JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2007, p. 3546–3548 Vol. 45, No. 11
0095-1137/07/$08.00+0 doi:10.1128/JCM.01555-07

Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed?[▽]

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Received 3 August 2007/Returned for modification 8 August 2007/Accepted 11 September 2007

Although several reports have shown that two to three 20-ml blood cultures are adequate for the detection of bacteremia and fungemia in adults, a recent study (F. R. Cockerill et al., Clin. Infect. Dis. 38:1724–1730, 2004) found that two blood cultures detected only 80% of bloodstream infections and that three blood cultures detected 96% of episodes. We reviewed data at two university hospitals to determine whether the recent observations by Cockerill et al. are applicable more widely. We assessed all blood cultures obtained from adult inpatients from 1 January 2004 through 31 December 2005 at Robert Wood Johnson University Hospital and Duke University Medical Center. All instances in which ≥3 blood cultures per patient were obtained during a 24-h period were included. The medical records of patients who met the inclusion criteria were reviewed retrospectively to determine the clinical significance of the positive blood culture (true infection versus contamination). Data were analyzed to determine the cumulative sensitivity of blood cultures obtained sequentially during the 24-h time period. Of 629 unimicrobial episodes with ≥3 blood cultures obtained during the 24-h period, 460 (73.1%) were detected with the first blood culture, 564 (89.7%) were detected with the first two blood cultures, 618 (98.2%) were detected with the first three blood cultures, and 628 (99.8%) were detected with the first four blood cultures. Of 351 unimicrobial episodes with ≥4 blood cultures obtained during the 24-h period, 257 (73.2%) were detected with the first blood culture, 308 (93.9%) were detected with the first two blood cultures, 340 (96.9%) were detected with the first three blood cultures, and 350 (99.7%) were detected with the first four blood cultures. Among unimicrobial episodes, *Staphylococcus aureus* was more likely to be detected with the first blood culture (approximately 90% detected with the first blood culture). There were 58 polymicrobial episodes in which ≥3 blood cultures were obtained. Forty-seven (81.0%) were detected with the first blood culture, 54 (93.1%) were detected with the first two blood cultures, and 58 (100%) were detected with the first three blood cultures. The results of this study indicate that two blood cultures in a 24-h period will detect approximately 90% of bloodstream infections in adults. To achieve a detection rate of >99%, as many as four blood cultures may be needed. The previously held axiom that virtually all bloodstream infections can be detected with two to three blood cultures may no longer be valid but may also depend on the definition of the "first" blood culture obtained (see Materials and Methods and Discussion in the text).

A blood culture is defined as a specimen of blood obtained from a single venipuncture or intravenous access device. There have been numerous changes in blood culture media and systems during the past 30 years (1, 3, 5, 6, 8). Newer media reportedly are more sensitive for the detection of microorganisms, and modern, automated, continuous-monitoring blood culture systems (CMBCSs) detect positive results 1 to 1.5 days earlier than previously used conventional blood culture systems (2, 4).

Studies reported in the 1970s, 1980s, and early 1990s suggested that two to three blood cultures from adults obtained during a 24-h period could detect >99% of all bloodstream infections (BSIs) (1, 5, 7, 8). However, a 2004 study from the Mayo Clinic using the BACTEC 9240 CMBCS found that two blood cultures detected only 80% of BSIs, that three detected 96% of BSIs, and that four were required to detect 100% of BSIs (3). This observation was unexpected given the use of a modern CMBCS and contemporary culture media. The authors hypothesized that newer systems may detect bacteremia at lower levels than older systems do and that more blood cultures are necessary to detect low-level bacteremia. To determine whether the observations were unique to the Mayo Clinic and its patient population, we systematically reviewed blood cultures at two geographically unrelated university medical centers to determine the cumulative sensitivity of blood cultures obtained sequentially during a 24-h period.

MATERIALS AND METHODS

All positive blood cultures from adult inpatients at Robert Wood Johnson University Hospital, New Brunswick, NJ, and Duke University Medical Center, Durham, NC, from 1 January 2004 through 31 December 2005 were evaluated for inclusion in the study. At Robert Wood Johnson University Hospital, the BACTEC 9240 blood culture system with aerobic resin and anaerobic lytic blood culture medium was used. At Duke University Medical Center, the BACTEC 9240 blood culture system with the same medium or the BACT/ALERT blood culture system with activated charcoal medium, FA and FN, was used. A blood culture consisted of 20 ml of blood obtained either by venipuncture or from an intravenous access device. All instances in which ≥3 blood cultures per patient were obtained during a 24-h period were included. The medical records of patients who met the inclusion criteria were reviewed by one of the investigators to determine the clinical significance (true infection versus contamination) of the positive blood culture. Only patients whose positive blood cultures were judged to represent true infection were included.

The method for collecting and coding data was deliberately designed to un-

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▽ Published ahead of print on 19 September 2007.

TABLE 1. Microorganisms isolated from unimicrobial and polymicrobial BSIs in which three or more blood cultures were obtained

Microorganism(s)	No. of isolates		
	Total per hospital		Total by microorganism
	Duke University Medical Center	Robert Wood Johnson University Hospital	
Staphylococcus aureus	97	85	182
Coagulase-negative staphylococci	36	88	124
Enterococcus spp.	41	65	106
Streptococcus pneumoniae	6	5	11
Viridans group streptococci	11	6	17
Other streptococci	13	7	20
Other gram-positive bacteria	4	13	20
Escherichia coli	47	20	67
Klebsiella pneumoniae	35	49	84
Other *Enterobacteriaceae*	46	26	72
Pseudomonas aeruginosa	26	13	39
Other gram-negative bacteria	18	21	39
Gram-positive anaerobes	7	2	9
Gram-negative anaerobes	7	4	11
Yeasts	31	45	76
Mycobacterium spp.	2	0	2

derestimate rather than overestimate detection sensitivity. For example, if a patient had two blood cultures obtained at 8 a.m. and two more blood cultures obtained at 4 p.m. on the same day and only the 4 p.m. blood cultures were positive, the first positive culture for that 24-h period would be coded as culture number 3. Thus, we accepted the possibility that the patient was not bacteremic at the time of the first two blood cultures but still coded culture number 3 as the first positive, thereby potentially underestimating the sensitivity of the blood culture system.

Unimicrobial and polymicrobial episodes were analyzed separately. Any new BSI episode in which the same microorganism(s) was grown within a 2-week period of the initial positive episode was excluded from analysis. For polymicrobial episodes, we analyzed the data by organism (i.e., which culture number was positive for each individual organism) and by episode (i.e., which culture number was the first one to detect the BSI episode).

RESULTS

During the 2-year study period, there were 629 unimicrobial BSI episodes and 58 polymicrobial BSI episodes in which ≥3 blood cultures were obtained during a 24-h period. Microorganisms isolated from these BSI episodes studied are shown in Table 1.

Of the unimicrobial episodes in which ≥3 blood cultures were obtained, 460 (73.1%) were detected with the first blood culture, 564 (89.7%) were detected with the first two blood cultures, 618 (98.2%) were detected with the first three blood cultures, and 628 (99.8%) were detected with the first four blood cultures. There were 351 episodes of unimicrobial BSI in which ≥4 blood cultures were obtained during a 24-h period. Of these, 257 (73.2%) were detected with the first blood culture, 308 (87.7%) were detected with the first two blood cultures, 340 (96.9%) were detected with the first three blood cultures, and 350 (99.7%) were detected with the first four blood cultures.

Table 2 shows the number of blood cultures needed to detect common bloodstream pathogens. *Staphylococcus aureus* bacteremia was detected with the first blood culture in approximately 90% of episodes, whereas *Pseudomonas aeruginosa* bacteremia and *Candida albicans* fungemia were detected by the first blood culture in only 60% of episodes.

There were 58 polymicrobial BSI episodes in which ≥3 blood cultures were obtained. Of these, 47 (81.0%) were detected with the first blood culture, 54 (93.1%) were detected with two blood cultures, and all 58 were detected with three blood cultures. Polymicrobial episodes were also analyzed according to the number of cultures needed to detect all microorganisms. One hundred twenty-one organisms were isolated from episodes in which ≥3 blood cultures were obtained during a 24-h period. Of these, 81 (66.9%) were detected with the first blood culture, 101 (83.4%) were detected with two blood cultures, 120 (99.1%) were detected with three blood cultures, and all 121 organisms were detected with four blood cultures.

DISCUSSION

For many years, it has been a standard and widely accepted recommendation that two to three blood cultures be obtained over a 24-h period for the optimal detection of BSIs in adults (5, 8). In 1975, Washington (5) reported that three 20-ml blood cultures were necessary to detect 99% of 80 bacteremic episodes: 64 (80%) with the first blood culture, 70 (88%) with the second blood culture, and 79 (99%) with three blood cultures. In 1983, Weinstein et al. (8) reported results from 282 bacteremic adults from whom 17 ml of blood was obtained per

TABLE 2. Number of blood cultures required to detect common microorganisms causing unimicrobial bacteremia and fungemia

Microorganism(s)	Four blood cultures obtained					Three blood cultures obtained			
	No. of BSI episodes	Cumulative % detected by culture no.:				No. of BSI episodes	Cumulative % detected by culture no.:		
		1	2	3	4		1	2	3
S. aureus	100	93	97	100		75	87	93	100
Coagulase-negative staphylococci	66	64	85	100		41	71	98	100
Enterococcus spp.	36	67	80	89	100	47	68	87	100
Streptococci	26	77	85	100		20	85	100	
Escherichia coli	43	72	91	95	100	20	65	90	100
Klebsiella pneumoniae	40	78	90	98	100	25	76	88	100
P. aeruginosa	15	60	85	100		16	62	94	100
C. albicans	20	60	85	95	100	15	60	83	100
Candida glabrata	8	75	88	100		10	80	100	

culture. In these patients, 258 (91.5%) episodes were detected with the first blood culture and 280 (99.3%) episodes were detected with the first two blood cultures (8). Both of the aforementioned reports were undertaken in hospital laboratories that utilized conventional manual blood culture methods.

Recently, Cockerill et al. (3), using the BACTEC 9240 CMBCS with aerobic and anaerobic resin-containing blood culture broth, reported that cumulative yield for consecutive cultures was less than had been reported by Washington (5) and Weinstein et al. (8). This study analyzed 181 BSI episodes in adults (163 nonendocarditis, 18 endocarditis) in which three or more 20-ml blood cultures were obtained during a 24-h period. Of these, 122 (67.4%) episodes were detected with the first blood culture, 148 (81.8%) episodes were detected with the first two blood cultures, 173 (95.6%) episodes were detected with the first three blood cultures, and all episodes were detected with four blood cultures (3). The results in the current study appear to support the observations by Cockerill et al. Although our results showed somewhat higher detection rates with consecutive cultures, they confirm the need for up to four blood cultures in order to exceed 99% detection rates.

Prior studies have not assessed the ability of consecutive blood cultures to detect specific microorganisms. Our data, shown in Table 2, indicate that among common bloodstream pathogens, *S. aureus* is the most likely microorganism to be detected with the initial blood culture. In contrast, *P. aeruginosa* and *C. albicans* are the least likely bloodstream pathogens to be detected with the initial blood culture.

The observation by Cockerill et al. that more than three blood cultures were needed for 99% test sensitivity was unexpected and seemed paradoxical, given multiple studies of newer blood culture media containing resins and activated charcoal that show improved yield and speed of microbial detection with these media and systems compared with earlier medium formulations and those that do not use these additives. Cockerill et al. (3) have speculated that the reason for the decrease in the cumulative yield of consecutive cultures in the current era may be that lower levels of bacteremia are being detected by modern systems. Accordingly, detecting such low-level bacteremia or fungemia may require a greater volume of blood, i.e., more blood cultures. An alternative explanation is that in the current era more patients are on effective antibiotic therapy at the time at which blood cultures are obtained and that more blood cultures may be required because these agents may impair microbial growth.

Another potential explanation for our observations as well as those by Cockerill et al. could be methodological artifact. As noted in Materials and Methods, we chose consciously to err on the side of underestimating rather than overestimating detection sensitivity. Thus, if a patient had two blood cultures obtained at 8 a.m. and two more blood cultures obtained at 4 p.m. on the same day and only the 4 p.m. blood cultures were positive, the first positive culture for that 24-h period would be coded as culture number 3. However, it is possible that the patient was not bacteremic at the time of the first two blood cultures, so that the coding rule underestimates the sensitivity of the system. Indeed, in the example given, it may well be that the bacteremic episode was detected on the first rather than the third culture of the episode. Whether the same methodological bias was used by Cockerill et al. is not stated in their report (3). Similarly, the method used by Washington (5) is not known. In the report by Weinstein et al. (8), the first positive blood culture defined a BSI episode, and the blood culture with the lowest accession number (if two or three samples arrived simultaneously in the laboratory) was arbitrarily considered to be the first one obtained (M. P. Weinstein, personal communication). Accordingly, there may have been methodological bias toward overestimating test sensitivity in that report (8).

In summary, our observations support the findings by Cockerill et al. (3) that as many as four blood culture sets over a 24-h period may be needed for >99% test sensitivity. Whether these observations are due to changes in microbiologic and laboratory phenomena or represent an artifact owing to differences in analytic methods between the recent and the earlier reports is uncertain.

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