An Unexpected Experimental Pitfall in the Molecular Diagnosis of Bacterial Endophthalmitis

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General primer-mediated ribosomal DNA amplification during endophthalmitis may improve the quality of diagnostic microbiology. However, extreme care needs to be taken not to introduce contaminating bacterial DNA during surgery procedures. The use of decontaminating iodine solutions can lead to such contamination due to the presence of DNA from Pseudomonas-like organisms.

Postoperative bacterial endophthalmitis is a rare complication of intraocular surgery or trauma. Bacterial toxins in particular are known to cause significant damage to retina and uvea, whereas the inflammatory process in itself also adds to the damage caused by the infection. Bacterial virulence is considered the main variable contributing to morbidity, and the absence of positive-testing cultures during endophthalmitis is a predictor for a positive outcome (1, 5). The present “gold standard” for the diagnosis of bacterial endophthalmitis is culture-based analysis of vitreous samples. Obtaining these samples, however, carries a risk for the patient and may lead to vitreous hemorrhage and retinal detachment. It would be a major improvement if vitreous samples could be replaced by samples derived from the anterior compartment of the eye. In general, however, these samples display a lower sensitivity in case of microbiological testing; consequently, a more sensitive laboratory tool for diagnosis is required (1). PCR could provide such a means, since this extremely sensitive method is capable of detecting limited numbers of DNA molecules (12). Several reports have described the application of PCR testing for the detection of bacterial endophthalmitis (9, 10, 11, 12, 13, 14). However, the general ribosomal DNA (rDNA) primed approach is highly sensitive to contamination. We applied bacterial rDNA amplification for diagnosing ocular infections and identified an unexpected source of bacterial DNA contamination.

From 1 September 2002 until 30 September 2002 we obtained undiluted vitreous or aqueous samples (n = 14) from several groups of patients requiring surgery at the Rotterdam Eye Hospital. This study has been approved by the institutional review board, and written informed consent was obtained from all patients.

Five patients presented with suspected bacterial endophthalmitis. Vitreal samples (from one of which an additional aqueous sample was obtained) from three patients were collected using a vitrectome after opening of the conjunctiva in the operation theatre, and a vitreous sample from two patients was obtained using a 27-gauge needle through the conjunctiva in the office. Eight control patients underwent vitrectomy for macular pucker (n = 3), macula hole (n = 1), or retinal detachment (n = 4). All control vitreous samples were obtained with the aid of a vitrectome after opening of the conjunctiva in the operation theatre.

All patients were treated twice with 0.3% Betadine iodine solution (Viatris, Diemen, The Netherlands) (10% stock) in a balanced salt solution (BSS; B. Braun, Meisingen, Germany) (0.9% NaCl) in the last 30 min before surgery. Immediately prior to surgery, bacterial decontamination of the eye was performed by generous application of a 1% dilution of Betadine iodine solution in BSS. The decontamination fluid was prepared fresh every day. Samples from the eye were collected during surgery, injected into sterile Eppendorf reaction vessels, and stored at −20°C.

Nucleic acids were extracted from the clinical specimens essentially according to the method described by Boom et al. (3). First, the samples were treated by lysozyme, lysostaphin, and proteinase K including 1% sodium dodecyl sulfate to destroy bacterial cell walls and bulk protein. DNA was stored in 10 mM Tris-HCl (pH 8.0) at −20°C. rDNA PCR amplification was performed using the primers EUR-L (5′-CTTTAGCGCCATTIAATCCG-3′) and EUR-R (5′-AGAGTTTGATCCTGTTCA-3′) according to the method of Wilson et al. (18). The PCR fragments thus generated were analyzed directly by electrophoresis and restriction fragment length polymorphism (RFLP) using the restriction enzyme AluI (Boehringer-Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. DNA extraction, amplification, and RFLP analysis of fluids used for decontamination and sterilization before surgery were performed similarly to the negative process controls. Control samples included chlorhexidine, 0.9% NaCl solutions, 10% Betadine, 0.3% BSS, and 1% Betadine in BSS and tap water. In addition, the degree of DNA contamination of the Eppendorf tubes used for sample storage was assessed.

All clinical samples gave rise to successful DNA isolation, and the rDNA PCR results were positive in all instances. This was surprising, since not all samples were collected from patients suffering from obvious bacterial endophthalmitis, as patients operated on for noninfectious reasons were included as well. In addition, when the 550-bp-long PCR product was digested with the restriction enzyme AluI, strikingly similar...
were most of the nonsterilized Eppendorf tube results. It has to shown); the chlorhexidine solution results were negative, as the same PCR signal, although it was far less intense (results not shown); the fluids used for eye decontamination as important sources of contaminating DNA. Nonsterilized tap water showed the same PCR fragment patterns. The BSS as well as the diluted iodine working solution appeared that extracts from the fluids used to decontaminate the eye before surgery gave rise to identical PCR RFLP patterns. The BSS as well as the diluted iodine working solution showed strongly positive in the PCR. This ultimately pointed to the fluids used for eye decontamination as important sources of contaminating DNA. Nonsterilized tap water showed the same PCR signal, although it was far less intense (results not shown); the chlorhexidine solution results were negative, as were most of the nonsterilized Eppendorf tube results. It has to be emphasized that microbiological cultivation of the decontamination fluids did not result in growth of the microorganisms (results not shown).

The finding that antisepsics and surgical materials may be contaminated with bacteria is not new, with the first reports dating back as far as the mid-1970s. By that time it had already been demonstrated by bacterial culturing that viable bacteria can be present in benzalkonium antiseptic (6, 8), povidone iodine (2, 4, 14, 17), or even chlorhexidine, which was shown not to be a source of contamination here (17). The organisms most frequently observed included Burkholderia cepacia and Enterobacter spp. Also, distilled water was to be used for injection or for the preparation of dilutions can be a source of infection (15). Modern sterilization technology obviously solved this latter problem. However, we here show that the sterilized products may still contain significant amounts of the DNA of the microorganisms that apparently thrived in the not-yet-stereilized formulation of the products. This issues a warning to those who would like to use PCR for the quality control of pharmaceuticals: in the end, an undesired large fraction of all materials may not pass quality control due to the presence of (harmless?) microbial DNA (7).

This short report highlights the finding that the procedure for collection of samples during common surgical procedures may severely compromise molecular diagnosis of bacterial endophthalmitis. We observed that the contamination process was independent of the location where surgery was performed, the clinical syndrome that was treated, and the nature of the sample obtained. This led to the hypothesis that various decontamination fluids were the most likely sources of the DNA contamination rather than, for instance, transient contamination of the exterior eye. The level of contamination was high, which resulted in competition between contaminating templates and the templates derived from the genuine infectious agents, false-negative results being the ultimate consequence. Before embarking on large-scale diagnostic studies, one needs to verify not only that the laboratory reagents are free of contaminants but also that the materials used before and during the operation are checked for unsuspected DNA contamination.

**REFERENCES**


