JOURNAL OF CLINICAL MICROBIOLOGY, July 1983, p. 208–210
0095-1137/83/070208-03$02.00/0

Vol. 18, No. 1

Enhanced Detection of Polymicrobic Bacteremia by Repeat Subculture of Previously Positive Blood Cultures

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Received 24 November 1982/Accepted 1 April 1983

Blood subcultures repeated 3 days after the cultures were first identified as positives increased our detection of polymicrobic bacteremia in 9.1 to 27% of clinically significant patient episodes. Reincubation and repeated subculture of previously positive blood cultures had a direct impact on the therapeutic management of patients with polymicrobic bacteremia.

Recent medical literature suggests that the incidence of polymicrobic bacteria is increasing. More deaths are associated with bacteremia caused by multiple species of bacteria than with monomicrobic bacteremia (3–5, 7).

We have used the BACTEC 460 radiometric blood culturing system for several years. Regardless of the methods used, however, most clinical microbiology laboratories follow a similar blood culture protocol: frequent blood culture examinations for early detection of positives, Gram stains and subcultures of suspected positives, and routine early subcultures and Gram stain of all blood cultures. Once a blood culture is recognized to be positive, the organism(s) is identified and susceptibility studies are performed. The isolates may or may not be saved for future studies. Subsequent subcultures of recognized positives are not performed.

In 1975, Harkness et al. (2) found little value in subculturing positive blood cultures 7 days after the initial positive subculture. In their study of 393 positive cultures, six bottles had an additional organism upon resubculture 7 days later. They believed that this additional yield of 1.5% did not justify performing such subcultures on a routine basis. However, the results of our study indicate that reincubating positive cultures and repeating subcultures and Gram stains at a later date enables the detection of significant numbers of additional organisms.

Radiometric cultures were processed by the recommendations of the manufacturer. Aerobic bottles were read twice on days 1 and 2 and daily once thereafter for a total of 7 days. Anaerobic bottles were routinely read once on days 2 through 7, unless the companion aerobic bottle was positive. Positive cultures were suspected when the growth index readings exceeded the preset threshold, when there was a delta change in consecutive readings of $\geq$10 growth index units, or when the septum was elevated. Gram-stained smears were immediately examined. Subsequent subcultures were performed on the basis of Gram stain characteristics of the bacteria seen and the atmosphere of incubation of the bottles. Aerobic and anaerobic bottles were subcultured to blood, chocolate, and Levine eosin methylene blue agars (BBL Microbiological Systems, Cockeysville, Md.) and incubated in 5% CO_2 at 35°C. When mixed gram-positive and -negative organisms were seen, phenylethyl alcohol blood agar (BBL) is also used. Anaerobic bottles are further subcultured to brucella blood agar with hemin and vitamin K, Centers for Disease Control blood, and phenylethyl alcohol agars (BBL) and incubated anaerobically at 35°C in a GasPak jar (BBL). Our procedure essentially adheres to the recommendations of Reller et al. (6) as outlined in *Cumitech 1A*.

Of 3,412 blood cultures bottles processed with the above protocol, 214 were positive; of these, 185 were judged to be clinically significant. Our criteria for judging significant results was based on the clinical decision to treat the patient with an antimicrobic intended to cover the organism(s) isolated from blood cultures. Seven bottles yielded multiple organisms and represented four patient episodes of polymicrobic bacteremia, whereas the remaining 178 positive bottles represented 40 patient episodes of what appeared to be monomicrobic bacteremia. These 178 positive bottles were reincubated for 60 to 72 h, at which time Gram stains and subcultures were repeated by the above protocol. An additional organism was isolated from 22 of the 178 previously positive bottles, representing eight additional patient episodes judged clinically to represent true polymicrobic bacteremia (Table 1). Table 2 summarizes the initial number of positive bottles and the number positive with the additional isolate upon repeat subcultures.

None of the original Gram stains suggested mixed morphotypes, and the Gram stains re-

TABLE 1. Additional cases of clinically significant polymicrobic bacteremia detected by repeat subculture of previously positive blood cultures

Case no.	Initial isolate	Additional isolate	Original antimicrobics	Probable source of polymicrobic bacteremia	Final antimicrobics
1	*Escherichia coli*	*Peptostreptococcus micros*	Gentamicin	Colonic bladder fistula	Gentamicin Clindamycin
2	*Klebsiella penumoniae*	*Streptococcus faecalis*	Amikacin Cefoxitin	Intra-abdominal abscess (post-surgery)	Amikacin Cefoxitin Ampicillin
3	*Escherichia coli*	*Klebsiella pneumoniae*	Ampicillin	Urinary tract obstruction	Gentamicin Cefamandole
4	Viridans group streptococci	*Veillonella parvula*	Penicillin	Lung abscess	Penicillin
5	*Morganella morganii*	*Streptococcus faecalis*	Gentamicin	Subphrenic abscess (post-surgery)	(Patient expired)
6	*Staphylococcus aureus*	*Streptococcus faecalis*	Nafcillin	Subphrenic abscess (post-surgery)	Nafcillin Gentamicin
7	*Fusobacterium mortiferum*	*Bacteroides thetaiotaomicron*	Penicillin	Decubitus ulcer	(Patient expired)
8	*Staphylococcus aureus*	*Streptococcus faecalis*	Nafcillin Chloramphenicol	Vertebral osteomyelitis	Nafcillin Streptomycin Penicillin

peated 3 days later suggested mixed morphotypes only in cases 1, 2, and 5 (Table 1). In case 1, the urinary tract was originally suspected as the source of the positive blood cultures. Gram stain of the urine demonstrated gram-negative bacilli and small gram-positive cocci in chains, but culture yielded only 10^5 CFU of *Escherichia coli*. Repeat subcultures of the positive blood cultures also grew *Peptostreptococcus micros*, and roentgenographic studies revealed a colonic bladder fistula. In cases 2 and 3, urinary tract infections were originally suspected as the sources of bacteremia. In case 2, when an additional organism was isolated from repeat subculture of the positive blood cultures, laparatomy was performed, and *Klebsiella pneumoniae* and *Streptococcus faecalis* were isolated from a subphrenic abscess. In case 3, two previous urine cultures grew 10^5 CFU each of *E. coli* and *K. pneumoniae*. When *K. pneumoniae* was isolated from repeat subculture of positive blood cultures, additional studies showed obstruction and pyelonephritis of the right kidney. In case 5, the probable source of the polymicrobic bacteremia was determined at autopsy, when *Morganella morganii* and *S. faecalis* were isolated from a subphrenic abscess. In case 7, the organisms were part of the mixed flora isolated from a decubitis ulcer.

Repeat Gram stain and subculture of previously positive blood culture bottles increased the detection of polymicrobic bacteremia from 3.8 to 15.7% of true-positive cultures. When translated to total patient episodes of polymicrobic bacteremia, there was an increase from 9.1 to 27%. Of the six living patients in whom polymicrobic bacteremias were detected upon repeat subculture, five required additional antimicrobics to cover the additional organisms.

Reincubation and repeat subculture of previously positive blood culture has improved blood culture techniques in our hospital. The additional time and expense is less than that expended in

TABLE 2. Initial positive blood culture bottles per patient and bottles with an additional organism isolated 3 days later

Case no.	No. of positive bottles/total with:	
	Initial isolate[a]	Additional isolate
1	4/4 (1)	2/4[b]
2	2/2 (1)	2/2
3	4/4 (2)	4/4
4	4/4 (1)	2/4[b]
5	4/4 (1)	4/4
6	4/4 (1)	4/4
7	2/4[b] (2)	2/4[b]
8	4/4 (1)	2/4[b]

[a] The day on which the bottle was found positive is given in parentheses.

[b] Anaerobic 7c bottles only.

performing routine terminal subcultures of radiometric negative blood cultures. We and others (1) have demonstrated that terminal subculturing lacks significant value in detecting bacteremia or fungemia. Published reports of fatal monomicrobic bacteremia might, in fact, include undetected and untreated polymicrobic episodes. Our findings suggest that such incidents could be reduced through additional time spent on positive blood cultures.

This study was supported by the Veterans Administration.

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