Blood Culture Cross Contamination Associated with a Radiometric Analyzer

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During a 9-day period in August 1980 in a New Jersey hospital, three pairs of consecutively numbered blood cultures from different patients were identified as positive for the same organism (two pairs of *Klebsiella pneumoniae* and one pair of group A *Streptococcus*). For each pair, both cultures were positive in the same atmosphere, both organisms had the same sensitivities, and the second of each pair grew at least 2 days after the first and was the only positive blood culture obtained from the patient. When the hospital laboratory discontinued use of its radiometric culture analyzer for 15 days, no more consecutive pairs of positive cultures occurred. Subsequent use of the machine for 9 days with a new power unit but the original circuit boards resulted in one more similar consecutive pair *(Staphylococcus epidermidis)*. After replacement of the entire power unit, there were no further such pairs. Examination of the machine by the manufacturer revealed a defective circuit board which resulted in inadequate needle sterilization. Laboratories which utilize radiometric analyzers should be aware of the potential for cross contamination. Recognition of such events requires alert microbiologists and infection control practitioners and a record system in the bacteriology laboratory designed to identify such clusters.

Pseudobacteremia has resulted from contamination introduced at almost every stage of specimen handling (5). As with any laboratory test, each report of a positive blood culture must be interpreted using the clinical setting as well as other supportive laboratory data.

Guidelines have been developed to distinguish between true-positive and false-positive blood cultures: true-positives are more likely to be positive in more than one culture bottle and to involve an organism that is almost always a pathogen rather than a common contaminant (4). Pseudobacteremia epidemics in hospitals are more easily recognized when the frequency of contamination is great or when there is an unusual organism involved (6). We describe here a small cluster of pseudobacteremia consisting of pairs of positive blood cultures (one true-positive and one false-positive) involving several different organisms. Cross contamination occurred through inadequately sterilized needles of a radiometric blood culture analyzer (BACTEC 460, Johnston Laboratories, Inc.) and was recognized through surveillance of microbiology laboratory records correlated with clinical findings.

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MATERIALS AND METHODS

Background. In August 1980, during an investigation of two cases of reported group A streptococcal bacteremia, the infection-control nurse at a New Jersey hospital noted that the positive blood cultures had consecutive numbers. The clinical picture of the second patient was not felt to be compatible with streptococcal disease. A review of the microbiology records revealed that two other pairs of consecutively numbered blood cultures (both *Klebsiella pneumoniae*) had been identified in August. Again, the diagnosis for the second of each pair was clinically suspect.

On 1 March 1980, the hospital began using a radiometric analyzer (BACTEC 460) for initial analysis of blood cultures. Aerobic and anaerobic culture bottles are placed in separate racks (usually in sequential order) and analyzed separately. The rubber stopper of each bottle is cleaned with 70% isopropyl alcohol before being placed in the machine. Two needles puncture the rubber stopper on the top of the bottle, and 100 ml of a gas mixture is pumped into the bottle through one needle and withdrawn through the other needle (Fig. 1).

The machine then determines the amount of $^{14}CO_2$ found, and an indicator light goes on if this value exceeds a predetermined threshhold level. If a culture exceeds this threshhold, it is taken out of the rack, Gram stained, and subcultured by conventional methods. Also, bottles that appear positive before or between samplings are removed from the rack, Gram stained, and subcultured. With the day of initial sampling defined as day 0, bottles were being sampled
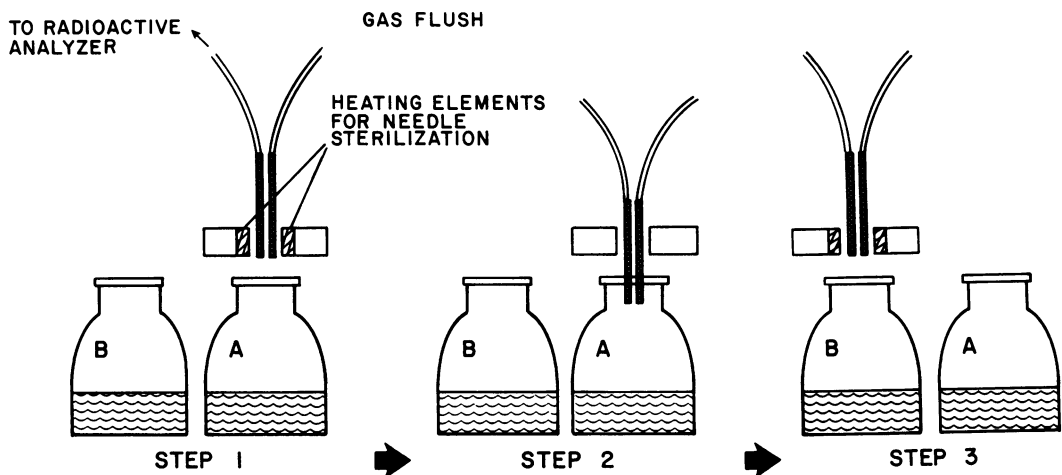
FIG. 1. Radiometric blood culture analyzer.

once each day on days 1 to 5 during the time of the outbreak.

After the two sampling needles are withdrawn from each bottle, they are sterilized by a heating coil before being introduced into the next bottle. The needles were changed twice a week, sterilized, and examined for microscopic deposits before being reused.

Between samplings, aerobic culture bottles were incubated in a shaker to gently agitate the bottles. The hospital first began doing this on 22 July 1980. With the use of a shaker, bubbles of liquid may accumulate directly under the stopper. These bubbles can be eliminated by inverting the bottles before sampling.

Methods. The hospital microbiology log books were reviewed for the periods 1 March through 29 August for the years 1979 and 1980. For each day, the number of blood cultures done and the number of positive cultures were recorded. For these time periods, the number of times each organism was isolated from separate individuals was recorded. All primary laboratory work was done by the hospital laboratory. Capsule typing for *Klebsiella* and biotyping for *Staphylococcus epidermidis* were done by the Bureau of Laboratories, Centers for Disease Control.

The following definitions were used: pair, blood cultures drawn on the same day from two different individuals were both positive for the same organism; consecutive pair, same as pair, plus the blood cultures were consecutively numbered; pair representing laboratory cross contamination, same as pair, plus both cultures were positive in the same atmosphere (aerobic or anaerobic), both organisms had the same antibiotic sensitivity pattern (and biotype or capsule type if done), the second culture of the pair became positive at least 1 day after the first, and the second culture of the pair was the only positive blood culture for that patient.

RESULTS

During the 6-month periods studied, 194 of 2,870 (6.8%) blood cultures done were positive in 1979, compared with 261 of 4,137 (6.3%) in 1980. There were 9 pairs (same organism on

same day from different individuals) in 1979 and 17 in 1980. Only one of these pairs in 1979 was a consecutive pair, compared with eight in 1980. In August 1979 there were no consecutive pairs, compared with five in 1980. Three consecutive pairs, all occurring in August 1980, met our criteria for cross contamination: the organisms isolated were group A hemolytic *Streptococcus*; *K. pneumoniae*, capsule type 25; and *K. pneumoniae*, capsule type 23. In 1980, *K. pneumoniae* accounted for 8% of all positive blood cultures, and group A *Streptococcus* accounted for 0.8%. If positive blood cultures occurred randomly, the probability of a consecutive pair of cultures positive for *K. pneumoniae* occurring by chance would be <0.0001, and the probability of a consecutive pair of group A *Streptococcus* would be <0.000001.

On 29 August, the use of the radiometric analyzer was discontinued and the manufacturer was notified of the problem. On 15 September, the sterilizer unit and the four circuit boards of the radiometric analyzer were replaced. The analyzer malfunctioned initially, and the old circuit boards were reinserted with apparent success. The new sterilizer unit was used with these old circuit boards from 15 September through 23 September, when the sterilizer unit and the four circuit boards were again replaced at the suggestion of the manufacturer. Since 29 August 1980, there has been only one pair of positive cultures meeting the criteria for cross contamination. This pair (*S. epidermidis*, biotype 3) occurred on 19 September, during the time when the original circuit boards were in use. Examination of the original analyzer components by the manufacturer revealed that the circuit board that powered the sterilizer was not functioning and that the needles were not being heated at all.

DISCUSSION

An initial investigation of these pairs of positive blood cultures led to a suspicion of a laboratory problem. Clinically, pseudobacteremia was suspected in all four patients who represented the second of each pair. In fact, only one of the four patients (a patient with renal failure and a vascular shunt with a positive culture for *S. epidermidis*) was treated with antibiotics for the organism reported. The lack of a significant change in the percentage of positive blood cultures and the variety of organisms made a common contaminated product (culture tube, disinfectant, medium, etc.) unlikely.

The Centers for Disease Control has reportedly investigated several outbreaks associated with the use of radiometric blood culture machines (3). One such investigation (2a) was of a cluster of cases of *K. pneumoniae* in a Chicago hospital in 1977. Blood cultures were divided into probable true-positives (>one positive culture per patient) and probable false-positives (only one positive culture per patient). It was observed that probable true-positive cultures became positive in a mean of 1.23 days, compared with 5.16 days for probable false-positives. Also, probable false-positives were located significantly closer to preceding bottles in the machine that were positive for *K. pneumoniae* than were probable true-positives. Cross contamination was demonstrated in a mock trial in which organisms inoculated into blood culture bottles grew in sterile bottles run on the same analyzer. Cross contamination in that outbreak and in the mock trial did not always occur in the next consecutive bottle, but usually skipped bottles. Although the mechanism of cross contamination was not proven, it was felt to be due to improperly maintained needles and not to a faulty sterilizer.

Our criteria for cross contamination are based on the hypothesis that the radiometric analyzer was the source. If the needle became contaminated after insertion in a true-positive blood culture bottle and inoculated a subsequent bottle, the culture analyzed later (the second of the pair) would always be the false-positive. The individual from whom this blood culture was drawn would have no other positive blood cultures. We would also expect that, as in the above-described outbreak, this culture would not register as positive until at least 1 day after the first. Since aerobic and anaerobic cultures are analyzed separately, the second (false-positive) culture would necessarily be positive in the same atmosphere as the first. As in any case of cross contamination, the organisms would have the same sensitivity patterns. The manufacturer's finding of a nonfunctional sterilizer and the meeting of the criteria for cross contamination only when the malfunctioning unit was in place support the conclusion that the radiometric analyzer was the source of the contamination.

The radiometric analyzer has an indicator light to warn users of specific types of sterilizer malfunction, such as wire breakage. The needles are allowed to heat for a set time interval, but the analyzer has no intrinsic feedback loop to detect whether the needles are heating to appropriate temperatures. Such a setup would, according to the manufacturer, require engineering changes which would make the needles difficult to clean and maintain properly. Aging and use may cause resistance in the wires to increase, which could then necessitate heating for longer time periods to attain the same temperatures. As a precaution, the manufacturer recommends replacing the complete sterilizer unit annually, even if it appears to be functioning properly.

If the sterilizer was truly not working at all, one wonders why more episodes of cross contamination did not occur. There are several possible explanations. Ordinarily, needles should not have direct contact with blood or contaminated media. Contact only occurs if bubbles are inadvertently left in the tops of bottles after they are shaken or if high pressure in the bottle causes medium to be aspirated. Also, bottles that are obviously positive by visual inspection are not put into the machine.

After recognizing this outbreak, we surveyed 11 of the 30 other hospitals in New Jersey that used this same type of analyzer for their blood cultures. Our survey of these 11 hospitals revealed for the most part good machine maintenance and clear record keeping. However, several microbiologists interviewed were unaware that cross contamination could occur through the machine. Almost half did not systematically check for cross contamination daily. In two hospitals, the record system in place would have made detection of cross contamination difficult because bottles were not placed in the machine in sequential order or positive cultures were not recorded on the original entry or in a positive blood culture workbook or both. Two laboratories had noted what they believed to be episodes of cross contamination.

Pseudobacteremia associated with hospital laboratories has previously been reported due to contamination by a laboratory technician (1), contaminated pencillinase in multidose vials (2), and contaminated thimerosal solution used to disinfect diaphragms of blood culture bottles in the laboratory (3). Because automated equipment is likely to take over more of the work in microbiology laboratories, it is important for laboratory workers to realize the limitations of the machines that they are using.

It may be that the use of machines reduces the

chances for specimen contamination in laboratories because of decreased handling. However, because machines themselves can introduce contamination, good maintenance and operating procedures, careful record keeping, and intelligent surveillance of all results need to be practiced.

LITERATURE CITED

1. **Centers for Disease Control.** 1979. Pseudobacteremia due to *Staphylococcus aureus*—New York. Morbid. Mortal. Weekly Rep. **28:**82–83.
2. **Faris, H. M., and P. F. Sparling.** 1972. *Mima polymorpha* bacteremia: false-positive cultures due to contaminated penicillinase. J. Am. Med. Assoc. **219:**76–77.
2a. **Greenhood, G. P., A. K. Highsmith, J. R. Allen, W. A. Caufey, C. M. West, and R. E. Dixon.** 1981. *Klebsiella pneumoniae* pseudobacteremia due to cross contamination of a radiometric blood culture analyzer. Infect. Control **2:**460–465.
3. **Lynch, J. M., A. Anderson, F. R. Comancho, A. K. Winters, G. R. Hodges, and W. G. Barnes.** 1980. Pseudobacteremia caused by *Clostridium sordellii.* Arch. Intern. Med. **140:**65–68.
4. **MacGregor R. R., and H. N. Beaty.** 1972. Evaluation of positive blood cultures. Guidelines for early differentiation of contamination from valid positive cultures. Arch. Intern. Med. **130:**84–87.
5. **Maki, D. G.** 1980. Through a glass darkly, nosocomial pseudoepidemics and pseudobacteremias. Arch. Intern. Med. **140:**26–28.
6. **Weinstein, R. A., and W. E. Staum.** 1977. Pseudoepidemics in hospital. Lancet **ii:**862–864.