Identification of microorganisms directly from blood culture bottles with polymicrobial growth: comparison of FilmArray and direct MALDI-TOF MS

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Bloodstream infections (BSIs) are related to high mortality and morbidity. Rapid administration of effective antimicrobial treatment is crucial for patient survival. Recently developed rapid methods to identify pathogens directly from blood culture bottles speed up diagnosis of BSIs. The present study compares the performance of two rapid identification methods, FilmArray and direct MALDI-TOF MS, on identifying microorganisms directly from positive blood culture bottles with polymicrobial growth. FilmArray and direct MALDI-TOF MS were performed directly on positive clinical and simulated polymicrobial blood culture bottles. Assay results were compared with standard culture methods.

In total, 110 polymicrobial blood culture samples, of which 96 samples contained two microorganisms while 14 samples contained three microorganisms, were studied. FilmArray was able to identify 215/234 (92.0%) of isolates detected by the standard culture method and successfully identified all microorganisms in 88/110 (80.0%) of blood culture bottles. In contrast, direct MALDI-TOF MS was only able to identify 65/234 (27.8%) of isolates and managed to identify all microorganisms in 2/110 (2.1%) of blood culture bottles. FilmArray is a rapid method for direct identification of polymicrobial blood culture samples that can complement the conventional identification methods. Direct MALDI-TOF MS has low performance with polymicrobial samples.

Key words: Bloodstream infection; MALDI-TOF MS; FilmArray; blood culture; polymicrobial growth.

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Bloodstream infections (BSIs) are related to high mortality and morbidity (1, 2). The associated mortality rate of 23.5–27.5 per 100,000 person-years means that BSIs contribute to more deaths than any other infectious disease in the developed countries (3). The mortality rates are even higher in polymicrobial BSIs (4). In the case of neonates, polymicrobial BSIs are associated with more than 3-fold increase of mortality compared to monomicrobial BSIs (5). Polymicrobial BSIs are not uncommon and range from 6% to 32% of all diagnosed BSIs (6–8). Studies have repeatedly demonstrated that the rapid administration of effective antimicrobial treatment is crucial for patient survival (9,10); hence, efficient diagnostics of BSIs is paramount.

Blood culture (BC) remains the gold standard for microbiological diagnostics of BSIs. However, the sensitivity of BC has been estimated to be as low as 50% (11,12). In addition, subculture of microorganisms after BC positivity takes around 24 h with conventional methods, and it may take another 24 h to identify microorganisms (13,14). Polymicrobial BSIs can be difficult to detect by conventional
culture methods and erroneously classifying them as monomicrobial may lead to inappropriate antimicrobial therapy treatment (5,15).

Recent improvements in rapid methods focused on identification of microorganisms from BC bottles have showed that it is possible to bypass the subculture step after BC positivity. The methods that have been proven successful in the context of monomicrobial BC samples include direct MALDI-TOF MS which identifies microorganisms based on ribosomal protein patterns (16–19), the Accelerate Pheno system which is based on fluorescence in situ hybridization (20), and PCR-based FilmArray and Verigen BC assays (19–24). Hitherto published studies have mainly focused on the performance of these rapid methods in identification of microorganisms from BC bottles with monomicrobial growth. We and others have previously shown that FilmArray has a 90% sensitivity and up to 77% specificity in identification of microorganisms from positive BC bottles (22,25,26). However, these studies included too few polymicrobial samples to conclude on the performance of FilmArray on polymicrobial blood cultures; hence, there are scarce data on BC bottles with polymicrobial growth. We previously showed that FilmArray could detect up to three different microorganisms from a positive BC bottle (22).

Rapid identification of microorganisms remains challenging in the case of polymicrobial BSIs (5,27–29), and those that performed well with monomicrobial BC samples have shown poorer performance in detection and identification of microorganisms from BC bottles with polymicrobial growth (23,28,30). Hence, the aim of this study was to evaluate the performance of these two rapid identification methods, direct MALDI-TOF MS and FilmArray, on the identification of microorganisms directly from positive BC bottles with polymicrobial growth.

**MATERIAL AND METHODS**

**Samples**

Both clinical and simulated polymicrobial blood cultures samples were included in the study. Only one bottle was included per patient. Simulated polymicrobial samples were created by mixing equal volumes of broth from two positive blood culture bottles that signaled positive on the same day. In total, 10 ml of broth from two different bottles was inoculated in a new bottle, and the simulated bottle was incubated in the blood culture system and treated further as a clinical sample. In addition, six simulated polymicrobial samples were created by spiking six clinical blood culture bottles (five monomicrobial bottles and one polymicrobial bottle containing E. coli and S. epidermidis) with 1000 CFU of *Candida albicans* ATCC 10231™. A flowchart illustrating how clinical and simulated polymicrobial bottles were obtained is depicted in Fig. 1.

**Blood culture system**

All blood culture bottles were incubated in the automated BacT/ALERT 3D blood culture system (bioMérieux, Marcy-l’Etoile, France) until positivity or for a maximum of 5 days before being examined by Gram stain. Only resin-based blood culture bottles were included in the study, that is, BacT/ALERT FA Plus, BacT/ALERT FN Plus, and BacT/ALERT PF Plus (bioMérieux, Marcy-l’Etoile, France) as charcoal interferes with downstream analyses in FilmArray as recommended by the manufacturer. The methods were evaluated according to their performance in identifying microbial species in positive blood cultures with polymicrobial growth.

**Reference method**

Standard culture was used as the reference method in this study. Blood culture bottles with polymicrobial growth were subcultured on appropriate plates that included blood, cysteine lactose electrolyte deficient (CLED), and chocolate agar plates. Plates were then placed in an appropriate atmospheric environment for overnight incubation at 37°C. Colonies that grew after incubation were subcultured and analyzed by conventional methods such as MALDI-TOF MS (Bruker Daltonik, Hamburg, Germany) and VITEK 2 XL (bioMérieux, Marcy-l’Etoile, France). Identification criteria recommended by the manufacturer for identification with MALDI-TOF MS were used for evaluating the results, where a score ≥2.000 indicates identification at species level, a score of 1.700-1.999 indicates identification at genus level, and a score of <1.700 indicated no identification. The results from these analyses were used as reference for evaluation of the study results.

**FilmArray**

The Biofire® FilmArray® Blood Culture Identification (BCID) kit (BioFire Diagnostics by bioMérieux, Salt Lake City, UT, USA) is an *in vitro* diagnostic system that identifies microorganisms through nucleic acid extraction, multiplex PCR, and post-PCR DNA melt curve analysis directly from clinical specimens (Table 1). Briefly, 200 µl of broth was taken from the BC suspension and mixed with 500 µl sample dilution buffer that was included in the kit. After mixing the broth with dilution buffer, the inoculum was injected into the FilmArray pouch. Finally, the FilmArray pouch was loaded in to the FilmArray system for analysis. The FilmArray software automatically provided results within 65 min.

**Direct MALDI-TOF MS**

An in-house preparation method for performing MALDI-TOF MS directly from BC broth was used. Briefly, a 5 ml aliquot of BC broth was centrifuged at 180 g for 10 min. The supernatant was discarded, and the pellet was resuspended with 100 µl 5% saponin and 5 ml of deionized water to lyse any human cells before
being centrifuged again at 180 g for 10 mins. The resulting pellet was washed with 1 ml of erythrocyte lysis buffer (0.15M NH₄Cl, 1mM KHCO₃, pH 7.4) and transferred to a microcentrifuge tube. After centrifuging for 1 min at 15,000 g, the supernatant was discarded and the pellet was washed with 1 ml of deionized water, recentrifuged, and finally suspended in an equal volume of 70% formic acid and 50% acetonitrile (Fisher Scientific, UK). Samples were spotted in triplicates to a steel MALDI-TOF MS target plate. MALDI-TOF MS analysis was performed using the Bruker Biotyper 3.1 software and library (version 4613, Bruker Daltonics, MA, USA) with the mass spectra ranging from 2,000 to 20,000 Daltons.

**Data analysis**

The number of different microorganisms per blood culture bottle and the genus and species of these microorganisms, as well as the level of identification (genus or species level) by reference method, FilmArray, and direct MALDI-TOF MS, was collated for each sample. Each method was evaluated for the number of successful identifications compared to the reference method.

**RESULTS**

**Sample characteristics**

A total of 110 polymicrobial samples (generated from 183 BC bottles) were included in the study, of which there were 44 clinical, and 66 simulated BC bottles samples (Fig. 1). Among the 44 clinical polymicrobial BC bottles, 37 BC bottles were found to contain two microorganisms and seven BC bottles to contain three microorganisms using the reference method. Of the 66 simulated samples, 59 contained two microorganisms, and seven simulated BC samples contained three microorganisms.

**Diversity of isolates**

We first examined the diversity and distribution of the microorganism isolates of the BC bottles in this study. Isolates included 50 coagulase-negative staphylococci (CoNS), 48 *Escherichia coli*, 21 *Staphylococcus aureus*, 20 alpha-hemolytic streptococci, 18 *Klebsiella pneumoniae*, 16 *Enterococcus faecalis*, 10 *Candida albicans*, 10 beta-hemolytic streptococci, 8 *Klebsiella oxytoca*, 7 *Enterobacter cloacae* complex, 5 *Enterococcus gallinarum* isolates. In addition, one each of *Acinetobacter pittii*, *Candida glabrata*, *Clostridium ramosum*, *Clostridium sp.*, *Enterococcus avium*, *Micrococcus luteus*, *Salmonella typhimurium*, *Serratia marcescens*, *Streptococcus pneumoniae*, gram-positive cocci, and anaerobic gram-negative rod (Tables S1, S2). In total, 43 different types of microorganisms were identified during the study period (Table 2, Fig. 2). The reference method could not further determine the identity of the remaining two isolates, and these were defined as gram-positive cocci and anaerobic gram-negative rod.
Identification of polymicrobial samples by FilmArray method

Overall, the FilmArray method successfully identified 215/234 (92.0%) microorganisms from the BC bottles. The total theoretical coverage rate of all microorganism species in the sample material by the FilmArray Blood Culture Identification (BCID) Panel was 224/234 (95.7%). When considering only microorganisms covered by the FilmArray BCID Panel, the assay could identify 215/224 (96.0%) of the isolates (Table 2). Interestingly, it was also observed that five clinical sample isolates were correctly detected by FilmArray but were not immediately identified by the reference method. These comprised of two isolates each of *K. oxytoca*, alpha-streptococci species, and one *C. albicans*. These isolates were first identified by FilmArray. When the culture-based reference method was repeated, the two isolates were also detected and confirmed by the reference method. There were also two false-positive results with FilmArray, one of each *K. pneumoniae*, and *E. coli* that were not confirmed by the reference method.

In the clinical polymicrobial samples containing two microorganisms, FilmArray successfully identified both microorganisms in 27/37 (73.0%) of BC bottles and one of two microorganisms in 10/37 (27.0%) of BC bottles. In the clinical polymicrobial samples containing three microorganisms as determined by the standard culture method, all

| No. Microorganism                                   | No. of unique isolates | No. of isolates identified in each method |
|----------------------------------------------------|------------------------|------------------------------------------|
|                                                    |                        | Reference method | FilmArray BCID | Direct MALDI-TOF MS |
| **Gram-positive**                                  | 130 (54.4%)            | 128 (54.7%)     | 112 (52.1%)   | 36 (55.4%)           |
| Coagulase negative staphylococci (CoNS\(^1\))     | 50                     | 50              | 40            | 10                   |
| 1 Staphylococcus aureus                            | 21                     | 21              | 20            | 17                   |
| Alpha-hemolytic streptococci\(^2\)                | 20                     | 18              | 18            | 0                    |
| 2 Enterococcus faecalis                           | 16                     | 16              | 15\(^G\)     | 5                    |
| Beta-hemolytic streptococci\(^3\)                 | 10                     | 10              | 10            | 2                    |
| 3 Enterococcus faecium                            | 5                      | 5               | 5\(^G\)      | 2                    |
| 4 Bacillus species                                | 2                      | 1\(^G\)         | Not in panel | 0                    |
| 5 Enterococcus gallinarum                         | 2                      | 2\(^G\)         | 0             | 0                    |
| 6 Enterococcus avium                              | 1                      | 1\(^G\)         | 0             | 0                    |
| 7 Gram-positive cocci                             | 1                      | 1               | Not in panel | 0                    |
| 8 Micrococcus luteus                              | 1                      | 1               | Not in panel | 0                    |
| 9 Streptococcus pneumoniae                        | 1                      | 1               | 0             | 0                    |
| **Gram-negative**                                  | 93 (38.9%)             | 91 (38.9%)      | 92 (42.8%)   | 29 (44.6%)           |
| 10 Escherichia coli                               | 48                     | 48              | 48            | 16                   |
| 11 Klebsiella pneumoniae                          | 18                     | 18              | 18            | 10                   |
| 12 Klebsiella oxytoca                             | 8                      | 6               | 8\(^G\)      | 2                    |
| 13 Enterobacter cloacae                           | 7                      | 7\(^G\)         | 1             | 1                    |
| 14 Pseudomonas aeruginosa                         | 4                      | 4               | 4             | 0                    |
| 15 Proteus mirabilis                              | 3                      | 3               | 3             | 0                    |
| 16 Acinetobacter baumannii                        | 2                      | 2               | 2             | 0                    |
| 17 Acinetobacter pittii                           | 1                      | 1               | Not in panel | 0                    |
| 18 Serratia marcescens                            | 1                      | 1               | 0             | 0                    |
| 19 Salmonella typhi                                | 1                      | 1               | 0             | 0                    |
| **Yeast**                                          | 11 (4.6%)              | 10 (4.3%)       | 11 (5.1%)    | 0 (0.0%)             |
| 20 Candida albicans                               | 10                     | 9               | 10            | 0                    |
| 21 Candida glabrata                                | 1                      | 1               | 1             | 0                    |
| **Anaerobic**                                      | 5 (2.1%)               | 5 (2.1%)        | 0 (0%)        | 0 (0%)               |
| 22 Bacteroides fragilis                           | 2                      | 2               | Not in panel | 0                    |
| 23 Clostridium ramossom                           | 1                      | 1               | Not in panel | 0                    |
| 24 Clostridium species                            | 1                      | 1               | Not in panel | 0                    |
| 25 Gram-negative rods, anaerobe                   | 1                      | 1               | Not in panel | 0                    |
| **Total**                                          | 239                    | 234             | 215           | 65                   |

N: Detection of microorganism to species level; N\(^G\): Detection of microorganism to genus level.

\(^1\)CoNS includes 33 *S. epidermidis*, 9 *S. hominis*, 3 *S. capitis*, 3 *S. warneri*, 1 *S. haemolyticus*, and 1 *S. sciuri*.

\(^2\)Alpha-hemolytic streptococci includes 5 *S. anginosus*, 3 *S. mitis*, 3 *S. salivarius*, 2 *S. sanguinis*, 1 *S. cristatus*, 1 *S. galloyticus*, 1 *S. oralis*, 1 *S. parasanguinis*, and 3 unspecified alpha-hemolytic streptococcus.

\(^3\)Beta-hemolytic streptococci includes 6 *S. pyogenes*, 3 *S. agalactiae*, and 1 unspecified group G beta-hemolytic streptococcus.
microorganisms were successfully identified in 4/7 (57.1%) bottles, two out of three microorganisms were successfully identified in 2/7 (28.6%) BC bottles, and one out of three microorganisms were successfully identified in 1/7 (14.3%) BC bottles (Table 3).

In simulated polymicrobial samples containing two microorganisms, FilmArray detected two of two microorganisms in 54/59 (91.5%) blood culture samples and one out of two microorganisms were successfully identified in 1/7 (14.3%) BC bottles (Table 3).

In simulated polymicrobial samples containing two microorganisms, FilmArray detected two of two microorganisms in 54/59 (91.5%) blood culture samples and one out of two microorganisms were successfully identified in 1/7 (14.3%) BC bottles (Table 3).

Overall, FilmArray correctly identified all microorganisms in 88/110 (80.0%) of the BC bottles (Fig. 3).

Identification of polymicrobial samples by direct MALDI-TOF MS

The MALDI-TOF MS panel covers all species found in the BC bottles of this study. However, our results show that direct MALDI-TOF MS was only able to identify 65/234 (27.8%) of isolates in this study that were detected by the reference method (Table 2). Also, direct MALDI-TOF MS performed poorly at the sample level and could identify the complete set of microorganism species in only 2/110 (2.1%) BC bottles. The two BC bottles that were completely identified were simulated samples that contained two microorganism species. One of the two simulated BC bottles that were completely identified contained S. hominis and E. coli, while the other contained S. aureus and K. oxytoca. In BC bottles containing three microorganisms, direct MALDI-TOF MS did not manage to identify more than one of the species contained in these bottles. Consequently, the microorganisms of a substantial number of polymicrobial BC bottles could not be identified by direct MALDI-TOF MS (Tables 3, 4, Fig. 3).

DISCUSSION

In the present study evaluating the performance of FilmArray and direct MALDI-TOF MS, we found that FilmArray performed significantly better than MALDI-TOF in identification of multiple microorganisms for the same sample.
FilmArray was able to identify over 90% of the isolates and correctly identify all microorganisms in 80% of the BC bottle samples in the present study. Moreover, FilmArray identified both bacteria and yeast from polymicrobial BC samples, while direct MALDI-TOF MS was not able to identify any of the yeast isolates detected by the reference method. In contrast to FilmArray, direct MALDI-TOF MS performed poorly as it only managed to identify 27.8% of isolates and detected only one of the microorganisms in the vast majority of these samples. Similar to our observations, another study on the FilmArray and direct MALDI-TOF by Fiori et al. (31) showed that FilmArray identified 94.7% of isolates and gave complete identity of 89.5% of polymicrobial BC bottles, while direct MALDI-TOF MS performed poorly as it only managed to identify 29.0% of isolates. Our findings are also in line with previous studies showing that direct MALDI-TOF MS could detect only one microorganism in around 65% of polymicrobial BC bottles (23,28,32).

For FilmArray, most of the microorganisms that were not detected by the BCID panel were those generally considered as contaminants from skin flora, with the exception of *E. faecalis* and alpha-streptococci that are generally considered as clinically significant findings. Non-detection of these species is a common limitation to rapid identification methods that are designed to identify only the most frequent clinically significant microorganisms (25,28,32). Another limitation of FilmArray is that it can only identify certain microorganisms to the genus level; hence, it is unable to differentiate between some clinically relevant microbes such as *E. faecalis* and *E. faecium*.

Low sample microbial loads in blood cultures can lead to failure to identify the microorganism by MALDI-TOF MS (34,35). We attempted to avoid this by using a higher volume, that is, 5 ml of starting material for direct MALDI-TOF MS. However, direct MALDI-TOF MS was still only able to identify 65/234 (27.8%) of bacteria isolates in polymicrobial bottles. Interestingly, Fiori et al. also used a higher starting volume of 8 ml to perform the MALDI-TOF MS on polymicrobial BC samples and obtained similar rates of identification of

### Table 4. Identification of microorganisms from simulated BC bottles with polymicrobial growth

| Method           | Bottles containing 2 microorganisms | Bottles containing 3 microorganisms |
|------------------|-------------------------------------|-------------------------------------|
|                  | 2 of 2                              | 1 of 2                              | 0 of 2                              | 3 of 3                              | 2 of 3                              | 1 of 3                              | 0 of 3                              |
| Reference        | 59 (100%)                           | 0 (0.0%)                            | 0 (0.0%)                            | 7 (100%)                            | 0 (0.0%)                            | 0 (0.0%)                            | 0 (0.0%)                            |
| FilmArray        | 54 (91.5%)                          | 5 (8.5%)                            | 0 (0.0%)                            | 3 (42.9%)                           | 2 (28.6%)                           | 2 (28.6%)                           | 0 (0.0%)                            |
| Direct MALDI-TOF MS | 2 (3.4%)                           | 25 (42.4%)                         | 32 (54.28%)                        | 0 (0.0%)                            | 0 (0.0%)                            | 3 (42.9%)                           | 4 (57.1%)                            |

![Fig. 3](image_url). Comparison of successful species identification of polymicrobial samples containing two microorganisms (a), and three microorganisms (b), by Reference method, FilmArray, and direct MALDI-TOF MS.

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bacteria (31). Hence, other reasons other than microbial load could have led to a lower identification rate of bacteria in polymicrobial samples. It has been demonstrated in polymicrobial urine samples of two bacteria species that MALDI-TOF MS tends to only report the dominant species if the number of cells of each species in the same sample differs more than two-fold from each other or report an unreliable identification (36). In addition, both the present study and Fiori et al. could not identify any yeast isolates using direct MALDI-TOF MS on polymicrobial samples. This is in contrast with other studies that successfully used direct MALDI-TOF MS to identify yeast from monomicrobial BC bottles despite using lower starting volumes (35,37). The exact reasons for why direct MALDI-TOF MS works better for fungi in monomicrobial compared to polymicrobial BC samples is unknown, but it could be due to the different growth rates between bacteria and yeast in the same bottle leading to bacteria being the dominating species as they grow at a faster rate.

There are several limitations to the present study. First, a significant number of samples were simulated polymicrobial samples. Analysis of clinical polymicrobial BC cultures would be considered the most representative of the clinical situation. However, the limited numbers of clinical polymicrobial samples could only be compensated by analysis of simulated samples. In preparation of simulated samples, we preferred to mix two different positive blood cultures. This approach was unique as it includes the isolates that grew directly in clinical BC samples rather than direct inoculation of microbes. Another limitation is that we did not have access to the clinical data from the patients with polymicrobial BC bottles. Therefore, it was not possible to discuss potential consequences of the rapid identification results on antimicrobial treatment. In addition, the clinical data would help us to define the possible contaminant microorganisms. However, the present study focuses on the analytical performance and shows clearly that the present direct MALDI-TOF MS method has significant limitations in direct identification of microorganisms from polymicrobial BC bottles.

In conclusion, the present study shows that the FilmArray performed well and could be used to complement the existing reference method. Further studies analyzing the clinical performance of the method in microbiological diagnosis of polymicrobial infections are warranted.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical blood culture bottles.
Table S2. Simulated blood culture bottles.