Predominance of *Enterobacteriaceae* Isolates in Early Positive Anaerobic Blood Culture Bottles in BacT/Alert System

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We collected and analyzed the time to detection (TTD) of blood cultures in the BacT/Alert automated system from 2002 to 2007. Among the 10,893 monomicrobial isolates from a total of 133,735 blood culture sets, the recoveries of aerobic bottles were compared with those of anaerobic bottles in this study. Significantly more Gram-positive cocci (except *Staphylococcus aureus* and enterococci), glucose non-fermentative Gram-negative bacteria, and yeast were recovered from aerobic bottles than from anaerobic bottles. The average TTD was 19.0 hr and 20.1 hr for the aerobic and anaerobic bottles, respectively, and 96.8% of the microorganisms were detected

within the first 72 hr. Of the 5,489 microorganisms recovered from both of the blood culture bottle pair, microbial growth was significantly more often detected first in the anaerobic bottles than the aerobic bottles for *Enterobacteriaceae* except *Serratia marcescens*, while *S. aureus*, coagulase-negative staphylococci and *Pseudomonas aeruginosa* were more often detected first in the aerobic bottles. According to these data, we conclude that the earlier positivity of anaerobic bottles is a useful marker for rapid presumptive identification of *Enterobacteriaceae* infection. J. Clin. Lab. Anal. 27:113–120, 2013.

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INTRODUCTION

Bacteremia is one of the most serious problems in all infectious diseases with a mortality rate above 20% among hospitalized patients (1, 2). Rapid detection and identification of the microorganisms in blood cultures enables early administration of appropriate antibiotic treatment, which is critically important for improving the mortality rate (3, 4). Despite recent developments, such as nucleic acid probes (5), polymerase chain reaction (6), and other new molecular techniques for microbiological diagnosis (7, 8), blood cultures still remain the most practical and reliable method for the diagnosis of bacteremia (9, 10).

Typically, a routine blood culture for adults consists of paired aerobic and anaerobic blood culture bottles.

An aerobic bottle allows for preferential growth of aerobic and facultative anaerobic microorganisms, whereas an anaerobic bottle allows for preferential growth of strictly anaerobic bacteria (11). A number of parameters have been evaluated to improve the performance and to reduce

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the cost related to the usage of blood cultures. Several studies have shown that inoculating the same volume of blood into two aerobic blood culture bottles, at least on a selective basis, could be more clinically beneficial than the traditional aerobic-anaerobic pair (12–15). Some have even challenged the need for the use of a routine anaerobic blood culture bottle. However, by comparing the TTD (time to detection) of aerobic bottles with anaerobic bottles, the TTD difference between the aerobic and anaerobic bottles was presumed related to the particular microorganism isolated. Thus, the use of an anaerobic bottle still seems warranted (16, 17).

The goal of this study was to compare the yields and TTD of various microorganisms isolated from the aerobic and anaerobic blood culture bottles by using the BacT/Alert system (bioMérieux, Lyon, France), and then the probability of predicting microorganisms by a combination of the Gram stain results and the first positive bottle type before final identification was evaluated.

MATERIALS AND METHODS

Blood Culture Collection

All blood samples in this study were obtained from adult patients from Tri-Service General Hospital, National Defense Medical Center (Taipei, Taiwan) between January 2002 and December 2007. The BacT/Alert blood culture system (bioMérieux) was used throughout the study period. All blood cultures were performed on the demand of clinical physicians. The blood samples were drawn after strictly cleansing the patient's skin with 70% isopropyl alcohol and applying 10% povidone-iodine for 1 min. The blood samples were inoculated at a volume of 8 to 10 ml into an aerobic and an anaerobic bottle (the standard aerobic with the anaerobic bottles or the BacT/Alert FAN aerobic bottle and BacT/Alert FAN anaerobic bottle.) as one set. The choice of bottle pairs was based on whether any antibiotic was used. All blood culture bottles must be sent to the laboratory and immediately loaded onto the BacT/Alert system according to the standard procedure in our hospital. The exact time of venipuncture was usually marked on the requisition sheet accompanied with the blood culture bottles. The variation of transportation period was however neglected, because we focused on comparing the initial detection intervals of the bottles in each pair of blood culture after loading them onto the BacT/Alert system.

Laboratory Processing

All bottles were stored under a temperature of 35°C in the BacT/Alert system for a 7-day incubation period and monitored in accordance with the manufacturer's rec-

ommendations. Whenever there was a sign of microbial growth, the TTD was recorded and Gram stain was performed. All positive bottles were systematically plated on Columbia colistin-nalidixic acid agar (CNA) with 5% sheep blood, MacConkey agar, chocolate agar, and anaerobic sheep blood agar. Subcultures were incubated at 35°C for 48 hr. When growth was detected, the Gram-negative bacteria (GNB) were identified by using the VITEK identification system (bioMérieux, Marcy l'Etoile, France) and the other bacteria and yeast were identified through manual procedures. All microorganisms were considered clinically significant, except that a single positive culture of *Corynebacterium* spp., coagulase-negative staphylococci, viridans group streptococci, *Bacillus* spp., aerobic diphtheroids, and *Propionibacterium* spp. in only one bottle was interpreted to be a contaminant. The TTD was determined the inoculation of the bottles to the positive signaling in the instrument. If the microorganism grew in both bottles of the blood culture, the TTDs of the aerobic bottles and the anaerobic bottles were compared. They were considered to be positive at the same time if the TTD difference between the bottles was less than 0.2 hr (12 min).

Data Analyses

Each pair of blood culture bottle was considered an independent sample without checking whether it is the first or recurrent positivity. We only compared single microbial culture results because of the limited information about growth interaction among microbials. Neither contaminated nor multimicrobial culture results were excluded from this study. Statistical analyses of aerobic bottle to anaerobic bottle comparisons were done by a Chi-square test, with statistical significance at the 0.05 level ($P < 0.05$). Positive predictive value (PPV) is the proportion of positive blood bottles that are true positive for *Enterobacteriaceae* isolates. Estimates were presented with 95% confidence intervals (CI). All statistical analyses were done by using the Statistical Package for the Social Sciences software, version 10 (SPSS, Inc., Chicago, IL).

RESULTS

Proportion of Positive Blood Cultures

In the present study, a total of 133,735 blood culture sets were collected during the study period. Of these, 13,106 (9.8%) grew microorganisms, including 10,893 (8.2%) grew single microorganism, 933 (0.7%) grew more than one microorganisms, and 1,280 (1.0%) grew contaminants. The comparative yield of blood cultures from aerobic and anaerobic bottles is summarized in Table 1. Among the 10,893 monomicrobial isolates, 4,032 (37.0%)

TABLE 1. Comparative Yields of Clinically Important Bacteria and Yeast in BacT/Alert Aerobic and Anaerobic Blood Culture Bottles

Group and microorganism	Number of isolates	% of total	Number of isolates recovered from ^a :			P -value
			Aer + / Ana –	Aer + / Ana +	Aer – / Ana +	
Gram-positive cocci	4,032	37.0	856	2,521	655	<0.001
<i>Staphylococcus aureus</i>	2,099	19.3	229	1,558	312	<0.001
Coagulase-negative staphylococci	1,104	10.1	403	476	225	<0.001
Streptococci	477	4.4	93	325	59	0.006
Enterococci	265	2.4	51	159	55	0.698
<i>Micrococcus</i> species	87	0.8	80	3	4	<0.001
Enterobacteriaceae	4,385	40.3	773	2,803	809	0.365
<i>Escherichia coli</i>	2,120	19.5	390	1,307	423	0.247
<i>Klebsiella pneumoniae</i>	1,108	10.2	200	742	166	0.076
Other Enterobacteriaceae	367	3.4	56	233	76	0.082
<i>Enterobacter cloacae</i>	265	2.4	42	184	39	0.739
<i>Serratia marcescens</i>	228	2.1	33	167	28	0.522
<i>Salmonella</i> species	190	1.7	36	105	49	0.159
<i>Proteus mirabilis</i>	107	1.0	16	65	28	0.070
Glucose nonfermentation GNB	1,237	11.4	1,121	93	23	<0.001
<i>Pseudomonas aeruginosa</i>	373	3.4	322	41	10	<0.001
Other GNF-GNB	367	3.4	329	29	9	<0.001
<i>Acinetobacter baumannii</i>	343	3.1	321	19	3	<0.001
<i>Stenotrophomonas maltophilia</i>	154	1.4	149	4	1	<0.001
Gram-positive bacilli	132	1.2	66	36	30	<0.001
Other bacteria	18	0.2	9	5	4	0.166
Fungi	837	7.7	807	23	7	<0.001
Anaerobic bacteria	252	2.3	5	8	239	<0.001
Anaerobic GNB	148	1.0	1	2	147	<0.001
Other anaerobic bacteria	104	1.4	4	6	92	<0.001
All microorganisms	10,893	100.0	3,637	5,489	1,767	<0.001

^a Aer +, positive aerobic bottle; Aer –, negative aerobic bottle; Ana +, positive anaerobic bottle; Ana –, negative anaerobic bottle.

were Gram-positive cocci, 4,385 (40.3%) were *Enterobacteriaceae*, 1,237 (11.4%) were glucose nonfermentative GNB, 837 (7.7%) were yeast, and 252 (2.3%) were anaerobic bacteria. The majorities of the identified microorganisms were ranked sequentially as *Staphylococcus aureus* (2099, 19.3%), coagulase-negative staphylococci (1104, 10.1%), *Escherichia coli* (2120, 19.5%), *Klebsiella pneumoniae* (1108, 10.2%), *Pseudomonas aeruginosa* (373, 3.4%), and anaerobic bacteria (252, 2.3%). These estimates remained stable across the 6-year research period (data not shown).

Comparative Yields from Aerobic and Anaerobic Bottles

The two bottles yielded 10,893 clinically important microorganisms, of which 5,489 (50.4%) were recovered from both bottles, 3,637 (33.4%) from the aerobic bottles only, and 1,767 (16.2%) from the anaerobic bottles only ($P < 0.001$). More coagulase-negative staphylococci, streptococci, *Micrococcus* species, glucose nonfermentative GNB, Gram-positive bacilli, and yeast were recovered from the aerobic bottles only, compared with anaerobic

bottles only ($P < 0.001$). Conversely, more isolates of *S. aureus* or anaerobic bacteria were recovered from the anaerobic bottles only rather than from the aerobic bottles only ($P < 0.001$). For the 252 anaerobic microorganisms, which amounted to 2.3% of all microorganisms, 94.8% of them were isolated only in the anaerobic bottles without any concomitant growth in the aerobic bottles (Table 1). There was no statistically significant difference in the isolation preference for the rest of 4,385 *Enterobacteriaceae*.

Comparing the TTD of Aerobic and Anaerobic Bottles

Table 2 shows the average TTD for each microorganism recovered from the aerobic and anaerobic blood culture bottles by using the BacT/Alert automated system. About 97.7 and 95.7% of those microorganisms could be detected in the aerobic and anaerobic bottles within 72 hr. The average TTD was 19.0 hr and 20.1 hr for the aerobic and anaerobic bottles, respectively. As indicated in Table 2, for most microorganisms the TTD difference between the two bottles was only about 1–3 hr, although the growth of

TABLE 2. Time to Detection of Pathogens Isolated from 16,382 Aerobic or Anaerobic Blood Culture Bottles

Group and microorganism	Aerobic bottles					Anaerobic bottles				
	n	Time to detection of growth (hr)			Percentage of positive recovery in 72 hr	n	Time to detection of growth (hr)			Percentage of positive recovery in 72 hr
		Mean	Median	Range			Mean	Median	Range	
Gram-positive cocci	3,377	20.5	16	2–163.2	98.1	3,176	23.5	18	2–158.4	96.4
<i>Staphylococcus aureus</i>	1787	17.1	14	2–156	99.3	1,870	21.2	17	2–141.6	97.8
Coagulase-negative staphylococci	879	29.1	23	2.2–163.2	96.7	701	35.1	28	5.3–158.4	91.7
Streptococci	418	15.3	11	2–163.2	98.3	384	18.5	11	2–132	96.4
Enterococci	210	13.3	11	2–115.2	99.5	214	13.7	12	2–76.8	99.5
<i>Micrococcus</i> species	83	47.4	36	12.7–132	84.3	7	40.2	46	16.7–62.4	100.0
<i>Enterobacteriaceae</i>	3576	12.7	9	2–141.6	98.8	3,612	14.1	9	2–158.4	97.0
<i>Escherichia coli</i>	1697	11.5	9	2–134.4	99.4	1,730	12.5	8	2–144	97.4
<i>K. pneumoniae</i>	942	13.6	9	2–141.6	98.2	908	14.6	9	2–136.8	96.8
Other <i>Enterobacteriaceae</i>	289	11.7	9	2–122.4	99.3	309	14.4	10	2–136.8	97.7
<i>Enterobacter cloacae</i>	226	12.4	10	2–108	98.2	223	14.2	10	2–112.8	98.2
<i>Serratia marcescens</i>	200	15.8	11	2–141.6	98.5	195	18.6	12	2–136.8	95.4
<i>Salmonella</i> species	141	16.7	12	2.2–105.6	97.2	154	20.2	11.5	2.2–129.6	94.8
<i>Proteus mirabilis</i>	81	16.4	13	2–136.8	98.8	93	19.0	12	2–158.4	94.6
Glucose nonfermentative GNB	1214	19.6	15	2–153.6	97.7	116	25.9	17	2.2–132	94.0
<i>Pseudomonas aeruginosa</i>	363	19.0	15	2.2–127.2	97.3	51	21.7	16	8.7–132	98.0
Other GNF-GNB	358	24.8	19	2.2–153.6	95.8	38	29.8	21	2.2–120	89.5
<i>Acinetobacter baumannii</i>	340	12.1	11	2–60	100.0	22	23.0	14	8–105.6	95.5
<i>Stenotrophomonas maltophilia</i>	153	25.4	21	5.5–136.8	98.0	5	50.3	60	20.5–81.6	80.0
Gram-positive bacilli	102	36.4	34.5	2–129.6	95.1	66	45.9	20.5	3.7–139.2	71.2
Other bacteria	14	41.8	21	3.8–117.6	71.4	9	49.0	26	19.3–100.8	77.8
Yeast	830	36.0	30	2–139	92.9	30	44.3	37	12–127.2	83.3
Anaerobic bacteria	13	67.8	70	2–129.6	53.9	247	50.0	38	2.2–151.2	76.9
Anaerobic GNB	3	47.9	50	2–91.2	66.7	149	39.3	33	5.3–122.4	89.3
Other anaerobic bacteria	10	73.8	72	19.5–129.6	50.0	98	66.1	58	2.2–151.2	58.2
All Microorganisms	9126	19.0	13	2–163.2	97.7	7256	20.1	13	2–158.4	95.7

coagulase-negative staphylococci and yeast was detected apparently earlier in the aerobic bottles (median at 23 and 30 hr) than in the anaerobic bottles (median at 28 and 37 hr). The growth of Gram-positive bacteria was detected earlier in the anaerobic bottles with a median of 20.5 hr.

The TTD of microorganism in each aerobic–anaerobic bottle pair is compared in Table 3. Of the 5,489 microorganisms recovered from both aerobic and anaerobic bottles, growth of 655 (11.9%) microorganisms was detected at the same time in both bottles, growth of 2,429 (44.3%) microorganisms was detected first in the anaerobic bottles, and growth of 2,405 (43.8%) microorganisms was detected first in the aerobic bottles ($P > 0.05$, not significant). For the individual microorganism, detectable growth was observed for *S. aureus* ($P < 0.001$), coagulase-negative staphylococci ($P = 0.006$) and *P. aeruginosa* ($P < 0.001$) from the aerobic bottles earlier than from the anaerobic bottles. On the other hand, detectable growth was observed for *E. coli* ($P < 0.001$), *K. pneumoniae* ($P < 0.001$), *Enterobacter cloacae* ($P < 0.001$), *Salmonella* species ($P < 0.001$), and *Proteus mirabilis* ($P < 0.001$) from the anaerobic bottles earlier than from the aerobic bottles.

Preferential Strategy for Predicting *Enterobacteriaceae* Isolation

There were total 5,772 GNB results of Gram stain in the study. Subtotal isolates of GNB from aerobic bottles only, anaerobic bottles only and both bottles were 1895, 979 and 2898, respectively. Of the 2,898 GNB recovered from both bottles, 514 GNB isolates were detected from both bottles concurrently, 739 GNB isolates were detected from the aerobic bottles earlier, and 1,645 GNB isolates were detected from the anaerobic bottles first. There were a total of 4,385 isolates of *Enterobacteriaceae* in this study. Subtotal isolates of *Enterobacteriaceae* from aerobic bottles only, anaerobic bottles only and both bottles were 773, 809 and 2,803, respectively. Of the 2,803 isolates of *Enterobacteriaceae*, 511 isolates were detected from both bottles concurrently, 675 isolates were detected first from the aerobic bottles, and 1,617 isolates were detected first from the anaerobic bottles. In the third phase of the study, we evaluated several strategies to ensure the presumptive identification of *Enterobacteriaceae* bacteremia by using the preferential earlier TTD from the anaerobic bottles (Table 4). The following three strategies were applied for selecting the potential blood culture bottles of

TABLE 3. Time to Detection (TTD) of Bacteria and Yeasts Growing in Aerobic and Anaerobic Bottles of the BacT/Alert system. Less than 0.2 hr between TTDs of Two Blood Culture Bottles Was Considered As No Difference Arbitrarily

Group and microorganism	Number of isolates	Average of TTD (hr)		Number of bottles until growth was detected			P-value
		Aerobic bottle	Anaerobic bottle	Aerobic bottle earlier	Aerobic bottle and anaerobic bottle at same time	Anaerobic bottle earlier	
Gram-positive cocci	2,521	17.7	20.4	1,623	139	759	<0.001
<i>Staphylococcus aureus</i>	1,558	16.1	18.2	1,166	66	326	<0.001
Coagulase-negative staphylococci	476	26.8	32.7	262	11	203	0.006
Streptococci	325	14.5	16.8	129	34	162	0.053
Enterococci	159	12.2	11.6	63	28	68	0.662
<i>Micrococcus</i> species	3	31.0	47.6	3	0	0	0.083
<i>Enterobacteriaceae</i>	2,803	10.9	11.1	675	511	1,617	<0.001
<i>Escherichia coli</i>	1,307	10.0	9.8	233	236	838	<0.001
<i>Klebsiella pneumoniae</i>	742	11.2	11.7	222	141	379	<0.001
Other <i>Enterobacteriaceae</i>	233	10.6	11.2	64	41	128	<0.001
<i>Enterobacter cloacae</i>	184	11.1	11.5	44	45	95	<0.001
<i>Serratia marcescens</i>	167	14.5	15.8	80	26	61	0.110
<i>Salmonella</i> species	105	12.7	13.1	25	9	71	<0.001
<i>Proteus mirabilis</i>	65	15.0	11.2	7	13	45	<0.001
Glucose nonfermentation GNB	93	17.2	23.4	63	3	27	<0.001
<i>Pseudomonas aeruginosa</i>	41	16.2	19.9	28	0	13	0.019
Other GNF-GNB	29	20.3	25.8	18	2	9	0.083
<i>Acinetobacter baumannii</i>	19	12.1	20.0	13	1	5	0.059
<i>Stenotrophomonas maltophilia</i>	4	29.4	57.7	4	0	0	0.046
Gram-positive bacilli	36	20.0	31.2	21	1	14	0.237
Other bacteria	5	43.2	36.5	3	1	1	0.317
Fungi	23	28.1	45.6	17	0	6	0.022
Anaerobic bacteria	8			3	0	5	0.480
Anaerobic GNB	2	46.6	23.9	1	0	1	1.000
Other anaerobic bacteria	6	78.6	64.0	2	0	4	0.414
All microorganism	5,489	14.4	15.9	2,405	655	2,429	0.730

Enterobacteriaceae isolation (Table 4) (1). Besides the GNB finding in Gram stain, the microorganisms detected solely or earlier from the aerobic bottles were selected as 2,634 (1,895 + 739) (2). Besides the GNB finding in Gram stain, the microorganisms detected solely or earlier from the anaerobic bottles were selected as 2,624 (979 + 1,645) (3). Besides the GNB finding in Gram stain, the microorganisms concomitantly detected from both blood

culture bottles at the same time were selected as 514. The PPV for predicting *Enterobacteriaceae* isolation was then represented by the rate of *Enterobacteriaceae* isolate in each selected set. The total isolation rate of *Enterobacteriaceae* was 40.3% (95% CI, 39.3–41.2%) without any selection, and the *Enterobacteriaceae* proportion was up to 76.0% (95% CI, 74.9–77.1%) among all bottles of GNB finding. However, we combined GNB finding with the

TABLE 4. Strategy for Predicting *Enterobacteriaceae* Isolations

Strategy	Number of <i>Enterobacteriaceae</i> (True <i>Enterobacteriaceae</i> / prediction)	% PPV (95% CI)
Strategy 1: The microorganisms were detected alone or earlier in aerobic bottle than anaerobic bottle and Gram stain results were GNB	1,448 / 2,634 (773 + 675) / (1,895 + 739)	55.0 (53.1–56.9)*
Strategy 2: The microorganisms were detected alone or earlier in anaerobic bottle than aerobic bottle and Gram stain results were GNB	2,426/2,624 (809 + 1,617) / (979 + 1,645)	92.5 (91.4–93.4) *
Strategy 3: The microorganisms were detected at the same time in both bottles and Gram stain results were GNB	511 / 514	99.4 (98.3–99.9) *

* χ^2 -test, $p < 0.001$.

CI, confidence intervals; PPV, positive predictive value.

information of sole or first positive bottle type to predict *Enterobacteriaceae*. The proportion of *Enterobacteriaceae* dropped to 55.0% (95% CI, 53.1–56.9%) while evaluating the GNB microorganisms solely or earlier detected from the aerobic bottles in strategy 1. Conversely, the proportion of *Enterobacteriaceae* accounted for 92.5% (95% CI, 91.4–93.4%) of the GNB isolates solely or earlier detected from the anaerobic bottles in strategy 2. Finally, the proportion of *Enterobacteriaceae* accounted for 99.4 (98.3%–99.9%) of the GNB isolates detected from both the aerobic and anaerobic bottles at the same time in the strategy 3. The three strategies generated significantly different results (χ^2 -test, $P < 0.001$)

DISCUSSION

Blood cultures are among the most frequently ordered and clinically important tests performed in any clinical microbiology laboratory. To date, a lot of information has been published regarding the performance of different blood culture systems (18–20), different bottle types (21, 22), and the individual bottle of the blood culture pair (13, 23) in detecting bloodstream pathogens. In this study, we analyzed and added more information about the different performance of individual bottles of the blood culture pair in recovering bacteria or yeast from patients subjected to routine clinical practices.

Our findings showed that the aerobic bottles significantly outperformed the anaerobic bottles in detecting more microorganisms of coagulase-negative staphylococci, streptococci, *Micrococcus* species, glucose nonfermentative GNB, and yeast. This is expected, since these microorganisms were recovered optimally from an aerobic medium. For these microorganisms, greater yields from the aerobic bottles can be explained by higher oxygen content in the bottle, given that glucose nonfermentative GNB and yeast are strictly aerobic microorganisms (21, 22). In contrast, lower oxygen content in the anaerobic bottles resulted in earlier detection of *S. aureus*, coagulase-negative staphylococci, and glucose nonfermentative GNB. We found that 97.7 and 95.7% of all clinically significant bacterial and fungal isolates were detected within the first 3 days of incubation using both the aerobic and anaerobic blood culture bottles. Previous studies have demonstrated that a BacT/Alert FAN aerobic bottle combined with a BacT/Alert standard anaerobic bottle could detect 95–97% of all clinically significant microorganisms within 3 days of incubation (15, 24). In another study, it was found that all clinically significant episodes of bacteremia were detected within 4 days of incubation when paired FAN aerobic and FAN anaerobic bottles were utilized (25). Most importantly, it was found the TTD is crucial for the management of septicemia patients. The earlier the pathogen is detected is

better, in terms of initiating an appropriate empiric therapeutic regimen. The average TTD of microorganism in the BacT/Alert system was 19.0 hr for the aerobic bottles and 20.1 hr for the anaerobic bottles in this study, while it was 15–24 hr in the previous studies (26, 27). Despite this difference, the TTD of microorganisms in this study showed a wide range in both the aerobic and anaerobic bottles, which is consistent with the previous studies (18, 22, 27). It is unlikely that differences found between the median times of detection would be clinically important since it was less than 3 hr for any group of microorganisms. One should be cautious about overinterpreting the statistical differences in TTD that might have little or no clinical meaning because of the wide range of TTD and the appreciable overlap in recovery between the two bottle types.

Although the routine use of anaerobic blood culture media has been questioned (23), many laboratories continue to use a combination of an aerobic and an anaerobic medium in their routine blood culture systems (16, 22). This routine practice was challenged in a series of articles (14, 15, 23). The study presented herein, however, showed that the anaerobic bottles outperformed the aerobic bottles on account of the higher recovery rate for *S. aureus* and anaerobic bacteria, and the shorter TTD for *Enterobacteriaceae* except *Serratia marcescens*. In this study, several advantages of the anaerobic bottles were found consistent with previous studies (13, 18, 22), including (i) high percentage of the microorganisms had a significant positive blood culture detected by the anaerobic bottle alone in the absence of any growth in all aerobic bottles; (ii) for these anaerobic microorganisms, the isolated bacteria were not obligate anaerobes suggesting that the usefulness of the anaerobic bottle is not restricted to the isolation of strictly anaerobic bacteria; and (iii) when found in the aerobic and anaerobic bottles of the same blood culture, *Enterobacteriaceae* (*Enterobacteriaceae* except *S. marcescens*) grew faster in the anaerobic bottles than in the aerobic ones.

Recent retrospective studies on *S. aureus* bacteremia have shown that TTD in blood culture alone may yield useful information regarding the source of bacteremia (28, 29). The TTD of *Candida* spp. in blood cultures may be a useful tool for the diagnosis of catheter-related candidemia (30). The rapid diagnosis of *Enterobacteriaceae* infection in peripheral blood has important implications for patient management (31–34). Most published methods involve the use of automated systems to decrease the turn around time for the detection of organisms in blood culture specimens and the release of negative results (35, 36). Considering *Enterobacteriaceae* are a family of rod-shaped, aerobic, facultatively anaerobic bacteria, this means that while these bacteria can survive in the presence of oxygen, they prefer to live in an anaerobic (oxygen-free) environment. Among the 10,893

monomicrobial isolates, *Enterobacteriaceae* were the most common microorganisms in our retrospective study (40.3%). Glucose nonfermentative GNB was frequently isolated in the aerobic bottles alone and anaerobic GNB was rarely isolated from blood cultures (1.0%). Given these reasons, we tested for the accuracy of a strategy for the members of *Enterobacteriaceae* based on the direct Gram stain result with the TTD between the two bottles and positive solely in the anaerobic bottles. The sensitivity of strategies 2 and 3 for the diagnosis of *Enterobacteriaceae* infection was high (92.5 and 99.4%) in our retrospective study.

The strengths of our study include analyzing on a 6-year large database, incorporating TTD data of each bottle between the instrument and laboratory information system, and providing another implication for predicting the blood culture isolates. Our study results and conclusion might be limited because of including the repeat positivity from the same patient, neglecting the variation of inoculation volume and simply analyzing on one blood culture system (i.e., BacT/Alert system). The isolates of *Enterobacteriaceae* were easily interpreted as Gram-negative rod bacteria because of its typical shape under microscopy. Our results also suggest that Gram stain results and the TTD of the two bottles may be a useful tool in the evaluation of patients with *Enterobacteriaceae* infection. The results of this study also support the continued, routine use of an anaerobic bottle in conjunction with an aerobic bottle in blood cultures with the BacT/Alert system in our hospital. Future studies should aim to confirm and extend our findings and to define the clinical impact of these results.

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