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ORIGINAL ARTICLE

A comparative evaluation of BACT/ALERT FA PLUS and FN PLUS blood culture bottles and BD BACTEC Plus Aerobic and Anaerobic blood culture bottles for antimicrobial neutralization

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Abstract

The performance of BACT/ALERT FA and FN PLUS (FA PLUS and FN PLUS) blood culture bottles with the BACT/ALERT VIRTUO (bioMérieux, Inc., Durham, NC) and BD BACTEC Plus Aerobic and Anaerobic (BD Aerobic and BD Anaerobic) blood culture bottles with the BD BACTEC FX (BD Diagnostics, Sparks, MD) for antimicrobial neutralization at peak serum concentration was evaluated. The following antibiotic agents and microbial strains were used: ampicillin, cefepime, cefotaxime, gentamicin, levofloxacin, meropenem, piperacillin-tazobactam, and vancomycin; methicillin-sensitive *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*. The detection rate of FA PLUS bottles was 69.1% (259/375) and that of BD Aerobic bottles was 75.5% (283/375) ($p = 0.050$). In the case of anaerobic culture, the overall detection rate of FN PLUS bottles was 77.0% (231/300) and that of BD Anaerobic bottles was 71.3% (214/300) ($p = 0.113$). The time to detection (TTD) from aerobic culture was 2.8 h shorter in FA PLUS bottles (12.4 h) compared to BD Aerobic bottles (15.2 h) ($p < 0.001$). And the TTD from anaerobic culture was 1.6 h shorter in FN PLUS bottles (18.1 h) compared to BD Anaerobic bottles (19.7 h) ($p = 0.061$). The FA PLUS bottles exhibited a lower detection rate compared to BD Aerobic bottles, while FN PLUS bottles showed a higher detection rate compared to BD Anaerobic bottles. The BACT/ALERT VIRTUO system exhibited shorter TTD compared to the BD BACTEC FX system for both aerobic and anaerobic cultures.

Keywords Blood culture · Antimicrobial neutralization · BACT/ALERT FA PLUS · BACT/ALERT FN PLUS · BD BACTEC Plus Aerobic · BD BACTEC Plus Anaerobic

Introduction

Blood culture should be performed as soon as possible when sepsis is suspected and before antibiotics are given to maximize the detection rate. However, antibiotics are often administered before blood collection for culture to manage urgent septic conditions. According to previous studies, over 40% of inpatients are administered antibiotics before blood collection for culture [1–3]. To minimize the inhibitory effect of antibiotics, some blood culture media contain substances like resin or charcoal intended to adsorb antimicrobial agents or other substances.

Not only the presence of antibiotics in the culture bottles but also the volume of blood collected in culture bottles is a critical parameter affecting the quality of blood culture. Although the recommend volume of each blood sample tested in a culture bottle is ≥ 10 mL [4], several studies have reported that the actual volume is far less than the recommended one, with the mean volume per bottle at around 5 mL [5, 6].

In the present seeded study, we assumed a clinical setting in which 5 mL of blood is collected after initiation of antibiotic treatment. With this utmost reflection of reality, we compared the neutralization effect of two automated blood culture systems that utilize resin-containing blood culture bottles, BACT/ALERT VIRTUO (bioMérieux, Marcy l'Etoile, France) and

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BD BACTEC FX (Becton Dickinson Diagnostics, Sparks, USA).

Materials and methods

Blood culture media and instrument BACT/ALERT FA PLUS (FA PLUS) bottles and BACT/ALERT FN PLUS (FN PLUS) bottles were used in combination with the automated BACT/ALERT VIRTUO blood culture system. BD BACTEC Plus Aerobic (BD Aerobic) bottles and BD BACTEC Plus Anaerobic (BD Anaerobic) bottles were used in combination with the automated BD BACTEC FX blood culture system.

Microorganisms and antimicrobial substances Microbial species were chosen among the frequently isolated ones in clinical microbiology laboratories. The following reference strains were used: methicillin-susceptible *Staphylococcus aureus* (MSSA) ATCC 29213, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *Bacteroides fragilis* ATCC 25285. Additionally, corresponding clinical strains sensitive to most of the antibiotics used in this study were tested (see Table S1 in the supplemental material). Colonies from BAP agar plate were serially diluted to a target suspension of 250 CFU/mL. Antibiotics in common use were chosen (Table 1) and peak serum concentration (C_{max}) achieved after standard adult dosing [7] were used to simulate patient blood levels.

Blood culture The blood culture bottles were inoculated with 5 mL of sheep blood containing antibiotics at peak serum concentration and with 0.5 mL of microorganism suspension (125 CFU/bottle). For each microorganism-antibiotics combination, incubations were performed six times for ATCC strains and in triplicate for clinical strains. Considering lower rates of neutralization with cefepime [8, 9], incubations were performed nine times for ATCC strains with cefepime. Non-fermentative Gram-negative bacilli (*A. baumannii* and *P. aeruginosa*) were inoculated only in aerobic bottles and the strict anaerobe (*B. fragilis*) was inoculated only in anaerobic bottles. Other microorganisms were inoculated in both of aerobic and anaerobic bottles. Positive controls without antimicrobials and negative controls without microorganisms were included for every combination. Bottles were incubated until flagged positive or declared negative after 5 days.

Table 1 Peak serum concentration for each antimicrobial substance

Antimicrobial substance	Peak serum concentration (μg/mL)
Ampicillin	47
Cefepime	164
Cefotaxime	100
Gentamicin	10
Levofloxacin	8.6
Meropenem	49
Piperacillin-tazobactam	209
Vancomycin	50

Data analysis and statistics Detection rates in aerobic bottles and anaerobic bottles between the two culture systems were compared using Fisher's exact test or Pearson's chi-square test. To analyze the differences of time to detection (TTD), Mann–Whitney tests were applied, and the median of TTD was compared since the data were not normally distributed. When calculating median TTD, the results from microorganisms that were not detected within 5 days were excluded. A p value of < 0.05 was considered significant.

Results

A total of 375 FA PLUS bottles and 300 FN PLUS bottles were tested in the VIRTUO system, and a total of 375 BD Aerobic bottles and 300 BD Anaerobic bottles were tested in the FX system. In the presence of antibiotics, 73.1% (987/1350) were declared positive (see Table S2 in the supplemental material). The overall detection rate of FA PLUS bottles was 69.1% (259/375), which was lower compared to the 75.5% (283/375) of BD Aerobic bottles ($p = 0.050$). In the case of anaerobic culture, the overall detection rate of FN PLUS bottles was 77.0% (231/300), which was higher compared to the 71.3% (214/300) of BD Anaerobic bottles ($p = 0.113$) (Table 2).

Gentamicin, piperacillin-tazobactam, and vancomycin demonstrated the highest detection rates as nearly all the tested strains were recovered in both culture systems. For the remainder of the antibiotic agents, some of the tested strains were not recovered. Cefepime and meropenem exhibited the lowest detection rates in both culture systems. In the case of cefepime, the VIRTUO system demonstrated a lower detection rate compared to the FX system in both aerobic culture (18.3% vs. 51.7%, $p < 0.001$) and anaerobic culture (35.4% vs. 50.0%, $p = 0.096$). In the case of meropenem, the VIRTUO system demonstrated a higher detection rate compared to the FX system in anaerobic culture (52.8% vs. 0%, $p < 0.001$). In aerobic culture, most of the microorganisms were not recovered with meropenem in either culture system (13.3% vs. 15.6%, $p = 0.764$).

Among the 58 microorganism-antibiotics combinations in which bacteria were detected in both aerobic culture systems, 54 (93.1%) demonstrated shorter TTD in FA PLUS bottles compared to BD Aerobic bottles. And among the 47 combinations in which bacteria were detected in both anaerobic culture

Table 2 Summary of detection rate (%) in the BACT/ALERT FA and FN PLUS blood culture bottles and BD BACTEC Plus Aerobic and Anaerobic blood culture bottles for ATCC and clinical strains

Strain types	Detection rate (%)					
	BACT/ALERT FA PLUS	BACT/ALERT FN PLUS	As a pair	BD BACTEC Plus Aerobic	BD BACTEC Plus Anaerobic	As a pair
ATCC strains	71.0 (181/255)	77.5 (158/204)	81.0 (248/306)	78.4 (200/255)	72.5 (148/204)	80.1 (245/306)
Clinical strains	65.0 (78/120)	76.0 (73/96)	73.6 (106/144)	69.2 (83/120)	68.8 (66/96)	72.9 (105/144)
Total	69.1 (259/375)	77.0 (231/300)	78.7 (354/450)	75.5 (283/375)	71.3 (214/300)	77.8 (350/450)

systems, 42 (89.4%) exhibited shorter TTD in FN PLUS bottles compared to the counterpart (see Table S3 in the supplemental material). The TTD from the above 58 combinations in aerobic culture was 2.8 h shorter in FA PLUS bottles (12.4 h) compared to BD Aerobic bottles (15.2 h) on average ($p < 0.001$). And the TTD from the above 47 combinations in anaerobic culture was 1.6 h shorter in FN PLUS bottles (18.1 h) compared to BD Anaerobic bottles (19.7 h) ($p = 0.061$).

Discussion

In this study, the BD Aerobic bottles exhibited a higher detection rate compared to FA PLUS bottles in the presence of antibiotics. For the anaerobic culture, the opposite was observed as the FN PLUS bottles showed a higher detection rate compared to the BD Anaerobic bottles. However, there were no statistically significant differences in both aerobic and anaerobic cultures. And when considering that blood culture bottles are to be run as a pair of aerobic and anaerobic, the two culture systems showed comparable results for the detection rate for which microorganisms were detected in at least one bottle of each pair. In a previous study which compared the blood culture media of BACT/ALERT FA system and BACTEC PLUS system, which are the previous version of blood culture system for bioMérieux and BD Diagnostics, the detection rate of bacterial pathogens in samples containing therapeutic levels of antibiotics was higher in the BACTEC PLUS system (95.1%) than in the BacT/ALERT FA system (25.1%) [10]. Our comparative study of VIRTUO system and FX system gives up-to-date information about detection rate in the presence of antibiotics.

When analyzing according to antimicrobial agents, ampicillin, gentamicin, piperacillin-tazobactam, and vancomycin were neutralized effectively in both culture systems. Cefotaxime and levofloxacin showed variable neutralization according to microorganisms and there were no statistical differences between the two culture systems. The antibiotics that exhibited the lowest neutralization effect were cefepime and meropenem. In the case of cefepime, the VIRTUO system demonstrated a lower detection rate compared to the FX system in both aerobic culture and anaerobic culture. The microorganisms which showed statistically significant differences were *S. aureus* and *P. aeruginosa*. Of note, *P. aeruginosa* was detected in nearly all the BD Aerobic bottles, whereas none was detected in FA PLUS bottles. In the case of meropenem, the VIRTUO system demonstrated a higher detection rate compared to the FX system in anaerobic culture. Notably, *S. aureus* and *B. fragilis* were detected in all the FN PLUS bottles but in none of the BD Anaerobic bottles.

When comparing with other studies that evaluated the neutralization effect of blood culture bottles, some differences were observed. Mitteregger et al. evaluated FA and FN PLUS bottles [9] and concluded that meropenem was more effectively neutralized in the anaerobic than in the aerobic, while the opposite was observed for cefotaxime and cefepime. In our study, cefepime was more effectively neutralized in anaerobic culture. Miller et al. evaluated the neutralization effect of BD Aerobic and Anaerobic culture bottles [8] and showed that *E. coli* and *P. aeruginosa* were not recovered in the presence of cefepime and levofloxacin. In our study, *P. aeruginosa* was detected in aerobic bottles. And, in their study, *E. coli* and *P. aeruginosa* were partially recovered when tested with piperacillin-tazobactam, whereas all of them were recovered in our study. MSSA also showed a low detection rate with vancomycin, whereas all of them were recovered in our study. In a recent study that evaluated the neutralization effect of FA PLUS and BD Aerobic bottles [11], *E. coli* and *P. aeruginosa* were not recovered with cefepime in both culture systems, which was inconsistent in that our study showed a high detection rate of *P. aeruginosa* in BD Aerobic bottles. In the case of meropenem, *E. coli* was recovered only in FA PLUS, whereas none was recovered in both aerobic bottles in our study and MSSA was not recovered in both aerobic bottles, which is consistent with our findings. The differences in detection rate between these studies are expected to be affected not only by the MICs of the test strains but also by the blood volume, the type of blood, the inoculated CFU per bottle, and the concentration of the antibiotics.

Early detection of positive culture is essential for the better prognosis of sepsis patients [12–14], and TTD is an important index for the performance of automatic blood culture systems. Overall, FA PLUS and FN PLUS bottles exhibited shorter TTD compared to BD Aerobic and BD Anaerobic bottles. And there was statistically significant difference in TTD for aerobic culture. The results are in line with a recent study that

evaluated TTD of VIRTUO system (aerobic, 11.6 h; anaerobic 10.1 h) in comparison with FX system (aerobic, 13.5 h; anaerobic 12.2 h) [15]. The differences seen are typically several hours and it is unclear if this will impact clinical decision making in a real-world setting. In our hospital, when the blood culture bottles signal positive, preliminary results are automatically reported as "presumptive growth of bacteria or fungi" until the final results are given. In an urgent septic patient, even such a limited information may be valuable in patient care. Additional clinical studies that evaluate the impact of shorter TTD on the actual initiation or changes made to the most appropriate antibiotic treatment will be needed.

There are some limitations in this study. First, sheep blood was used instead of human blood for simulation. Secondly, 5 mL of blood was inoculated which is less than the recommended volume for blood culture. However, as the blood volume collected in culture bottles has been reported to be far less than 10 mL [5, 6], we believe that this study design has well reflected the actual clinical setting. Lastly, only a limited number of bacterial species was evaluated in our study, and the other species frequently isolated in a clinical setting including *Candida* spp., coagulase-negative staphylococci, and enterococci were not evaluated. Further studies with more extensive microbial species will be able to provide additional information about a comparative evaluation of blood culture bottles for the antimicrobial neutralization.

In conclusion, we simulated the clinical setting in which blood culture is performed after initiation of antibiotic treatment and compared the neutralization of antibiotics in the FA and FN PLUS bottles and BD Aerobic and Anaerobic bottles. The data demonstrated comparable results between the VIRTUO system and the FX system for the overall detection rate. Specifically, in aerobic culture, the BD Aerobic bottles exhibited a higher detection rate compared to the FA PLUS bottles, while the FN PLUS bottles showed a higher detection rate compared to the BD Anaerobic bottles. Further comparison studies with blood samples from patients under antibiotic treatment will be able to provide additional information about the performance of the two culture systems for antibiotic neutralization.

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