J Microbiol Immunol Infect 2010;43(2):126–132

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Original Article

Effect of Overnight Storage of Blood Culture Bottles on Bacterial Detection Time in the BACTEC 9240 Blood Culture System

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BACKGROUND/PURPOSE: Identifying the pathogens present in blood stream infections is crucial to initiate appropriate antimicrobial therapy and avoid morbidity and mortality. The aim of this study was to evaluate the effect of overnight storage of aerobic and anaerobic BACTEC 9240 blood culture bottles on the detection time for common pathogens.

METHODS: From November 2007 to July 2008, a total of 2,105 isolates were positively detected using the BACTEC 9240 system. The time to positive detection (TTD) was calculated by subtracting the time of receipt in the laboratory from the time required to detect a positive culture. The mean TTD values were calculated using the TTD value of the first positive culture bottle only. Overnight delay at the National Cheng Kung University Hospital, Taiwan was 15 hours (from 5 pm to 8 am).

RESULTS: Of the 2,105 total isolates, 972 (46.1%) were Gram-positive bacteria, 1,024 (48.6%) were Gram-negative bacteria and 109 (5.1%) were fungi. Among the top 10 pathogens, 24.7% grew only in the aerobic bottle and 15.1% in the anaerobic bottle, including *Staphylococcus* spp., *Enterococcus faecium*, *Enterobacteriaceae*, and Gram-positive bacilli. Due to the overnight delay in loading a blood culture bottle into the instrument, for most of the pathogens (including *Staphylococcus* spp. and *Enterobacteriaceae*), a decrease in TTD by ≤4.4 hours was observed. An increase in TTD by 20.8 hours was observed for Gram-positive bacilli. We also found that the difference between TTD in aerobic versus anaerobic bottles during the day was higher in coagulase-negative *staphylococcus* (12 hours) and lower in *Escherichia coli* and *Staphylococcus aureus* (<2 hours). TTD was longer than 72 hours in 20.5% of Gram-positive bacilli and 7.3% of *Candida albicans*.

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Article History:
Received: Feb 17, 2009
Revised: Apr 20, 2009
Accepted: May 13, 2009

CONCLUSION: No difference in the TTD of major pathogens was observed in bottles processed during the day and after overnight delay, suggesting that the delayed entry of the blood culture bottle into the instrument may affect the detection time. Since high numbers of facultative anaerobes were detected in anaerobic bottles only, use of a single aerobic bottle might have a detrimental effect on the clinical therapy outcome.

KEYWORDS: BACTEC, bacteremia, blood culture bottles, overnight delay culture

Introduction

Identifying the pathogens present in blood stream infections is crucial to initiate appropriate antimicrobial therapy and avoid morbidity and mortality. To detect the presence of pathogens in blood, commercially available BACTEC blood culture bottles are widely used.[1–6] Several factors influence the time to positive detection (TTD) of the pathogens, including delays in loading the bottles into the instrument, the incubation temperature, contamination, inoculum size, type of culture bottle, and the detection system used at the hospital.[1,3,4,7,8] In Taiwan, no studies have analyzed the factors influencing the TTD of pathogens with the BACTEC 9240 system. At the National Cheng-Kung University Medical Center hospital, a 5-day protocol for the BACTEC 9240 system is followed for both aerobic and anaerobic bottles for the detection of positive blood cultures.[5] The aim of the present study was to evaluate the effect on detection time of delays in loading blood culture bottles into the instrument.

Methods

Blood culture collection

From November 2007 to July 2008, a total of 2,105 isolates were positively detected using the BACTEC 9240 automatic blood culture detection system (Becton-Dickinson Diagnostic Systems, MD, USA) at the Microbiology Laboratory, Department of Pathology, National Cheng-Kung University Medical Center, Tainan, Taiwan. The blood culture bottles were processed by microbiologists when the laboratory was open (from 8 am to 5 pm, Monday to Sunday). Overnight delay at the hospital was 15 hours (from 5 pm to 8 am). A blood culture set, consisting of aerobic and anaerobic bottles, was used. Blood culture bottles regularly used are BACTEC Standard 10 Aerobic/F, BACTEC LYTIC/10 Anaerobic/F, BACTEC PLUS Aerobic/F, BACTEC PLUS Anaerobic/F, and BACTEC Peds PLUS/F (Becton-Dickinson Diagnostic Systems). The skin was disinfected using standard techniques.[9] Following venipuncture, 8–20 mL of blood was withdrawn from adult patients into a sterile syringe, and equal volumes of blood were aseptically transferred to one aerobic and one anaerobic bottle.[5]

Blood culture processing

Bottles placed in the instruments were processed and tested according to the manufacturer's instructions. The bottles were incubated at 35°C with rocking agitation for a total of 5 days. The 9240 unit tested each bottle every 10 minutes. All bottles were analyzed in the microbiology laboratory over the course of 5 days by the BACTEC 9240 system. Bottles flagged as positive were removed from the data units and processed as described previously.[5] Briefly, an aliquot of the blood-broth mixture was aseptically removed with a needle and syringe. The aliquot was divided, and one part was used for Gram staining and the other for subculture. The subcultures were incubated according to the results of the Gram stains using a standard protocol. Isolates were identified using the Vitek API system (bioMérieux SA, Marcy-l'Etoile, France) according to the manufacturer's instructions. When morphologically identical organisms were recovered from both bottles of a blood culture set, only one isolate was identified by conventional microbiological methods. The instrument-negative bottles from sets in which another bottle was positive were not processed, but were left in the

data units until they were flagged as positive, or until the end of the 5-day incubation period. Individual blood culture bottles were removed from the automated system when growth was detected, and the time to detection was recorded.

The TTD was calculated by subtracting the time of receipt in the laboratory from the time required to detect a positive culture. When growth was detected in both aerobic and anaerobic bottles, only the TTD value of the first positive culture bottle was considered when calculating the mean TTD values. When a second clinically important isolate was detected in a bottle, the episode was categorized as a mixed or polymicrobial infection. The TTD values from mixed infection bottles were not considered while calculating the mean TTD of a species in a blood culture bottle.

Results

Of the 2,105 isolates, 972 (46.2%) were Gram-positive bacteria, 1,024 (48.6%) were Gram-negative bacteria and 109 (5.2%) were fungi. The 10 leading pathogens were coagulase-negative *staphylococcus* (CoNS) (22.0%), *Escherichia coli* (15.0%), *Klebsiella pneumoniae* (10.0%), *Staphylococcus aureus* (8.0%), *Pseudomonas aeruginosa* (3.2%), *Acinetobacter baumannii* (3.0%), Gram-positive bacilli (2.7%), *Enterobacter cloacae* (2.6%), *Candida albicans* (2.2%), and *Enterococcus faecium* (1.8%). Among the 2,105 isolates, 1,565 isolates (74.3%) were from patients with infection due to single species, and 540 isolates (25.7%) were from patients with infection due to more than one species. Only 1,565 isolates were analyzed for growth in both aerobic and anaerobic blood culture bottles. Among these, 105 different species were found, and the top 10 species accounted for 71.4% of the isolates (the remaining 95 species accounted for 28.6% of the isolates). Of the 1,565 isolates, 580 (37.1%) were detected in the aerobic bottle only, 355 (22.7%) in the anaerobic bottle only, and 630 (40.3%) in both the aerobic and anaerobic bottles.

As shown in Table 1, among the top 10 pathogens, 386 isolates (24.7%) grew only in the aerobic bottle, 236 (15.1%) in the anaerobic bottle only, and 496 (31.7%) in both the aerobic and anaerobic bottles. CoNS predominantly grew in the aerobic bottles (46.3%). Growth of *C. albicans* and *A. baumannii* was not observed in the anaerobic bottles. Almost all the isolates of *P. aeruginosa* grew in the aerobic bottle; only one isolate grew in an anaerobic bottle. For *E. coli* and *S. aureus*, 16.1% and 13.7%, respectively, were positive in the aerobic bottle only; and 18.9% and 19.3%, respectively, were positive in the anaerobic bottle. We also found that 1.4% of the 1,565 isolates were obligate anaerobes, including *Bacteroides* spp. (20 isolates) and *Clostridium perfringens* (3 isolates).

The TTD values of the aerobic and anaerobic bottles processed during the day were combined and compared with the aerobic and anaerobic bottles processed after overnight delay (Table 2). Due to overnight delay (~15 hours) in loading blood culture bottles into the instrument,

Table 1. Top 10 pathogens detected in BACTEC blood culture bottles at the National Cheng Kung University Hospital

Pathogen	No. of isolates	Positive detection		
		Aerobic bottle	Anaerobic bottle	Both bottles
Coagulase-negative Staphlococcus	322	149 (46.3)	116 (36.0)	57 (17.7)
Escherichia coli	254	41 (16.1)	48 (18.9)	165 (65.0)
Klebsiella pneumoniae	163	24 (14.7)	26 (16.0)	113 (69.3)
Staphylococcus aureus	161	22 (13.7)	31 (19.3)	108 (67.0)
Pseudomonas aeruginosa	52	46 (88.5)	1 (1.9)	5 (9.6)
Candida albicans	41	37 (90.2)	0	4 (9.8)
Gram-positive bacilli	39	33 (84.6)	3 (7.7)	3 (7.7)
Enterobacter cloacae	39	7 (18.0)	5 (12.8)	27 (69.2)
Acinetobacter baumannii	26	24 (92.3)	0	2 (7.4)
Enterococcus faecium	21	3 (14.3)	6 (28.6)	12 (57.1)

Table 2. Time to positive detection in all the isolates during the day and night

Pathogen	*n*	No. of isolates	No. of aerobic	No. of anaerobic	Average time (hr)	Difference (hr)
Coagulase-negative *Staphlococcus*	321	140/181	75/119	65/62	29.0/25.9	3.1
Escherichia coli	248	96/152	42/53	54/99	9.8/9.3	0.5
Klebsiella pneumoniae	155	57/98	20/43	37/55	13.0/9.4	3.6
Staphylococcus aureus	158	55/103	38/51	17/52	17.6/17.4	0.2
Pseudomonas aeruginosa	52	19/33	18/33	1/0	19.2/14.7	4.4
Candida albicans	41	23/18	22/17	1/1	39.1/40.3	–1.2
Gram-positive bacilli	39	11/28	10/26	1/2	26.9/47.7	–20.8
Enterobacter cloacae	36	15/21	7/9	8/12	13.9/10.5	3.4
Acinetobacter baumannii	26	15/11	15/10	0/1	11.6/7.8	3.8
Enterococcus faecium	20	10/10	3/3	7/7	12.9/9.3	3.6

Table 3. Time to positive detection in aerobic and anaerobic bottles during the day

Pathogen	*n*	Aerobic		Anaerobic		Difference (hr)
		No. of isolates	Average time (hr)	No. of isolates	Average time (hr)	
Coagulase-negative *Staphylococcus*	140	75	23.4	65	35.4	–12.0
Escherichia coli	96	42	10.9	54	9.0	1.9
Klebsiella pneumoniae	57	20	18.6	37	9.9	8.7
Staphylococcus aureus	55	38	17.4	17	18.2	–0.7
Pseudomonas aeruginosa	19	18	17.4	1	50.9	–[a]
Candida albicans	23	22	40.0	1	18.5	–[a]
Gram-positive bacilli	11	10	26.1	1	35.4	–[a]
Enterobacter cloacae	15	7	19.7	8	8.8	10.9
Acinetobacter baumannii	15	15	11.6	0	0	–[a]
Enterococcus faecium	10	3	9.4	7	14.4	–5.0

[a]Not significant (in the anaerobic blood culture bottle=1 isolate was detected).

for 10 leading pathogens, a decrease in TTD detection by ≤4.4 hours was observed, whereas for Gram-positive bacilli TTD increased by 20.8 hours. In general, the TTD values of blood culture bottles processed after overnight delay were short for *Enterobacteriaceae*, including *E. coli* (9.3 hours), *K. pneumoniae* (9.4 hours), *A. baumannii* (7.8 hours), and *E. faecium* (9.3 hours), and long for *C. albicans* (40.3 hours) and Gram-positive bacilli (47.7 hours).

During the day, either immediate or delayed loading (≤2 hours) of the blood culture bottles into the BACTEC 9240 automatic blood culture detection system may occur at the hospital. The difference between TTD in aerobic versus anaerobic bottles was higher in CoNS (12 hours) and lower in *E. coli* and *S. aureus* (<2 hours) (Table 3). We also found that the average TTD for *K. pneumoniae* was shorter in the anaerobic bottles (9.9 hours) compared with aerobic bottles (18.6 hours); similar to *E. cloacae*. Growth was detected predominantly in the aerobic bottles for *A. baumannii, P. aeruginosa,* Gram-positive bacilli and *C. albicans*.

As shown in Table 4, the TTD was longer than 72 hours in 20.5% of cases of Gram-positive bacilli, 7.3% of *C. albicans*, 5.0% of *K. pneumoniae*, and 2.4% of *S. aureus*. Isolates were detected before 72 hours for most of the pathogens including, *P. aeruginosa, E. cloacae, A. baumannii* and *E. faecium*.

Table 4. Isolates with time to positive detection longer than 72 hours among the top 10 pathogens

Pathogen	*n*	TTD > 72 hr
Coagulase-negative *Staphylococcus*	322	5 (1.5)
Escherichia coli	254	2 (0.8)
Klebsiella pneumoniae	163	8 (5.0)
Staphylococcus aureus	161	4 (2.4)
Candida albicans	41	3 (7.3)
Gram-positive bacilli	39	8 (20.5)

Data presented as *n* or *n* (%). TTD = Time to positive detection.

Discussion

In this study, TTD values of the aerobic and anaerobic bottles processed in the daytime were combined and compared with overnight delayed and processed bottles. We found that due to overnight delay in loading the bottles into the instrument, TTD was decreased by 4.4 hours for the top 10 organisms compared with the bottles that were loaded during the day. The TTD value of CoNS was 29 hours during the day and 25.9 hours due to the overnight delay in loading. We predict that, with an overnight delay of ~ 15 hours, the overall time to detect the pathogen may increase from 25.9 hours to 40 hours, which might have an influence on clinical therapy. Interestingly, due to overnight delay in three of the major pathogens, *E. coli, S. aureus* and *C. albicans* (9.4 hours, 17.4 hours, and 40.3 hours, respectively), no difference in the TTD was observed in bottles processed during the day and after overnight delay, suggesting that the delayed entry of the blood culture bottle into the instrument may affect the detection time. Chapin and Lauderdale, using seeded BACTEC blood culture bottles, reported that due to an 8 hour delay at room temperature, the TTD reduced by 1 hour (to 6 hours) for *Enterobacteriaceae* pathogens, *Staphylococcus* spp. *Streptococcus* spp. and *Candida albicans,* and a delay of 24 hours led to a 6–15-hour decrease in TTD.[1] A few reports showed that delays in loading, the storage temperature, and the inoculum size and aeration were some of the factors involved in influencing the TTD of several pathogens; however, we cannot make direct comparisons with our data due to differences in study design, handling, and delays in loading and detection time.[1,3,4,8]

Several studies have reported the use of BACTEC series bottles for Gram-positive bacilli.[5,10] However, very few studies report the TTD values for all Gram-positive bacilli. Wilson et al reported that the TTD was 32.9 hours (6 isolates) in BACTEC NR6 aerobic bottles and 30.1 hours (2 isolates) in BACTEC NR7A anaerobic bottles.[11] In the present study, due to overnight delay in loading the blood culture bottles, the TTD increased from 26.9 hours to 47.7 hours; with an additional 15 hours due to overnight delay we predict that actual TTD would be 62.7 hours. Further investigations are needed to analyze the clinical significance of an increase in TTD on the outcome of clinical therapy. Since there is only one shift for the microbiology laboratory in most hospitals in Taiwan and South-eastern Asia, immediate loading is recommended for early detection of blood culture bottles.

In the present study, 37.1% of the isolates were detected in aerobic bottles only, 22.7% of the isolates in anaerobic bottles only, and 40.3% of the isolates in both the aerobic and anaerobic bottles. The previous study detected a lower percentage of pathogens in aerobic or anaerobic bottles only, compared with our study. Using the BACTEC 9240 system, Ciobutaro et al detected growth in aerobic bottles (19.6%) and anaerobic bottles (8.5%) and both bottles (72.0%).[2] In this study, we found that a single aerobic bottle was sufficient to detect *C. albicans, A. baumannii,* and *P. aeruginosa* isolates in this region. Horvath et al also reported that growth of *C. albicans* was positive in the aerobic bottle only.[3,4] Strict aerobes like *C. albicans* and *P. aeruginosa* account for 5.9% of the 1,595 isolates, which is lower than reports from Australia (10%) and the United States (15%).[8,12] We also found that 36% of the CoNS isolates were detected in anaerobic bottles only. A high number of isolates positive for *E. faecium* (28.6%), *S. aureus* (19.3%), *E. coli* (18.9%) and *K. pneumoniae* (16.0%) were also detected in anaerobic bottles only. This clearly indicates that use of aerobic bottles alone would result in a high number of false negative bottles. Chiarini et al reported that a lower percentage of CoNS (28.8% aerobic and 6.5% anaerobic) and *S. aureus* (10.6% aerobic and 4.5% anaerobic) isolates were detected in only one type of incubation compared with our study.[13] For *S. aureus* isolates, Khanna and Collignon reported that 14% of the isolates were detected in the aerobic bottle only, similar to our results, but a lower percentage was detected in the anaerobic bottles only (9%).[14]

We also found that only 1.4% of the total isolates were obligate anaerobes. Previous reports also have shown that the level of obligate anaerobe-mediated bacteremia is very low in the United States (2.2%), in Israel (1.5%) and in Japan (0.1–1.4%).[2,5,6] Although use of anaerobic bottles is debated, our data supports several studies that recommend the use of both bottles.[14,15] The use of both aerobic and anaerobic bottles increases the volume of blood cultured, and thus increases the isolation and early detection of facultative anaerobes, or the isolation and detection of those that are only positive when cultured in the anaerobic blood culture bottle.[16]

In our study, we also compared the TTD values in aerobic and anaerobic bottles that were processed during the day, and found a 12-hour difference between the aerobic and anaerobic bottles (23.4 hours *vs.* 35.4 hours) in CoNS. Murray et al reported that, in CoNS, the TTD was 22.9 hours for aerobic bottles and 19.2 hours for anaerobic bottles, with a possible delay during transportation.[8]

In a controlled study designed to detect *K. pneumoniae* in blood culture bottles, it was reported that, when comparing immediate loading versus an 8 hour delay in loading, the TTD was 11.0 hours and 8.6 hours respectively in the aerobic bottles, and 10.7 hours and 7.5 hours respectively in the anaerobic bottles.[1] In the present study, a higher TTD was found for *K. pneumoniae* in aerobic (18.6 hours) and anaerobic (9.9 hours) bottles. Since the difference in TTD between aerobic and anaerobic bottles for *E. coli* is 1.9 hours, its clinical significance might be minimal. However, since the difference between aerobic and anaerobic bottles was > 8 hours for CoNS and *K. pneumoniae*, its clinical significance needs to be evaluated. A TTD of 40 hours was observed for *C. albicans* isolates in aerobic bottles. Meyer et al reported a similar average detection time of 39.9 hours for *C. albicans* using Plus aerobic/F bottles.[17] Horvath et al reported that in a simulated candidemia by *C. albicans*, the TTD was 23.52 ± 2.64 hours in aerobic bottles, while in clinical isolates the TTD was 20.63 ± 1.57 hours.[3,4] However, no growth was observed in anaerobic bottles in either of these studies, but we detected growth in 10% of the isolates in anaerobic bottles in our study.

Finally, if a 3-day processing of the blood culture bottles in the instrument had been employed at NCKU hospital, 20% of Gram-positive bacilli isolates, and 7.3% of *C. albicans,* would have been false negatives. However, blood bottles are currently processed for 5 days at the NCKU hospital.[5] A study by Reisner and Wood showed that 4 days of incubation was sufficient to recover all clinically relevant bacteria using the BACTEC 9240 system.[18] Although 3% of the isolates were detected after 3 days of incubation in this study, we did not analyze whether a change in therapy was recommended based on the 4th or 5th day detection of pathogens in the blood culture bottles; hence we cannot recommend the use of a 3 day incubation period.

In conclusion, we found that due to overnight delays in loading the bottles into the instrument, the TTD was only ≤ 4.4 hours for most of the pathogens, which might be clinically significant. Hence, if there is no delay in entering the blood culture bottle into the instrument, it will shorten the TTD in the overnight bottles. In addition, 15.1% of the top 10 pathogens were only detected in anaerobic bottles. Since high numbers of facultative anaerobes were detected, the use of a single aerobic bottle might have a detrimental effect on the clinical therapy outcome.

Acknowledgments

This work was partly supported by grants NSC-2320-B-006-085 from the National Science Council, Taiwan and NCKUH 9703004 from the National Cheng-Kung University Hospital, Taiwan.

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