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Tracking Laboratory Contamination by Using a *Bacillus cereus* Pseudoepidemic as an Example

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From 1 March to 31 May 1990, *Bacillus cereus* was recovered from 24 of 5,534 (0.49%) blood cultures and 22 of 1,088 (2.02%) other body fluid cultures. The rarity of this organism as a pathogen and comparison with previous baseline rates led to the conclusion that it was a pseudoepidemic involving some form of culture contamination. Generalized precautions taken without specific knowledge of the contaminant source reduced the recovery rate of the organism. Recovery rates for the organism returned to normal baseline prevalence after environmental cultures and epidemiological analysis led to the sterilization of a contaminated water bath used for boiling thioglycollate media. The problems encountered in this investigation are examined, and a systematic approach to clinical laboratory epidemiology is outlined.

Pseudoinfections caused by contaminated cultures are a grudgingly accepted occurrence of modern medicine and clinical microbiology (5). Laboratory standards are established to keep this problem to a minimum. Even under ideal conditions, contamination from skin, air, or reagents used in processing may occur. Because of the expanding list of organisms that can be considered pathogens in various patient populations, such contamination can cause misdiagnosis and require unnecessary or improper treatment as a precaution until a repeat culture can be performed (10). The clinical microbiologist at times may be called upon to help distinguish between contamination and true infections. Because of the laboratory's limited clinical perspective and the subjectivity involved in such a determination, the microbiologist should exercise considerable caution in such cases, remembering that the final decision is the physician's responsibility.

When an organism is isolated above the normal baseline rate, it is incumbent upon laboratory personnel, with the aid of attending physicians, to determine if the unusual rate is due to laboratory contamination or true infection. A recurrent source of contamination may appear as a nosocomial epidemic. One study by the Centers for Disease Control determined that 11% of all nosocomial outbreaks were in fact pseudoepidemics (10). Recognizing and tracking the source of such outbreaks can be a difficult task (5). Standard epidemiological techniques do not always translate properly to the clinical laboratory setting. Using an investigation of a recent pseudoepidemic due to contaminated blood cultures in our laboratory, we examine problems that often arise in such investigations and outline a more systematic approach.

MATERIALS AND METHODS

Specimen collection and processing. Blood cultures were collected by house officers using the Du Pont Isolator culture tube. Blood samples received in the laboratory were centrifuged for 30 min and processed within a biological safety cabinet. During the initial setup, the supernatant fluid was withdrawn aseptically from the Isolator tube and the remaining sediment was inoculated onto Columbia 5% sheep blood agar, onto chocolate agar, and into a 45-ml Vacutainer brand supplemented peptone broth (SP) culture bottle (Becton Dickinson Company, Rutherford, N.J.). The broth was used to enhance the recovery of anaerobes and other fastidious bacteria. This SP broth had replaced laboratory prepared thioglycollate (TGC) broth for blood cultures at the beginning of February. Other body fluids, such as pleural, cerebrospinal, and synovial fluids, were plated onto 5% sheep blood and chocolate agar plates and inoculated into tryptic soy broth and TGC broth.

Organisms recovered from any culture were identified by conventional biochemical methods. Antimicrobial susceptibility tests were performed by a microtube method and interpreted according to standard M7-A2 of the National Committee on Clinical Laboratory Standards.

Environmental sampling. Environmental cultures were performed throughout the investigation of the contamination problem. Air plates (15-cm diameter; Columbia blood agar) were exposed to the environment for 30 min and 24 h. Plates were incubated at 35°C for 24 h. Laboratory surfaces and towels were sampled with a dry swab which was inoculated onto a Columbia blood agar plate and incubated at 35°C for 24 h. No attempt was made to remove residual disinfectants from environmental surfaces. Since water baths are used to boil TGC media, these baths were cultured by removing approximately 0.5 ml of water and inoculating a Columbia blood agar plate, which was then incubated at 35°C for 24 h. Sterility cultures were performed on commercially and laboratory prepared media by incubating the respective tubes and bottles at 35°C for 1 week.

Data analysis. A master list of all isolations of the suspect organism was compiled. Information on these isolates was obtained from culture work sheets and hospital records. Sorting by several factors was performed, including date and time of inoculation, media from which the organism was recovered, patient location, and antibiotic susceptibility results.

RESULTS

An outline of the investigation process is presented in Table 1. Three segments of the investigation can be distinguished, as follows.

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TABLE 1. Summary of various observations and interventions which led to detection and resolution of contamination problem with *B. cereus* in blood cultures and sterile body fluids

Time period	Observation	Investigation	Result	Conclusion
Initial outbreak (3rd wk in March)	Increase in *Bacillus* sp. noted by technologists (anecdotal)	Clinical status of patients	*Bacillus* sp. not linked to clinical sepsis	Increase in *Bacillus* sp. was due to contamination
		Medium sterility checks	Medium sterility checks are negative	
		Compilation of media and patient data	No patterns in hospital locations	Contamination occurring in laboratory
	Primary work area cleanup	Environmental culturing	No environmental source found	Source is unknown
	No contaminated pediatric cultures	Differences in pediatric culture technique	Peptone inoculated with syringe	Peptone uncapping theory postulated
	Greater awareness among technicians	Pediatric exception investigated	Break in laboratory policy: peptone was uncapped	Peptone uncapping theory confirmed
Interim period (late March to mid-April)	Drop in contamination to ≤3 isolates/wk			Cleanup and awareness reduced contamination rate
		Material and work flow analysis	Contamination correlated with inoculation time	Need for expanded environmental cultures noted
		Environmental cultures of secondary work areas	*Bacillus* sp. found in secondary work areas	Culture contamination occurring in secondary work areas
	Disinfection of secondary work areas	Expanded environmental cultures	*Bacillus* sp. found in other laboratory areas	Original source unknown
Second outbreak (mid-April to mid-May)	*Bacillus* sp. reappears: 4–5 isolates/wk	Environmental cultures	*Bacillus* sp. recovered from boiling water bath in secondary work area	Boiling water bath source of contamination and need for daily cleaning
	Last of previously boiled media removed		Previously unknown reserves of boiled media found	Persistence of contamination due to failure to find all involved media
	Last contamined blood culture observed			Contamination problem solved

(i) Initial outbreak. The initial outbreak was first noted in the third week of March, 1991, by technologists working the blood/sterile body fluid bench of the laboratory. Our laboratory identified the suspect organism as *Bacillus cereus*. This identification was later confirmed by the North Carolina State Microbiology Laboratories, Raleigh, N.C. Antibiotic susceptibilities (MIC in micrograms per milliliter) performed on the organism showed uniform resistance to ampicillin (>2.0), oxacillin (>4.0), penicillin G (>2.0), and trimethoprim-sulfamethoxazole (>2 and 78). It should be noted that monthly statistical control charts tracking *Bacillus* isolates from blood cultures did not exceed limits until data were compiled for April (Fig. 1). While *B. cereus* has been reported as a blood-borne pathogen in rare cases (1, 7), it is generally considered an airborne contaminant (11). Examinations of the patients' clinical statuses through consultations with attending physicians suggested contamination rather than true sepsis with this organism. Subsequently, the outbreak was investigated as a possible laboratory contamination problem.

Early attempts to determine a point source for the contamination involved the incubation of uninoculated culture media and compilation of patient and culture historical data. The number of contaminated cultures was tracked graphically by collection date (Fig. 2), providing a historical perspective of the problem. From culture data, it was immediately apparent that all contaminants were recovered from either the SP bottle used in blood cultures (the organism was usually recovered on the 6-day blind subculture) or the TGC broth used in other body fluid cultures. The lack of contamination in uninoculated media, and the lack of any positive correlation with patient locations, indicated that

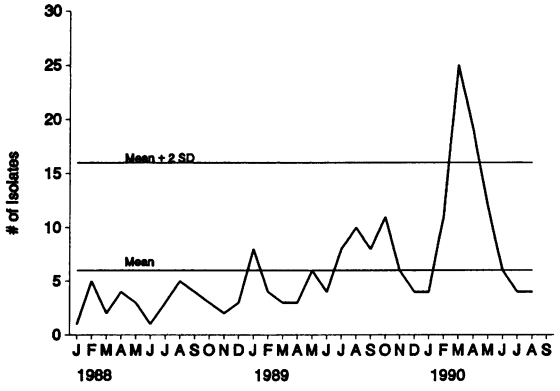

FIG. 1. *Bacillus* species isolated from blood by month during the period January 1988 through August 1990. SD, standard deviation.

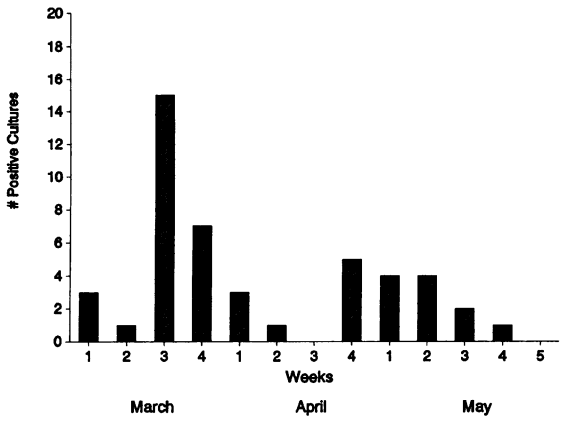

FIG. 2. *B. cereus* isolated per week from blood and normally sterile body fluids during the months of March, April, and May 1990.

these media were most likely being contaminated in the laboratory.

While there were no positive correlations with patient locations, the absence of contaminated pediatric blood cultures was noted. Pediatric specimens were inoculated through the rubber septum of the SP broth via a needle and syringe; adult specimens, by contrast, were first centrifuged, and then the sediment was inoculated with a pipette into the SP broth after the metal cap was removed. This procedure came under suspicion when a contaminated pediatric blood culture appeared, and investigation showed that the SP bottle had, contrary to policy, been uncapped prior to inoculation.

The initial environmental cultures of the primary work areas performed during this period failed to recover the contaminant organism. The reason for this may have been due to the use of dry swabs rather than premoistened swabs in our culture technique. Nonetheless, a general laboratory cleanup was performed, and there was increased awareness among laboratory personnel of the need for strict adherence to aseptic technique during culture inoculation.

(ii) Interim investigative period. During the period of late March to mid-April, the number of contaminated cultures declined. Since no contaminant source had been found, this decline was attributed to the greater awareness and resulting greater attention to aseptic technique among laboratory personnel.

A more extensive analysis of the compiled contaminated culture data showed that over half of the contaminated blood cultures were received within an hour of 8:00 a.m., normal opening time for the laboratory. All but two of the contaminated blood cultures not received during this time period were inoculated on the weekends. No such pattern existed for the receipt time of other contaminated body fluids.

This finding led to an examination of laboratory materials and work flow. It was discovered that the high volume of blood cultures during the 8:00 a.m. to 9:00 a.m. period required the use of a secondary work area, which had not been cultured during the initial outbreak. This secondary work area contained a boiling water bath in which TGC broths, used for other body fluids, were placed to remove dissolved oxygen. Cultures of the work surfaces and equipment in this area demonstrated the suspect *Bacillus* sp.; however, the organism was not found in the boiling water bath.

After a general disinfection of this secondary work area, extensive environmental cultures were performed throughout the laboratory, and the contaminant organism was recovered in smaller numbers from other work areas not involved in the primary culture process. While the evidence appeared to point to this secondary work area, the exact mode of contamination was not yet fully understood.

(iii) Second outbreak. In late April, the contamination of five other body fluid cultures signalled the return of the problem. Environmental cultures were repeated in the secondary work area, from which the contaminant organism was again recovered. Significantly, the organism was recovered in large numbers from the boiling water bath itself. It was theorized that initial cultures of the bath had been performed too soon after the boiling process, when the organism numbers had been reduced, or that reseeding had not occurred.

The mode of contamination was now felt to be understood. The morning boiling of TGC broths (performed at various times during the weekend) was the source of the contaminant organism. Handling of the wet tubes and subsequent uncapping of this medium and the SP broths processed at this time were the mode of transmission.

New laboratory protocols were established for changing the water in the boiling water bath daily and for the sterilization of the bath itself. Media processed prior to the institution of these protocols were removed from the laboratory, although in mid-May it was discovered that ancillary laboratories had retained some of the contaminated media. After all involved media had been discarded, no further isolations of the characteristic *Bacillus* sp. occurred. However, continued dissatisfaction with the uncapping of the SP broth led to the adoption of a protocol calling for the direct syringe inoculation of a portion of all blood samples prior to centrifugation.

DISCUSSION

Our previous experience with recurring culture contamination included a case of contaminated penicillinase and a commercial mycobacterial medium supplement contaminated during manufacture. Contaminant sources reported by others include contaminated disinfectants (4, 8), contaminated collection tubes (2, 8), and, recently, contaminated rubber gloves (12). The complexity of the problem observed with the *Bacillus* sp. in the current situation was a significantly greater challenge than our previous cases because of both the low frequency of contamination and the 6-day delay in its recognition. Indeed, our investigation led us to the conclusion that there was a preexisting contamination problem of even lower frequency of an ampicillin-sensitive *Bacillus* sp. that went unrecognized until the higher-frequency resistant *Bacillus* sp. focused attention on the problem. This is consistent with pseudoepidemics that can often "smolder" at low levels for long periods of time without being recognized (6).

The most important factor in recognizing pseudoepidemics is the ability to track a baseline recovery rate for all organisms (6). In many laboratories, such baseline rates may exist only as impressions in the minds of technologists. While very valuable and, as in our case, much faster than once-a-month statistical control charts (Fig. 1), which were not available until over a month after the investigation started, such impressions cannot be viewed as an adequate replacement for a formal epidemiological data base.

Several circumstances unique to microbiology make cooperation between the microbiologist and laboratory informa-

tion system manager important. These include the often-debatable pathogenicity of organisms and changes in nomenclature and identification techniques that result in new population patterns. Furthermore, since baseline rates in both formal and informal systems may include low-level contamination problems, each rate should be examined carefully to determine if it represents true patient infections. A difficult problem is distinguishing between the inevitable contamination during collection of the specimen and sporadic contamination during culture processing. For blood cultures, which are an effective measure of overall laboratory technique, it is generally recognized that phlebotomy contamination rates run from 3 to 5% (9). Contamination rates above this level are cause for investigation.

When contamination is suspected, the clinical microbiologist has two goals: first, halting the contamination by whatever means, and second, tracking and eliminating the point source. These goals can be conflicting when, in the interest of expediency, global changes in procedure are made before any specific source is found. Thus, as in the case of the *Bacillus* outbreak, the disappearance or abatement of the pseudoepidemic may not be attributable to any one change, and the underlying source of the pseudoepidemic may remain hidden and cause problems at a later date. For this reason, it is important to follow a systematic investigation through to its conclusion regardless of the apparent spontaneous or unexplained resolution of the problem.

As in all systematic approaches to problem solving, steps may be performed concurrently or out of order. In well-understood cases, steps may be entirely omitted. However, it is important to stress that unless all elements of a systematic investigation have been performed, either formally or informally, the potential exists for the incomplete or incorrect solution of the problem. Such a systematic approach would include the following steps.

(i) Gathering data. A complete list of contaminated cultures should be made, listing all external laboratory data (e.g., hospital location, patient service, and phlebotomist) and internal laboratory data (time of processing, technologists involved in processing, and medium lot numbers). Contaminants should be identified not only by species but by their antibiograms or biotypes.

(ii) Determining commonalities. Static patterns involving labeled factors such as medium lot or patient location will be most easily discerned. Dynamic patterns involving material or personnel flow will require more detailed study and may involve changes in laboratory procedure (such as initialling culture media upon inoculation). Formal statistical analysis, while the most complete method, often requires a considerable amount of time for gathering uncollated baseline data. While such data may be important in retrospective documentation, for the laboratorian intent on solving a contamination problem, time is better spent investigating "obvious" commonalities. An important aspect of this phase of the investigation is the direct observation of procedures involved in the problem, particularly if the investigator does not routinely perform such procedures. Quite often this will lead to the discovery of variances from standard protocols. In addition, volume overflow and backup procedures, which may not be listed in official protocols, should be included in the investigation.

(iii) Evaluating commonalities and their exceptions. Commonalities, combined with a knowledge of the entire culture process, are the primary tool of any investigation of pseudoepidemics. For instance, our lack of pediatric cases was similar to the pattern found by Semel et al. in their investigation of an outbreak of *Pseudomonas maltophilia* pseudosepticemia (8). In both instances, different collection tubes used for pediatrics circumvented the contamination problem and provided the investigators with a clue as to the source of the problem. Two factors should be considered when evaluating the data: contaminant source and contaminant transmission. When the connection between the two is not direct (as in the case presented), solutions aimed at either factor may reduce or eliminate the problem as a whole. Nonetheless, the optimal solution addresses both factors. The study of exceptions to the patterns can be very productive in the investigation of contamination problems. Exceptions have long been used in investigations of genuine epidemics (3), where they can serve as a valuable confirmation of hypotheses.

(iv) Performing environmental cultures. By using the evaluation of contamination patterns to focus the search, attempts should be made to isolate the contaminant from reagents, individuals, or equipment involved in the suspect process. With low-level contamination problems, environmental cultures may have to be performed repeatedly over a period of time before a source can be ruled out. If the organism is recovered from an environmental source, similar and proximal sources should be cultured as well. Except in the case of contaminated, sealed reagents, the initial isolation of a contaminant organism is often not the only or original source.

(v) Changing or enforcing existing procedures where necessary. As already noted, generic changes or simply raised consciousness due to recognition of the problem may in and of themselves solve the problem (usually by affecting the mode of transmission) before the formal investigation even begins. The delays inherent in recognition of reduced contamination require that each corrective step be documented by date and/or medium lot. Without this documentation, no clear conclusions can be drawn about the effectiveness of new corrective procedures. Broad generic solutions should also be considered even when a specific source and transmission route has been found. While specific solutions are to be included, a contamination problem should always spur a reexamination of the depth of quality control and procedural standards throughout the laboratory. In the case presented, for example, although the other water baths throughout the laboratory were not thought to be directly involved with the problem, it was considered prudent to establish routine cleaning schedules.

(vi) Monitoring subsequent contamination rates. While reduction of the contamination rate is the laboratory's main goal, it should be recognized that a return to baseline rates in the absence of a complete understanding of the problem cannot be considered a success. This is an especially common predicament in cases where the source of contamination is eliminated before the contamination makes itself evident in the culture. In such cases, a schedule of environmental and medium sterility checks should be established in an attempt to document the source should it recur.

While every microbiology laboratory suffers from a contamination problem at some time, the use of these guidelines should aid in the rapid resolution of the contamination and prevent subsequent recurrences.

REFERENCES

1. **Dalton, H. P., and M. S. Allison.** 1967. Etiology of bacteremia. Appl. Microbiol. **15**:808–814.
2. **Hoffman, P. C., P. M. Arnow, D. A. Goldmann, P. L. Parrott,**

W. E. Stamm, and J. E. McGowan, Jr. 1976. False-positive blood cultures: association with nonsterile blood collection tubes. JAMA **236:**2073–2075.
3. **Kelsey, J. L.** 1986. Methods in observational epidemiology, p. 236. Oxford University Press, Oxford.
4. **Lynch, J. M., A. Anderson, F. R. Camacho, A. K. Winters, G. R. Hodges, and W. G. Barnes.** 1980. Pseudobacteremia caused by Clostridium sordellii. Arch. Intern. Med. **140:**65–68.
5. **Maki, D. G.** 1980. Through a glass darkly: nosocomial pseudoepidemics and pseudobacteremias. Arch. Intern. Med. **140:** 26–28. (Editorial.)
6. **Maki, D. G., F. S. Rhame, D. C. Mackel, and J. V. Bennett.** 1976. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. Am. J. Med. **60:**471–485.
7. **Pearson, H. E.** 1977. Human infections caused by organisms of the Bacillus species. Am. J. Clin. Pathol. **53:**506–515.
8. **Semel, J. D., G. M. Trenhome, A. A. Harris, J. E. Jupa, and S. Leven.** 1978. Pseudomonas maltophilia pseudosepticemia. Am. J. Med. **64:**403–406.
9. **Washington, J. A.** 1978. Conventional approaches to blood culture, p. 41–87. *In* J. A. Washington (ed.), The detection of septicemia. CRC Press Inc., West Palm Beach, Fla.
10. **Weinstein, R. A., and W. E. Stamm.** 1977. Pseudoepidemics in hospitals. Lancet **ii:**862–864.
11. **Wilson, G. S., and A. Miles.** 1975. Topley and Wilson's principles of bacteriology, virology, and immunity, 6th ed., p. 1095. Edward Arnold Ltd., London.
12. **York, M. K.** 1990. *Bacillus* species pseudobacteremia traced to contaminated gloves used in collection of blood from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol. **28:**2114–2116.