Mechanism of Cross-Contamination of Blood Culture Bottles in Outbreaks of Pseudobacteremia Associated with Nonsterile Blood Collection Tubes

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Several studies have identified nonsterile blood collection tubes as the reservoir for contaminating microorganisms in outbreaks of pseudobacteremia. Microorganisms are thought to be transferred from the nonsterile tubes to the blood culture tubes when blood is drawn for cultures, but the validity of this hypothesis has not previously been tested. We recently investigated an outbreak of *Ewingella americana* pseudobacteremia in which an epidemiological association was found between pseudobacteremia and the practice of drawing blood for culturing at the same time that blood for coagulation studies is drawn. The nonsterile coagulation tubes, prepared in the hospital, were contaminated with *E. americana*. We studied three different procedures that personnel reported using to inoculate blood culture tubes after dispensing blood into the coagulation tubes. Although the failure to change or remove the needle before inoculating the blood culture tubes resulted in the highest percentage of cross-contamination, we found that all the techniques we simulated carried some risk of cross-contamination when coagulation tubes were filled first.

Several studies have identified nonsterile blood collection tubes as the reservoir for contaminating microorganisms in outbreaks of pseudobacteremia (1, 3, 5-7). Microorganisms are thought to be transferred from the nonsterile tubes to the blood culture tubes when blood is drawn for cultures, but the validity of this hypothesis has not been tested. We recently investigated an outbreak of *Ewingella americana* pseudobacteremia in a children's hospital. We found that citrated coagulation tubes, prepared in the hospital, were contaminated with *E. americana* and that cases of pseudobacteremia were associated with the practice of taking concurrent blood specimens for culturing and a coagulation profile. Although standard practice is to fill blood culture tubes first with a sterile needle, some personnel, fearing that the blood would clot, filled the coagulation tubes first.

From our interviews, we identified three techniques practiced by personnel at the hospital when they filled coagulation tubes and blood culture bottles consecutively (Fig. 1). In the first technique, personnel removed the needle after venipuncture, inserted the hub of the syringe into the rim of the coagulation tube, and allowed blood to drip from the hub of the syringe into the coagulation tube before attaching a new sterile needle and inoculating the sterile blood culture bottles. In the second and third techniques, personnel changed needles after venipuncture and inserted the new sterile needle into the coagulation tube to fill it. Subsequently, they used either the same needle or a new sterile needle to dispense blood into the blood culture bottles. After the first blood culture bottle was inoculated, the needle was always changed before any additional blood culture bottles were inoculated.

This laboratory study was undertaken to test our hypothesis that some of the techniques used when blood was drawn simultaneously for a coagulation test and a blood culture were responsible for the inadvertent inoculation of blood culture media with small amounts of contaminated

citrate solution. Another goal of the study was to closely simulate the procedure of filling coagulation tubes before instilling blood into blood culture bottles to evaluate the importance of procedural variations (i.e., the effect of needle removal versus needle retention) on the cross-contamination of blood culture bottles from coagulation tubes contaminated with *E. americana*.

MATERIALS AND METHODS

E. americana from the hospital was grown in citrated and anticoagulant solution. This culture was diluted 1:100 with membrane-filtered, citrated anticoagulant solution to yield a final concentration of 2.6×10^4 *E. americana* per ml. This suspension became the stock solution. Ninety sterile BACTEC blood culture bottles (aerobic bottles containing tryptic soy broth; Johnston Laboratories, Inc., Cockeysville, Md.) were randomly assigned to three groups, designated A, B, and C. Outdated citrated human whole blood from two standard 500-ml blood donor packs (supplied by the Atlanta Red Cross Blood Transfusion Service, Atlanta, Ga.) was placed by a sterile technique into a sterile beaker, and the beaker was then covered with sterile aluminum foil. Coagulation tubes were prepared from 5-ml round-bottomed tubes (75 by 12 mm) with red "push-in" stoppers (Walter Sarstedt, Princeton, N.J.) and were contaminated by the introduction of 0.3 ml of the stock solution of *E. americana* into each.

Group A (needle removal). Blood (8 ml) was aspirated aseptically into a sterile 10-ml syringe via an 18-gauge needle. The 18-gauge needle was removed from the syringe, and 3 ml of the blood from the syringe was introduced into a contaminated 5-ml round-bottomed tube by allowing the blood to drip from the hub of the syringe (placed inside the rim and in contact with the side of the coagulation tube). A new sterile 18-gauge needle was attached to the syringe, and the remaining 5 ml of blood was instilled into a blood culture bottle. This procedure was repeated until 21 blood culture bottles in group A had been inoculated.

Group B (needle retention). Blood (8 ml) was aspirated

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FIG. 1. Alternate manipulations postulated to be responsible for *E. americana* pseudobacteremia.

aseptically into a sterile 10-ml syringe via an 18-gauge needle. The 18-gauge needle on the syringe was replaced with a new sterile 18-gauge needle. A 3-ml portion of blood from the syringe was introduced via the needle into a contaminated 5-ml round-bottomed tube. (The needle was placed inside the rim and was in contact with the side of the coagulation tube). Care was taken so that the hub of the syringe did not touch the tube during this maneuver. With the same 18-gauge needle attached to the syringe, the remaining 5 ml of blood was instilled into a blood culture bottle. This procedure was repeated until 21 blood culture bottles in group B had been inoculated.

Group C (needle changing). A procedure similar to that described for group B was performed. However, after 3 ml of blood was instilled from the syringe into a contaminated coagulation tube, the needle was replaced with a new sterile 18-gauge needle before the remaining 5 ml of blood was instilled into a blood culture bottle. This procedure was repeated until 21 of the group C blood culture bottles had been inoculated.

Control groups. Three blood culture bottles were assigned to each of the groups A, B, and C to serve as negative controls. Each bottle was directly inoculated with 5 ml of blood without the needle being changed or other manipulations. Three blood culture bottles were assigned to each of the groups A, B, and C to serve as positive controls, and each was directly inoculated with 10^2 to 10^3 *E. americana* per ml. Three blood culture bottles were assigned to each group to serve as control blood culture bottles; these were

not inoculated with any citrated human whole blood but were treated identically with respect to culturing.

Blood culture bottles were incubated at 35°C. After 5 days of incubation, all blood culture bottles were sampled with a sterile tuberculin syringe and attached needle. A 0.1-ml sample from each bottle was placed on a MacConkey agar plate and subsequently streaked for isolation. The plates were then incubated for 18 to 24 h at 35°C and inspected to confirm that they were contaminated. Suspect colonies were picked and inoculated onto triple sugar iron agar, and further confirmation was made by standard biochemical methods (2). All bottles not showing growth at 5 days were reincubated and sampled by this method after 12 days of incubation. A positive blood culture was the detection of *E. americana* in the blood culture bottles.

For quantitative determinations, we also cultured each of the blood-filled coagulation tubes within 1 h after the addition of blood. We pipetted 0.1 ml of the contents of each tube onto the surfaces of blood agar plates and spread the samples with sterile glass L-rods. We also removed 0.01 ml of the contents with a calibrated loop and separately streaked it onto the surfaces of blood agar plates. The plates were then treated similarly to the MacConkey agar plates (see above), and *E. americana* was again identified by standard biochemical methods (2).

RESULTS

We were able to demonstrate cross-contamination in 2 (9.5%) bottles in group A (needle removal), 16 (76.2%) bottles in group B (needle retention), and 1 (4.8%) bottle in group C (needle changing). All 9 (100%) blood culture bottles directly inoculated with *E. americana* were found to be positive. All 9 (100%) control blood culture bottles that were not manipulated and 9 of 9 (100%) blood culture bottles directly inoculated with blood alone were negative for *E. americana* by the criteria described above. Quantitative cultures of the blood-filled coagulation tubes averaged 1.5×10^4 CFU/ml.

DISCUSSION

Pseudobacteremia, the occurrence of false-positive blood cultures, may result from the introduction of contaminating microorganisms into blood culture specimens either at the time of blood drawing or during their subsequent processing in a microbiology laboratory (4). The association of contaminated nonsterile coagulation tubes with pseudobacteremia has previously been described (1, 3, 5–7). The hypothesized method of contamination, however, was not previously tested under controlled conditions.

Based on our personnel interviews and the results of our mock transfer experiment, we postulate that the cross-contamination and pseudobacteremia in this outbreak most likely resulted when the coagulation tube and the blood culture bottle were filled with the same needle (group B). In one instance, transfer resulted even after the needle that had contacted the contaminated citrate solution was replaced with a new sterile needle before the blood culture medium was inoculated (group C). This observation suggests that, once the first needle has contacted contaminated citrate solution in a coagulation tube, changing the needle after instilling blood into the coagulation tube may be insufficient to prevent the transfer of contaminating microorganisms, possibly through retrograde contamination of blood in the syringe.

Blood drawing for multiple investigative tests is often indicated in the management of acutely ill hospitalized

patients. In addition, a concern for the comfort of patients and clinical expedience frequently lead to the simultaneous drawing of blood for these investigations. We have shown that even minor breaks in technique can result in the contamination of blood cultures from nonsterile collection tubes, a hypothesis that has been used to explain several outbreaks of pseudobacteremia. Given the potential risks associated with unnecessary antibiotic therapy, false-positive blood cultures may pose a risk to the health of hospitalized patients. Furthermore, breaks in specimen collection techniques may incur additional costs to hospital laboratories which may not be reimbursed under some new third-party payment mechanisms. These factors suggest a need for continued efforts to have all personnel adhere to standard laboratory protocols for obtaining specimens for culturing.

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