosoxidans* (Table).

The repetitive-element polymerase chain reaction assay demonstrated that all *P. aeruginosa* strains isolated from clinical samples and trypan blue solution samples had the same DNA fingerprint (Figure).

DISCUSSION

In our hospital, the prevalence of endophthalmitis following cataract surgery was 0.8% in the year before the outbreak, but during the epidemic period it was 1.3%. The attack rate for *P. aeruginosa* postoperative endophthalmitis during this outbreak was 0.65%. Many cases of *P. aeruginosa* postoperative endophthalmitis have been reported, and this microorganism has been described as a cause of endophthalmitis with rapid progression and a poor visual prognosis.[3]

In the epidemic cases described in the literature, the source of infection has been the saline solution used for moistening the cornea during surgery, the indomethacin ophthalmic preparation, or implanted contaminated lenses.[3,4,6,7] In the outbreak we describe, we suspected that endophthalmitis was caused by contaminated trypan blue solution, because this solution was used in all cases, and because Morel et al.[12] previously described contamination of trypan blue with *Bukholderia cepacia* in a cornea bank. We found *P. aeruginosa* in the water of the autoclave, in the sterilized trypan blue stock solution, and in the 4 trypan blue solution vials used in surgery, as well as in the 4 vitreous fluid samples obtained from all patients with a diagnosis of postcataract-surgery endophthalmitis.

The repetitive-element polymerase chain reaction assay demonstrated that all clinical *P. aeruginosa* isolates and all trypan blue isolates had the same DNA fingerprint. This finding allowed us to conclude that this outbreak was caused by an exogenous common source, which was the trypan blue solution prepared in the hospital's pharmacy service. We assume that viable bacteria probably gained access to the trypan blue stock solution during cooling after autoclaving, as has been described elsewhere,[13] although this could not be confirmed because the *P. aeruginosa* strains isolated from autoclave water and the clinical isolates had different repetitive-element polymerase chain reaction fingerprints. This discrepancy probably occurred because the autoclave water

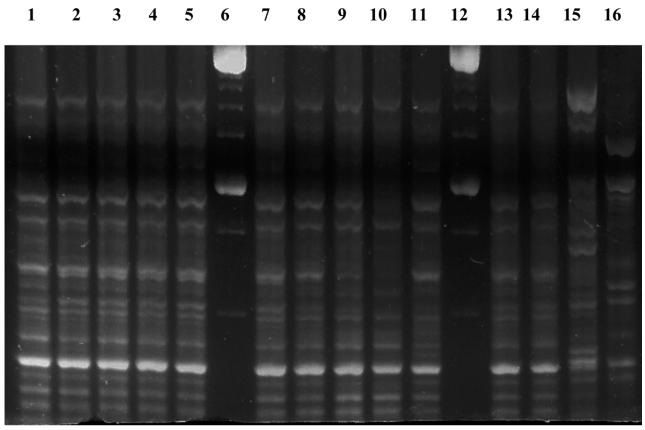

FIGURE Repetitive-element polymerase chain reaction patterns for the *Pseudomonas aeruginosa* isolates studied. *Lanes 1-5* and *7-9,* clinical isolates (duplicated run); *lanes 10-11* and *13-14,* trypan blue isolates; *lanes 6* and *12,* 100-bp molecular size markers; *lanes 15-16,* autoclave water isolates.

samples were cultured 1 month after the first case of endophthalmitis was diagnosed. The isolation of 3 species of gram-negative, nonfermentative bacilli, including *P. aeruginosa,* from the autoclave's water allows us to assume that contamination could take place during autoclaving. Additionally, an inquiry revealed that the trypan blue solution in vials prepared in the surgery service from trypan blue stock solution was not passed through a filter with 0.22-μm pores, as it should have been.

Thus, this outbreak occurred because of 2 incorrect procedures: defective autoclave processing and failure to filter the trypan blue solution before use. After this study and recall of the trypan blue solution used, no new cases of endophthalmitis occurred.

The temporal cluster of *P. aeruginosa* endophthalmitis cases was readily perceived on the basis of clinical and microbiological findings, and an exogenous source of contamination was unequivocally identified by means of the DNA fingerprinting of the strains involved.

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