

Inactivation of Clinically Frequently Used Antimicrobial Agents By BacT/ALERT FAN Plus and BACTEC Aerobic and Anaerobic Culture Media in Simulated Blood Cultures: First Comparative Evaluation in China Mainland

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Purpose: To evaluate the antimicrobial inactivation capabilities of BacT/ALERT (FA Plus and FN Plus) and BACTEC (Plus Aerobic/F and Lytic/10 Anaerobic/F) media.

Patients and Methods: The inactivation capabilities of the commercial blood culture media were compared using 21 microorganism-antimicrobial combinations in simulated adult blood cultures.

Results: BacT/ALERT culture media demonstrated higher detection rates than the BACTEC culture media. The recovery rates of the aerobic bottles were 74/115 (64.3%) for FA Plus bottles and 64/115 (55.7%) for BACTEC Aerobic Plus bottles. The BacT/ALERT FAN Plus culture media exhibited a shorter time to detection (TTD). The TTD of FA Plus media was 14.7 h, 4.85 h shorter than the BACTEC Aerobic media (19.55 h), while the TTDs of FN Plus media and BACTEC Anaerobic media were 16.8 h and 18.4 h, respectively.

Conclusion: BacT/ALERT (FA Plus and FN Plus) media showed relative, but not absolute, advantages, as it had higher detection rates and shorter TTD and thus can be selectively applied to patients with prior use of antimicrobial agents before blood culture samples are taken.

Keywords: antimicrobial inactivation, blood culture, automated system, BacT/ALERT FAN plus media, comparative evaluation

Introduction

Accurate and timely diagnoses of bloodstream infections (BSIs) are crucial for the treatment and prognosis of BSI patients, as well as to decrease the associated high morbidity and mortality.^{1,2} As the gold standard of BSI detection, blood culture samples should be collected the first time patients are suspected to have sepsis, and before antimicrobial agents are given to improve the detection rate as much as possible. However, antimicrobials are often administered before blood culture samples are collected in the urgent septic conditions. It is reported that nearly half of inpatients are administered with antimicrobials before blood culture samples are collected.³ As a result, the time to detection (TTD) of the commercial automated blood culture systems may be significantly prolonged. Even worse, due to the antimicrobial activity in blood culture samples, microbial growth in blood culture bottles could be undetectable.⁴

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Substances such as resin or charcoal which can absorb antimicrobial agents are added to some commercial blood culture media in order to minimize the inhibition of antimicrobials on microbial growth in blood culture.⁵ In 2013, BacT/ALERT FA Plus (aerobic) and FN Plus (anaerobic) bottles (bioMérieux) were developed to further improve the detection of microorganisms from sepsis patient samples. Until now, several blood culture simulation studies (involving blood culture bottles injected with whole blood, antimicrobial agents, and microorganisms) have compared the antimicrobial inactivation efficiency of BacT/ALERT FAN Plus media and BACTEC Plus media, including in both anaerobic and aerobic blood culture bottles.^{6,7} However, since the BacT/ALERT FAN Plus media was only recently approved in the Chinese mainland, data on the performance of antimicrobial agent inactivation of BacT/ALERT FAN Plus media from China mainland is not yet available.

In the present study, we aimed to evaluate the antimicrobial inactivation capabilities of BacT/ALERT FAN Plus media and BACTEC aerobic and anaerobic culture media by measuring the organism detection rate and TTD for several antimicrobial-microorganism combinations in simulated blood cultures. Specifically, the blood antimicrobial concentrations of patients with antimicrobial treatment were mimicked.

Patients and Methods

Ethics Clarification

No ethical review process was required for this simulated blood culture study, as it did not involve any animal or human experiments. In addition, as a simulated study, no specimens, microbial isolates, or any other materials from patients or healthy human bodies were involved. All the materials used in this study, including reference ATCC microorganism strains, antimicrobial agents, and horse blood were commercially available and purchased.

Blood Culture Media and Blood Culture Instrument

Resin-containing BacT/ALERT FA Plus (FA Plus) aerobic bottles, FN Plus (FN Plus) anaerobic bottles, and resin-free BacT/ALERT SA aerobic bottles and SN anaerobic bottles were applied to the automated BacT/ALERT 3D blood culture system. Resin-containing BD BACTEC Aerobic/F Plus (BD Aerobic Plus) bottles and resin-free Lytic/10

Anaerobic/F (BD Lytic/10 Anaerobic) bottles were applied to the automated BD BACTEC FX blood culture system.

Microorganisms

Microorganism species were chosen according to the frequency of recovery from positive blood cultures in our lab, and the following reference ATCC microorganism strains were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacteroides fragilis* ATCC 25285, methicillin-susceptible *Staphylococcus aureus* (MSSA) ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Candida parapsilosis* ATCC 22019. Strains were stored in skim milk and frozen at -80°C until use. Before being spiked into blood culture bottles, strains were subcultured twice on Columbia blood agar plates, and incubated at 37°C overnight. Minimum inhibitory concentrations (MICs) were tested by the Clinical Laboratory Standards Institute (CLSI) broth microdilution method in triplicate, and susceptibility categorization was determined based on the latest CLSI breakpoints (Table 1).⁸ Colonies from Columbia blood agar plates were suspended in phosphate-buffered saline, and serial dilutions of each organism were prepared to a target concentration of 100 CFU/mL, and 0.1 mL of the final dilutions were plated on the Columbia blood agar plate and incubated at 37°C overnight to verify the CFUs.

Antimicrobials

In this study, eight of the most commonly used antimicrobial agents to treat specific blood stream infections were chosen. The commercially available standards of meropenem, imipenem, levofloxacin, cefoxitin, piperacillin-tazobactam, vancomycin, voriconazole (Meilunbio, China), and linezolid (Sigma, USA) were reconstituted according to the manufacturer's instructions. The peak serum concentration (C_{max}), achieved according to standard dosing of the antimicrobial under the condition of a 70-kg adult with normal renal function, was used to simulate patient blood levels (Table 1). If no growth was detected, antimicrobials were then injected at concentrations of $C/2$ and/or $C/4$ to obtain positive results in any culture system. Each antimicrobial agent was diluted in sterile water from stock solutions to ensure that 0.3 mL of the dilution contained the desired final antimicrobial concentration of a blood culture bottle.

Blood Culture Preparation

In our simulated blood culture research, each blood culture bottle was spiked with 10 mL of sterile horse blood, 0.3 mL of microbial suspension (described above), and

Table 1 Microorganism and Antimicrobial Combinations Tested by the Simulated Adult Blood Culture Model

Species (Strain)	Antimicrobial	MIC ($\mu\text{g/mL}$) ^a	Antimicrobial Concentration ($\mu\text{g/mL}$) ^b		
			C _{max}	C/2	C/4
Gram-negative bacilli					
<i>Escherichia coli</i> (ATCC 25922)	Imipenem	0.25	40	20	10
	Meropenem	0.06	50	25	12.5
	Cefoxitin	4	110	55	27.5
	Piperacillin-tazobactam	4	242/24	121/12	60.5/6
	Levofloxacin	0.06	8.6	4.3	2.15
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	Imipenem	1	40	20	10
	Meropenem	0.25	50	25	12.5
	Piperacillin-tazobactam	4	242/24	121/12	60.5/6
	Levofloxacin	1	8.6	4.3	2.15
<i>Bacteroides fragilis</i> (ATCC 25285)	Imipenem	0.06	40	20	10
	Meropenem	0.06	50	25	12.5
	Piperacillin-tazobactam	0.25	242/24	121/12	60.5/6
Gram positive cocci					
<i>Staphylococcus aureus</i> (ATCC 29213)	Vancomycin	0.5	50	25	12.5
	Linezolid	2	20	10	5
	Imipenem	0.03	40	20	10
	Meropenem	0.12	50	25	12.5
	Levofloxacin	0.12	8.6	4.3	2.15
<i>Enterococcus faecalis</i> (ATCC 29212)	Vancomycin	2	50	25	12.5
	Linezolid	2	20	10	5
	Levofloxacin	1	8.6	4.3	2.15
Yeast					
<i>Candida parapsilosis</i> (ATCC 22019)	Voriconazole	0.03	3	1.5	0.75

Notes: Italicized text for genus and species name of microorganism. ^aMICs of the antimicrobial drugs for each strain tested were determined by CLSI broth microdilution and susceptibility categorization was assigned based on published CLSI breakpoints. ^bFor each antimicrobial tested, concentrations corresponding to the plasma peak (C_{max}), half (C/2), and quarter (C/4) levels are indicated.

0.3 mL of antimicrobial agent solution (described above) in turn. For each microorganism-antimicrobial combination, incubations were performed in quintuplicate for each type of blood culture media. Microorganisms were simultaneously spiked into aerobic and anaerobic bottles as pairs, except for *P. aeruginosa* and *C. parapsilosis*, which were only evaluated under aerobic conditions, and *B. fragilis* only tested under anaerobic conditions. To ensure the activities of the antimicrobials tested, for

each microorganism-antimicrobial combination, triplicate BacT/ALERT resin-free aerobic and/or anaerobic bottles (SA/SN) were incubated with horse blood, antimicrobial solution at peak serum concentrations and bacterial suspension. In addition, a negative control (containing only horse-blood) and a positive control (antimicrobial-free) were set for each combination. The negative controls were used to confirm the sterility of the spiked horse blood, which were simultaneously incubated on

Columbia blood agar plate and incubated at 37°C overnight. Subsequently, the spiked bottles were immediately loaded into the corresponding blood culture instrument and incubated until positive or negative signals were alarmed. A positive signal indicates the signal alarm given by the blood culture instrument when microbial growth was detected in any blood culture bottles loaded in the instrument, whereas a negative signal was observed when no microbial growth was detected up to 5 days (120 h). The negative and positive signals as culture endpoints were used to calculate the time to detection (TTD) of each blood culture bottle. After this, broth from blood culture bottles were subcultured onto Columbia blood agar plates overnight to confirm negative or positive detection as well as to exclude contamination.

Data Analysis and Statistics

Statistical analyses were performed using GraphPad Prism 7. Detection rates between the two culture systems and between aerobic and anaerobic bottles were compared using Fisher's exact test. Mann–Whitney tests were utilized to analyze the differences in TTD and to compare the mean TTD. When calculating mean TTD, blood culture bottles with no microorganisms recovered within 5 days (120 h) were excluded. A p value of < 0.05 was considered statistically significant.

Results

In this study, we tested 770 BacT/ALERT (FA Plus and FN Plus) and BACTEC (Aerobic/F Plus and Lytic/10 Anaerobic/F) blood culture bottles to evaluate the antimicrobial inactivation functions (Table 1). In general, the

detection rate of BacT/ALERT Plus media was 320/385 (83.1%), while that of BACTEC media was 251/385 (65.2%) ($p < 0.001$). Among the antimicrobial-free control bottles, all the microorganisms tested were recovered (340/340). In the presence of antibiotics, 231/430 (53.7%) bottles were declared positive, and the detection rate of BacT/ALERT system was significantly higher than that achieved for the BACTEC system (150/215 [69.8%] versus 81/215 [37.7%]; $p < 0.001$). The detection rate of FA Plus media were higher than that of BD Aerobic Plus bottles; however, the difference was not statistically significant ($p = 0.22$) (Table 2). To ensure the activities of antimicrobials involved, for each microorganism-antimicrobial combination, triplicate BacT/ALERT resin-free aerobic and/or anaerobic bottles (SA/SN) were incubated with horse blood, bacterial suspension and antimicrobial solution at peak serum concentrations, and all showed no microorganism growth (data not shown). Similar to resin-free anaerobic media, the BACTEC Lytic/10 Anaerobic/F media showed 17/80 (21.3%) recovery when evaluated with antimicrobial agents at peak serum concentrations.

Both culture systems showed complete inactivation of voriconazole tested with *C. parapsilosis* (Table 4). As shown in Tables 3 and 4, the BacT/ALERT system showed 100% recovery with ceftazidime, piperacillin-tazobactam, vancomycin and linezolid, which were significantly higher than those of the BACTEC system (ceftazidime, $p = 0.03$; piperacillin-tazobactam, $p = 0.047$; vancomycin, $p < 0.001$; linezolid, $p = 0.047$). The lowest detection rates were observed with carbapenems (imipenem and meropenem) in both systems. For imipenem, the recovery rates of the BACTEC and the BacT/ALERT system were 7/40 (17.5%)

Table 2 Recovery in Aerobic (BacT/Alert FA Plus and BACTEC Aerobic/F Plus) or Anaerobic (BacT/Alert FN Plus and BACTEC Lytic/10 Anaerobic) Bottles Containing Antibiotics by Microorganism

Strains	Recovery (%)			
	BacT/Alert FA Plus	BacT/Alert FN Plus	BACTEC Aerobic/F Plus	BACTEC Lytic/10 Anaerobic
<i>E. coli</i> (ATCC25922)	16/45 (35.6)	21/45 (46.7)	11/45 (24.4)	5/45 (11.1)
<i>P. aeruginosa</i> (ATCC27853)	19/25 (76.0)	–	19/25 (76.0)	–
<i>B. fragilis</i> (ATCC25285)	–	15/15 (100.0)	–	0/15 (0.0)
<i>S. aureus</i> (ATCC29213)	19/25 (76.0)	25/25 (100.0)	15/25 (60.0)	10/25 (40.0)
<i>E. faecalis</i> (ATCC29212)	15/15 (100.0)	15/15 (100.0)	14/15 (93.3)	2/15 (13.3)
<i>C. parapsilosis</i> (ATCC22019)	5/5 (100.0)	–	5/5 (100.0)	–
Total	74/115 (64.3)	76/100 (76.0)	64/115 (55.7)	17/100 (17.0)

Note: Italicized text for genus and species name of microorganism.

Table 3 Times to Detection of Gram-Negative Bacillus Recovered from Resin-Containing BacT/Alert or BACTEC Aerobic (AE) and Anaerobic (AN) Media at Each Antimicrobial Concentration Tested in Blood Culture Quintuplicates

Strains and Antimicrobials	Concentration ^a	BacT/Alert Plus Media						Bactec Media					
		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)	
		AE	AN	AE	AN	AE	AN	AE	AN	AE	AN	AE	AN
<i>E. coli</i>													
Imipenem	C _{max}	0/5	5/5	>120	12.7	NA ^d	0.5	0/5	0/5	>120	>120	NA	NA
	C/2	1/5	5/5	14.2	12.3	2.3	0.1	1/5	0/5	19.5	>120	8.1	NA
	None	5/5	5/5	11.9	12.2			5/5	5/5	11.4	10.2		
Meropenem	C _{max}	0/5	0/5	>120	>120	NA	NA	0/5	0/5	>120	>120	NA	NA
	C/2	0/5	0/5	>120	>120	NA	NA	0/5	0/5	>120	>120	NA	NA
	C/4	0/5	1/5	>120	15.4	NA	3.6	0/5	0/5	>120	>120	NA	NA
	None	5/5	5/5	11.4	11.8			5/5	5/5	11.6	10.0		
Cefoxitin	C _{max}	5/5	5/5	11.0	11.0	0.2	0.2	5/5	0/5	12.2	>120	0.6	NA
	None	5/5	5/5	10.8	10.8			5/5	5/5	11.5	10.0		
Piperacillin-tazobactam	C _{max}	5/5	5/5	11.6	12.7	2.1	2.4	5/5	5/5	13.9	13.7	3.0	3.6
	None	5/5	5/5	9.7	10.3			5/5	5/5	10.9	10.1		
Levofloxacin	C _{max}	1/5	0/5	17.3	>120	5.3	NA	0/5	0/5	>120	>120	NA	NA
	C/2	4/5	0/5	16.8	>120	4.8	NA	0/5	0/5	>120	>120	NA	NA
	None	5/5	5/5	12.0	11.8			5/5	5/5	11.3	9.8		
<i>P. aeruginosa</i>													
Imipenem	C _{max}	5/5	- ^e	18.4	-	2.1	-	5/5	-	19.6	-	2.1	-
	None	5/5	-	16.3	-			5/5	-	17.5	-		
Meropenem	C _{max}	1/5	-	23.0	-	6.5	-	0/5	-	>120	-	NA	-
	C/2	3/5	-	19.2	-	2.7	-	4/5	-	21.5	-	3.7	-
	None	5/5	-	16.5	-			5/5	-	17.8	-		
Piperacillin-tazobactam	C _{max}	5/5	-	15.8	-	-0.3	-	5/5	-	17.8	-	0.5	-
	None	5/5	-	16.1	-			5/5	-	17.2	-		
Levofloxacin	C _{max}	5/5	-	15.2	-	-0.6	-	5/5	-	17.5	-	0.2	-
	None	5/5	-	15.9	-			5/5	-	17.3	-		
<i>B. fragilis</i>													
Imipenem	C _{max}	-	5/5	-	30.7	-	4.3	-	0/5	-	>120	-	NA
	None	-	5/5	-	26.4			-	5/5	-	21.6		
Meropenem	C _{max}	-	5/5	-	33.1	-	9.6	-	0/5	-	>120	-	NA

(Continued)

Table 3 (Continued).

Strains and Antimicrobials	Concentration ^a	BacT/Alert Plus Media						Bactec Media					
		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)	
		AE	AN	AE	AN	AE	AN	AE	AN	AE	AN	AE	AN
	None	-	5/5	-	23.5			-	5/5	-	25.1		
Piperacillin-tazobactam	C _{max}	-	5/5	-	32.6	-	0.9	-	0/5	-	>120	-	NA
	None	-	5/5	-	31.7			-	5/5	-	30.5		

Notes: *Italicized text for genus and species name of microorganism.* ^aFor each antimicrobial tested, C_{max}, C/2, and C/4 concentrations are listed in Table 1. ^bFor each microorganism-antimicrobial combination tested, the median time to detection (TTD) was calculated by summing the TTDs of single blood culture replicates. TTD values are rounded to the nearest decimal. ^cFor each microorganism-antimicrobial combination tested, ΔTTD is the difference in TTD of the microorganism-antimicrobial-containing blood culture by that of the antimicrobial-free blood culture (antimicrobial-free control). ^dNA, not applicable (no microorganism growth in any bottle). ^eLack of data because the microorganism was cultured only in anaerobic (*B. fragilis*) or aerobic (*P. aeruginosa* or *C. parapsilosis*) bottles.

Abbreviations: AE, aerobic media; AN, anaerobic media.

and 28/40 (70.0%), respectively ($p < 0.001$). Similar lower detection rates with meropenem were also observed in the BACTEC and the BacT/ALERT system (6/55 [10.9%] vs 16/55 [29.1%]) ($p = 0.03$).

A total of 23 microorganism-antimicrobial combinations were found to get organisms recovered in both aerobic culture systems, and shorter TTD in FA Plus culture media than BD Aerobic Plus culture media were found in 16 (69.6%) combinations. For the two anaerobic culture systems, there were 20 microorganism-antimicrobial combinations observed to get organisms recovered, with 15 (75.0%) combinations showing shorter TTD in FN Plus bottles than the corresponding BACTEC anaerobic bottles (Tables 3 and 4). In addition, the TTD of FA Plus bottles (14.70 h) was 4.85 h shorter than that of the BD Aerobic Plus bottles (19.55 h) ($p = 0.047$). Similarly, the TTD of FN Plus bottles was 16.8 h, which was 1.6 h shorter than that of BD Lytic/10 Anaerobic bottles (18.4 h) ($p = 0.88$).

Tables 3 and 4 also summarize the differences in TTDs of the bottles spiked with microorganism-antimicrobial combinations compared with of the antimicrobial-free bottles (ΔTTD).⁷ For gram-negative bacteria, the highest ΔTTD (≥ 3 h) was observed for meropenem-*E. coli*, levofloxacin-*E. coli*, meropenem-*P. aeruginosa*, imipenem-*B. fragilis*, and meropenem-*B. fragilis* in BacT/ALERT system, and imipenem-*E. coli*, piperacillin-tazobactam-*E. coli*, and meropenem-*P. aeruginosa* in BACTEC system (Table 3). For gram-positive bacteria, the highest ΔTTD (≥ 3 h) was observed for linezolid-*S. aureus*, imipenem-*S. aureus*, and meropenem-*S. aureus* at the antibiotic C_{max} in the BacT/

ALERT system, and vancomycin-*S. aureus*, linezolid-*S. aureus*, imipenem-*S. aureus*, meropenem-*S. aureus*, and vancomycin-*E. faecalis* at the antibiotic C_{max} in the BACTEC system (Table 4). Interestingly, negative ΔTTDs were observed in some microorganism-antimicrobial combinations, especially in voriconazole-*C. parapsilosis* (-2.2 h) at the antibiotic C_{max} in BacT/ALERT FA Plus bottles (Table 4).

Discussion

In our study, significantly higher microorganism recovery rates were observed with the BacT/ALERT FAN Plus media in the presence of antimicrobial agents; however, the difference between the two aerobic blood culture bottles was not statistically significant. When one aerobic bottle and one anaerobic bottle were paired as a set of blood cultures, and microorganisms detected in at least one bottle of a pair are considered to be positive, the BacT/ALERT system showed a more satisfying detection rate. However, the recovery rates of the two systems were both lower than those reported elsewhere,^{6,7,9} which was probably due to the smaller inoculum (30 CFU/bottle) and susceptible ATCC strains used in our study. It is worth noting that the anaerobic bottles contained different media, which could influence the recovery of many pathogens, especially when exposed to antimicrobial agents.¹⁰ In addition, the resin-free BACTEC Lytic/10 Anaerobic/F bottle evaluated here is the only type of BD anaerobic blood culture media approved by the Chinese National Medical Products Administration (NMPA), which to

Table 4 Times to Detection of Gram-Positive Coccus and Yeast Recovered from Resin-Containing BacT/Alert or BACTEC Aerobic (AE) and Anaerobic (AN) Media at Each Antimicrobial Concentration Tested in Blood Culture Quintuplicates

Strains and Antimicrobials	Concentration ^a	BacT/Alert Plus Media						Bactec Media					
		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)		Recovery (No. of Replicates/ Total)		Median TTD ^b (h)		ΔTTD ^c (h)	
		AE	AN	AE	AN	AE	AN	AE	AN	AE	AN	AE	AN
<i>S. aureus</i>													
Vancomycin	C _{max}	5/5	5/5	14.6	21.4	1.6	0.8	2/5	0/5	22.4	>120	6.9	NA ^d
	None	5/5	5/5	13.0	20.6			5/5	5/5	15.5	14.0		
Linezolid	C _{max}	5/5	5/5	14.8	22.1	0.6	3.4	5/5	5/5	19.8	17.5	3.9	3.3
	None	5/5	5/5	14.2	18.7			5/5	5/5	15.9	14.		
Levofloxacin	C _{max}	5/5	5/5	13.7	18.7	1.2	-0.3	5/5	5/5	16.1	19.3	2.7	4.1
	None	5/5	5/5	12.5	19.0			5/5	5/5	13.4	15.2		
Imipenem	C _{max}	2/5	5/5	22.9	19.4	4.3	0.0	1/5	0/5	21.7	>120	8.0	NA
	None	5/5	5/5	18.6	19.4			5/5	5/5	13.7	112.1		
Meropenem	C _{max}	2/5	5/5	22.5	25.0	1.7	3.4	2/5	0/5	19.7	>120	5.9	NA
	None	5/5	5/5	20.8	21.6			5/5	5/5	13.6	12.5		
<i>E. faecalis</i>													
Vancomycin	C _{max}	5/5	5/5	13.5	14.9	1.1	0.3	4/5	2/5	19.7	20.4	4.8	6.5
	None	5/5	5/5	12.4	14.6			5/5	5/5	14.5	13.9		
Linezolid	C _{max}	5/5	5/5	11.5	20.6	0.2	1.9	5/5	0/5	11.8	>120	0.4	NA
	None	5/5	5/5	11.3	18.7			5/5	5/5	11.4	10.6		
Levofloxacin	C _{max}	5/5	5/5	12.5	20.0	0.0	0.3	5/5	0/5	11.5	>120	0.3	NA
	None	5/5	5/5	12.5	19.7			5/5	5/5	11			
<i>C. parapsilosis</i>													
Voriconazole	C _{max}	5/5	- ^e	32.2	-	-2.2	-	5/5	-	32.6	-	-0.1	-
	None	5/5	-	34.6	-			5/5	-	32.7	-		

Notes: Italicized text for genus and species name of microorganism. ^aFor each antimicrobial tested, C_{max}, C/2, and C/4 concentrations are listed in Table 1. ^bFor each microorganism-antimicrobial combination tested, the median time to detection (TTD) was calculated by summing the TTDs of single blood culture replicates. TTD values are rounded to the nearest decimal. ^cFor each microorganism-antimicrobial combination tested, ΔTTD is the difference in TTD of the microorganism-antimicrobial-containing blood culture by that of the antimicrobial-free blood culture (antimicrobial-free control). ^dNA, not applicable (no microorganism growth in any bottle). ^eLack of data because the microorganism was cultured only in anaerobic (*B. fragilis*) or aerobic (*P. aeruginosa* or *C. parapsilosis*) bottles.

Abbreviations: AE, aerobic media; AN, anaerobic media.

some extent has lead to the relative lower positive rate of BACTEC system. In comparison, the resin-free BacT/ALERT SN anaerobic bottles that functioned to ensure the activities of antimicrobials involved showed 0/48 (0.0%) recovery when evaluated with antimicrobial agents at peak serum concentrations. In this study, besides the

peak concentration (C_{max}), two other descending concentrations (C/2 and C/4), which were higher than the midpoint (M) and the trough (T) concentrations used in some other studies,¹⁰⁻¹² were selected for some antimicrobial-microorganism combinations. Therefore, even in the case that the tested blood culture media showed the best

antimicrobial inactivation efficiency, spiked microorganisms could still be recovered in blood culture bottles in the presence of residual active antimicrobial agents (concentrations above the MIC).¹³

Complete recovery of all microorganisms tested at antimicrobial C_{max} was observed in aerobic and anaerobic BacT/ALERT Plus and BACTEC aerobic culture media in at least one replicate, except for levofloxacin, imipenem, and meropenem. Variation in the inactivation of levofloxacin was found, and the difference between the two blood culture systems was not statistically significant. Complete inactivation of levofloxacin at the C_{max} was observed in all strains in BacT/ALERT aerobic and anaerobic culture media and BACTEC aerobic culture media, except for *E. coli* ATCC25922. In the case of *E. coli* ATCC25922, 20% recovery and 80% recovery were observed at C_{max} and $C/2$, respectively, in BacT/ALERT FA Plus bottles, and no inactivation was observed with BacT/ALERT FN Plus and BACTEC aerobic/anaerobic media. In another comparative evaluation with inoculum of 50–100 CFUs per bottle, complete recoveries of *E. coli* ATCC25922 incubated with levofloxacin at C_{max} and $C/2$ were observed in BacT/ALERT (FA Plus and FN Plus) and BACTEC (Plus Aerobic/F and Plus Anaerobic/F) culture media.⁷ This difference in detection rate could be attributed to the lower CFUs used. In addition, in our study, the blood culture simulation model was performed using bottles spiked with horse blood, bacterial suspension, and antibiotic solution. However, in the study mentioned above, antibiotic solution was added prior to bacterial suspension. It has been reported that the concentration of free levofloxacin quickly fell to under the MIC range of *E. coli* ATCC25922 in BacT/ALERT FA Plus media within 10 min of incubation.¹⁴ Thus, the majority of levofloxacin may have been absorbed before bacterial suspension was added.

Carbapenems, in particular meropenem, exhibited the lowest detection rates in our study. There are two reasons for the low detection rates of carbapenems, especially, meropenem. First, according to the manufacturer's instructions, the inhibition of carbapenems in FN Plus media was more significant than that of FA Plus media. Second, the low detection of carbapenems was largely due to the low recovery rate of *E. coli* ATCC25922; the possible explanation for this is the low MIC and high C_{max}/MIC quotient for the strain-antimicrobial combination. In our study, we further evaluated *E. coli* in the presence of meropenem at a concentration of $1/10 C_{max}$, and found 100% recovery

(median TTD of 15.4 h) in FA Plus and FN Plus bottles. In contrast, no recovery at $1/10 C_{max}$ was observed in the BACTEC (Plus Aerobic/F and Lytic/10 Anaerobic/F) culture media (data not shown).

The overall performance of BacT/ALERT FN Plus media was better than that of FA Plus media, caused by the more powerful antibiotic inactivation of FN Plus media and the difference in oxygen consumption niche of spiked bacteria. However, no statistically significant difference was observed, except for that of *S. aureus* ATCC29213 ($p=0.02$). The difference was largely due to the lower recovery rates with carbapenems caused by the weak inhibition of carbapenems by FA Plus media as well as the low MIC and high C_{max}/MIC quotient. The presence of voriconazole had no effect on *C. parapsilosis* detection in two blood culture systems, probably due to the fungistatic effect rather than the fungicidal effect of voriconazole against *Candida*.¹⁵ Therefore, the differences in detection rate among different blood culture systems are supposed to be decided not only by the antibiotic-inactivation ability of the culture media but also by the concentration of the antibiotics, the MICs of the evaluated strains, and the CFUs per bottle.

The first-time detection of positive blood culture in clinical microbiology laboratories is crucial for the treatment and prognosis of patients with sepsis, making TTD a significant parameter to evaluate the performance of commercial automatic blood culture systems. In general, the BacT/ALERT FA Plus and FN Plus bottles demonstrated shorter TTDs than the BACTEC aerobic and anaerobic bottles, respectively. In addition, the difference in the TTDs of aerobic culture media was statistically significant. These results are consistent with a recent study that compared the TTD of the VIRTUO and FX automated blood culture systems.⁷ It is not clear whether the typical several-hour differences will finally lead to the delay in clinical decision-making in clinical settings. Further prospective clinical studies evaluating the influence of differences in TTD on the initiation or change of antibiotic treatment strategies will be needed.

In terms of ΔTTD , another key parameter to evaluate the antimicrobial-inactivation ability of the automatic blood culture media examined here, delays of at least 3 h compared to positive results in control (antimicrobial-free) blood culture bottles were observed in nine gram-negative bacteria-antibiotic-concentration pairs and ten gram-positive bacteria-antibiotic-concentration pairs, respectively. Among the gram-negative bacteria, six cases (6/9, 66.7%) involved *E. coli*

ATCC25922. Among gram-positive bacteria, seven cases (7/10, 70.0%) involved MSSA. In terms of antibiotics, 9/19 (47.3%) cases involved carbapenems (imipenem and/or meropenem). Moreover, longer Δ TTDs were more frequently observed with BACTEC Plus Aerobic/F media than with FA Plus media. Thus, it is possible that the type and/or species of microorganisms, as well as the interaction between the microorganism and a specific antimicrobial in complex surroundings such as blood culture bottles can to some extent influence the outcome of antimicrobial inactivation.

However, there are still some limitations. First, as simulated blood culture, horse blood was spiked instead of human blood. Second, since the BD BACTEC Plus Anaerobic/F media is not permitted in mainland China, the BACTEC Lytic/10 Anaerobic/F media was evaluated instead with BACTEC Plus Aerobic/F media as pairs. Third, only susceptible ATCC strains were evaluated and resistant clinical isolates were not involved. However, as microorganisms with higher MICs grow faster and more easily in the presence of corresponding antibiotics and clinical isolates with MICs as low as the ATCC strains were not common in our hospital, it is supposed that our study design could well reflect the actual situations in clinical settings. Finally, only limited types and numbers of microorganisms and antimicrobials were evaluated in our study. Further studies with added microbial species/strains and antimicrobial types and further prospective comparative studies with blood culture specimens from patients undergoing antimicrobial treatment will provide more actual information about the capabilities of these commercial automatic blood culture systems for antimicrobial inactivation.

Conclusion

In conclusion, even with the limitations of the simulated study,⁹ relative but not absolute advantages were observed with BacT/ALERT (FA Plus and FN Plus) media. The majority of the tested broad-spectrum antimicrobial agents under most clinically meaningful concentrations can be effectively inhibited, with the exception of *E. coli* with meropenem in FA Plus and FN Plus bottles and *E. coli* with levofloxacin in FN Plus bottles. In addition, shorter TTD was also observed. In order to maximize the chances of recovery for patients who have previously received antimicrobial treatments, BacT/ALERT FAN Plus bottles with a relatively higher cost, can be selectively used. Nevertheless, optimizing the time of blood culture collection just before the next antimicrobials dose and with

complete aerobic and anaerobic sets is critical to maximize the efficiency of all blood culture bottles.

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Disclosure

The authors report no conflicts of interest in this work.

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