Pseudomonas putida Septicemia in a Special Care Nursery Due to Contaminated Flush Solutions Prepared in a Hospital Pharmacy


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Received 1 July 2005/Accepted 7 July 2005

Pseudomonas putida bloodstream infections were reported in two preterm neonates from a special care nursery. An unopened container of preservative-free heparin flush, compounded several weeks earlier in the hospital pharmacy and from the same batch that was administered to the patients, grew P. putida with a pulsed-field gel electrophoresis (PFGE) pattern identical to that of the patients’ isolates. Intrinsic contamination was ruled out by the absence of similar reports from other hospitals and by sterility testing of unopened stock solutions. We investigated the in vitro persistence of P. putida in heparinized saline; even under refrigerated conditions, inocula of 10^2 and 10^3 CFU/ml exhibited growth at 21 and 35 days, respectively. These findings highlight the need for compliance with current standards of aseptic technique and quality assurance during the preparation of compounded sterile products.

Pseudomonas species are opportunistic pathogens that primarily cause nosocomial infections (5). Pseudomonas aeruginosa is the most important pseudomonad species, but other species, including Pseudomonas putida, have been associated with clinical infections, particularly among children (4–7, 11, 17). In February 2001, P. putida bloodstream infections were reported in two preterm neonates from the special care nursery (SCN) of hospital A. Hospital A is a private community hospital with a maternity center that performs approximately 1,500 deliveries annually. The SCN is a level II unit that provides care to approximately five neonates each month who require specialized care but are not critically ill.

Patient 1 was an infant, born at 32 weeks of gestation, admitted to the SCN on the first day of life with respiratory distress and hypoglycemia. Empirical treatment with ampicillin and gentamicin was begun. A peripheral blood sample that was drawn at admission was culture negative. An umbilical artery catheter (UAC) was placed on the third day, and the infant developed sepsis 2 days later. P. putida was cultured from blood drawn peripherally and from the catheter. Clinical improvement followed modification of the antimicrobial therapy; the umbilical artery catheter was removed on day 10, and the patient was discharged at age 3 weeks.

Patient 2 was an infant born 8 days after patient 1, at 34 weeks of gestation. Patient 2 was admitted to the SCN with respiratory distress immediately following delivery; empirical treatment with ampicillin and gentamicin was started. An umbilical artery catheter was placed 20 min after birth and discontinued on the next day. P. putida was cultured from blood drawn from the catheter following its initial insertion and flushing. Because peripheral blood cultures were negative and the patient never developed clinical signs of sepsis, the positive blood culture was interpreted as pseudobacteremia. The patient was discharged at age 2 weeks.

The isolation of P. putida from patient 2’s blood sample followed the positive culture from patient 1 by 7 days; at that time, these neonates were the only patients in the SCN. No additional reports of Pseudomonas infection among hospital A patients were identified from our review of clinical microbiology laboratory records and SCN patient medical records from late 2000 through March 2001.

Environmental cultures from the SCN were obtained and included samples from lotions, soaps, work surfaces, syringes, and catheter tips. Similarly, cultures were done with samples obtained from the hospital pharmacy, including work surfaces, sinks, equipment, vials, and stock solutions. Environmental cultures of samples from faucets and surfaces were obtained from other selected areas of the hospital, including intensive care units. Cultures were also obtained for samples from the hands and nails of pharmacy workers and SCN nurses. Among the more than 130 samples tested, only one yielded P. putida. The positive sample represented an unused vial of umbilical artery catheter heparin flush solution compounded in the hospital pharmacy and was from the same batch as that used on patients 1 and 2. Genomic DNA from P. putida isolates was compared using standard methods for pulsed-field gel electrophoresis (12). P. putida isolates from the flush solution and multiple isolates from both patients all had identical DNA banding patterns (Fig. 1). Intrinsic contamination (6) was ruled out by the absence of similar reports from other hospitals and by sterility testing of unopened stock solutions from the same lots of saline (n = 2) and heparin (n = 10) used to prepare the flush solution.

The preservative-free umbilical artery catheter heparin flush...
solution used in the SCN was routinely compounded by the hospital pharmacy in a batch format. Each batch resulted in four 30-ml vials containing 0.5 unit of heparin per ml. In addition to the unopened vial referred to above, the batch associated with this incident was dispensed as follows: two vials were used for patient 1 (starting 22 days after the batch was compounded), and one was used for patient 2 (28 days after the compounding). The pharmacy technician who had prepared the implicated batch of UAC flush solution was no longer employed by hospital A and was not available for hand or nail cultures. Use of the hospital pharmacy for the provision of solutions to the SCN was suspended, and an alternate external source was used until the investigation was concluded.

We reviewed hospital A’s pharmacy practices related to the preparation of sterile products, including the observation of a mock preparation of UAC heparin flush solution. Although the exact source or mechanism of contamination could not be identified, several pharmacy practices may have led to unrecognized extrinsic contamination. The flush solution was prepared in a class 100 laminar-flow hood by pharmacy technicians who were typically not wearing gloves or other protective attire (e.g., laboratory jacket, gown, or mask), as required by state regulations and related guidelines when the anticipated dispensing time is >28 h away (2, 3). Each vial was prepared by using sterile syringes to transfer, separately, 1.5 ml of a 10-unit/ml concentration of preservative-free heparin flush (obtained from 1-ml vials) and 28.5 ml of 0.5 N saline into a sterile 30-ml vial. The saline was obtained from a 500-ml bag, which was opened <24 h before use. Notably, the heparin vial and saline bag stoppers were wiped using pads that were stacked in an open container of 70% alcohol, rather than the standard individually foil-wrapped alcohol pads. This might have facilitated the introduction of bacterial contaminants from a technician’s hand or other source. Completed vials of flush solution were sealed and held under refrigeration until dispensed, with a 30-day expiration limit. Sterility testing of the final product was not performed. We also found that vial labeling did not always identify the technician who compounded the sterile product and that log records for prepackaged compounded solutions were sometimes incomplete.

To evaluate the effects of potential extrinsic contamination during the preparation of preservative-free flush solutions, we investigated the in vitro persistence of *P. putida* in a half-strength normal saline plus heparin solution. The saline-heparin solution was prepared by taking 28.5 ml of sterile 0.5 N saline and adding 1.5 ml of a 10-unit/ml concentration of heparin flush solution. This saline-heparin solution was then filter sterilized with a 0.45 μm filter and added to sterile glass bottles in 30-ml aliquots. Colonies of *P. putida* obtained from overnight growth on sheep blood agar were suspended in the same sterile saline-heparin solution to achieve a 0.5 MacFarland standard. Dilutions of this suspension were then inoculated into the bottles of the saline-heparin solution to yield approximate initial concentrations of $10^2$ and $10^3$ CFU/ml. Four bottles of each concentration were incubated at either room temperature (21°C to 22°C) or refrigerator temperature (4°C to 5°C). A 0.1-ml sample of the inoculated saline-heparin solution was removed from each bottle on days 0 to 7, 14, 21, and 35 by using a sterile syringe and plated onto sheep blood agar; colony counts were determined using a standard method (15) after 24 h of incubation at 32°C. The in vitro survival curves for *P. putida* incubated at either room or refrigerator temperature demonstrated persistence, with generally stable or increasing colony counts following an initial decrease during the first week (Fig. 2). Even under refrigerated conditions, the $10^2$- and $10^3$- CFU/ml inocula exhibited growth at 21 and 35 days, respectively.

To help maintain patency, low doses of heparin (0.25 to 0.5
unit/ml) are recommended for flushes and other infusates administered through umbilical artery catheters (6). Although the majority of heparin solutions contain preservatives with antimicrobial activity (e.g., benzyl alcohol), the use of preservatives in solutions for use in newborns was discontinued in the 1980s because of concerns regarding neurologic toxicity (8, 9). In part because preservative-free heparin solutions are not commercially available at concentrations of <10 units/ml, flush solutions intended for neonates are typically prepared in the hospital setting. Pharmacists should prepare flush solutions for neonates on demand whenever possible. If these solutions are prepared in batches or must be prepackaged for later use, adherence to elevated levels of quality assurance procedures is called for (2). Extended expiration periods should be avoided to minimize the opportunity for the growth of contaminants introduced during compounding. Under the most recently published practice standards, batch-prepared flush solutions may not be refrigerated for more than 7 days unless sterility testing of the final product is performed (10).

Lack of adherence to sterile practices by pharmacies compounding injectable medications represents an important patient safety concern (7, 14, 16). To address this problem, a series of recommendations and guidelines that address pharmacy-prepared sterile products have been developed (1, 2). Adherence to these guidelines, however, has left much room for improvement and suggests that conditions similar to those described in the hospital A pharmacy were not atypical of many hospital pharmacies in the United States (13). The publication in 2004 of enforceable practice standards for compounding sterile preparations was an encouraging development (10). Growing awareness and implementation of these standards are needed to further improve the safety of sterile products administered to neonates and other patients.

We thank Henrietta Hardin, Amy Woron, Carol Worthington, and Teresa Wright for laboratory assistance; Mary Ellen Chesser, Sharon Gomer, Jack Cronk and William Moore for investigative assistance; and Geri Foster and Steve Huffines for advice related to pharmacy practices.

FIG. 2. In vitro survival curves for *Pseudomonas putida* in 0.5 N saline plus heparin. Survival was monitored at room temperature (21°C to 22°C) (dash-dot line) and at refrigerator temperature (4°C to 5°C) (solid line) for two levels of inocula: 10^2 CFU/ml (fine lines) and 10^3 CFU/ml (heavy lines).

REFERENCES


