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ORIGINAL ARTICLE

Evaluation of the Bruker® MBT Sepsityper IVD module for the identification of polymicrobial blood cultures with MALDI-TOF MS

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Abstract

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) considerably reduces timeframe required from initial blood culture positivity towards complete bacterial identification. However, rapid identification of polymicrobial blood cultures remains challenging. We evaluated the performances of the Bruker® MBT Sepsityper IVD module on MALDI-TOF MS for the direct identification of polymicrobial blood culture bottles. This module has the ability to give a strong indication that a sample contains a mixture of organisms and to identify two of them. Blood culture bottles considered as polymicrobial using routine subculture were collected and processed using the Sepsityper kit. MALDI-TOF MS identification was performed using the MBT Compass IVD software including the Sepsityper module. From 143 polymicrobial blood culture bottles tested, 34.3% (49/143) were completely identified by the module. Both microorganisms were more easily detected by the module in samples containing two pathogens than in samples containing two contaminants (36.8% vs 29.4%). Additionally, in more than half of the samples, the module detected 1 of the different microorganisms contained in the same vial. In these cases, with a pathogen and contaminant in the same sample, the module detected the pathogen in more than 80%. The Sepsityper module identified 14 microorganisms which were not recovered by conventional culture methods. The Bruker® MBT Sepsityper IVD module contributed to a valuable identification of polymicrobial blood cultures in more than a third of all cases. Conventional culture methods are still required to complete the results and to carry on susceptibility testing.

Keywords MALDI-TOF MS · Direct identification · Polymicrobial blood culture · Bacteremia

Introduction

Bloodstream infections (BSI) are a major healthcare problem associated with important morbidity and mortality [1–3]. Although most BSI are monomicrobial with *Escherichia coli*, *Staphylococcus aureus*, and coagulase-negative staphylococci being the most frequent causative agents [1, 2, 4–6], incidence of polymicrobial bloodstream infections (PBSI) is increasing [7]. According to largest series, PBSI account for 4–32% of all BSI [8–11]. Several conditions such as recent hospitalization, parenteral nutrition, neutropenia, corticosteroids, and previous antimicrobial therapy have been identified as risk factors for PBSI [6, 7, 12–15]. Royo-Cebrecos et al. further identified biliary stenting and cholangitis as predisposing elements of PBSI supporting that the main origin of PBSI are intra-abdominal infections with Gram-negative bacilli being the most frequently isolated microorganisms [6]. Having more than one microorganism in a blood culture vial delays identification and susceptibility testing of all microorganisms causing longer inappropriate empirical treatment and therefore poorer outcome [6, 8, 15]. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification of cultured microorganisms widely accelerated results timeframe

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[16]. In addition to the classical identification from subcultured colonies, many authors validated MALDI-TOF MS performances from young subcultures as well as directly from the blood of positive blood culture bottles [17–21]. Nevertheless, the shortcoming of these latter identification approaches is the failure to detect polymicrobial blood cultures. Subculture on solid medium during an 18- to 24-h incubation period is often required prior to the visualization of distinct colony morphologies. Recently, Bruker Daltonik (Bremen, Germany) released an updated version of the MBT Sepsityper module software able to detect mixed blood cultures through MALDI-TOF MS identification directly from a positive blood culture using the MALDI Sepsityper kit (Bruker Daltonik, Bremen, Germany). The software hereby generates a double identification result so called a "mixed culture hint" to be considered as a strong indication that a sample contains a mixture of organisms. This study aimed at evaluating the performances of the MBT Sepsityper module for the direct identification of polymicrobial blood culture bottles.

Material and methods

The study was conducted at the Cliniques universitaires Saint-Luc, a tertiary 964-beds hospital in Brussels. The clinical laboratory manages approximately 70,000 blood culture bottles a year with a positivity rate of 7.4% within a 5-day incubation period. Positive blood cultures are processed every day from 8 AM to 0 AM while bottles detected outside this timeframe are treated the following morning. Incubation is 24 h a day, 7 days a week.

Sample collection

From January 2017 to May 2017 polymicrobial blood culture bottles were collected among patient routine samples from different Belgian hospital sites including Cliniques universitaires Saint-Luc - UCL Brussels (H1), Clinique Saint-Joseph – CHC Liège (H2), Clinique et maternité Sainte-Elisabeth Namur (H3), and Cliniques de l'Europe - Site Sainte-Elisabeth Uccle (H4). H1 extended polymicrobial sample collection for an additional 7 months and further included 50 monomicrobial blood culture bottles for the aim of evaluating the specificity of the detection of multiple microorganisms by the Sepsityper IVD module. Blood culture media used in H1 and H2 were BD BACTEC Plus Aerobic, Lytic Anaerobic, and Peds Plus medium (Beckton Dickinson, Franklin Lanes, NJ, USA). Blood culture bottle types used in H3 were BacT/ALERT FA and BacT/ALERT SN while those used in H4 were BacT/ALERT FA, FN and PF media (bioMérieux, Marcy l'Etoile, France).

A blood culture bottle was considered as polymicrobial if routine subculture on solid media had generated distinct colony morphologies and more than one identification with MALDI-TOF MS. A blood culture was considered as monomicrobial if routine subculture on solid media had generated single colony morphology with a unique MALDI-TOF MS identification result. Only one bottle of each positive blood culture episode was enrolled.

Positive blood culture bottle MBT Sepsityper identification process is outlined in Fig. 1

One milliliter of blood was collected from each blood culture bottle and was processed using the Sepsityper kit (Bruker Daltonik, Bremen, Germany). Briefly, 200 µl lysis buffer was added and the mixture was centrifuged (2 min at 13,000 rpm) after vortexing for 10 s. The supernatant was discarded and the bacterial pellet was suspended in 1 ml washing buffer. After centrifugation (1 min at 13,000 rpm) and supernatant removal, 300 µl deionized water and 900 µl ethanol were added to resuspend the pellet. Subsequently, a formic acid/acetonitrile extraction was performed as follows:

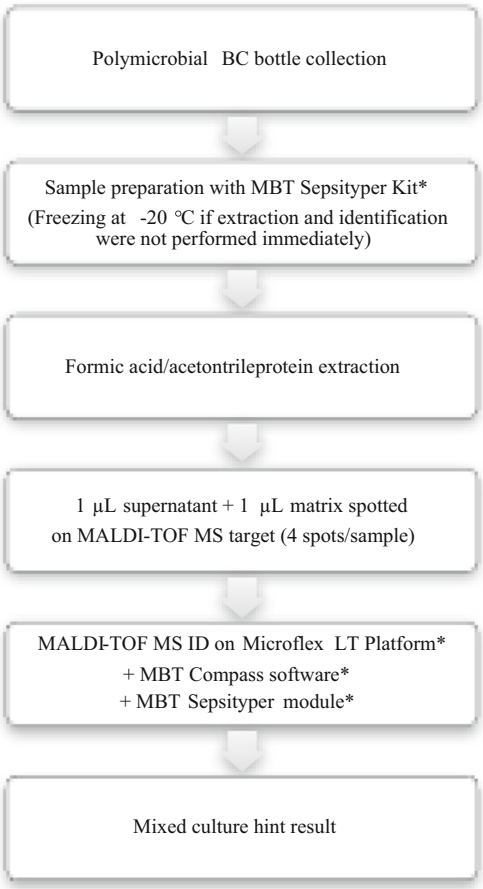

Fig. 1 Workflow of the polymicrobial blood culture bottle identification process. *BC* blood culture, *ID* identification, *MALDI-TOF MS* matrix-assisted laser desorption ionization time-of-flight mass spectrometry, *MBT* MALDI Biotyper. * Components distributed by Bruker Daltonik (Bremen, Germany)

the sample was centrifuged at 13,000 rpm for 2 min, supernatant was discarded, and sample was centrifuged again. Once the pellet was completely dried, 50 μl of formic acid (70%) were added prior vortexing and then 50 μl of acetonitrile were finally added. The mixture was mixed and centrifuged a final time (2 min at 13,000 rpm). Ultimately, four spots of 1 μl of supernatant were made on a steel target plate and allowed to dry at room temperature before adding 1 μl of matrix (alpha-cyano-4-hydroxy-cinamic acid, HCCA). MALDI-TOF MS identification was performed on a Microflex LT (Bruker Daltonik, Bremen, Germany) using the MBT Compass software including the MBT Sepsityper module (version 4.2.80). Each sample was registered as a "Sepsityper sample type" enabling the module to a specific data processing taking into account the undesired mass spectrum peaks originating from blood cells. Acquired spectra were compared with spectra from the reference database and an identification result was associated with an identification score used to express the degree of spectral concordance. Confidence score value thresholds ≥ 1.6 and ≥ 1.8 were accepted for identification to respectively genus and species level. Additionally, the module was also updated with the ability to detect a potential combination of two single spectra. Concerning the latter, the module first compared individual acquired spectrum of both bacteria with the database and a confidence score was given. Then, a virtual combined spectrum was created and compared with the combined spectrum obtained from the sample. If the confidence score of the combined spectrum was higher than that obtained for both single spectra, it was assumed that the sample was a mixture of the two species. The module's ability to detect a mix of strains was strictly limited to the identification of maximum two microorganisms associated with a unique confidence score. The Sepsityper sample processing prior formic acid/acetonitrile extraction had to be performed within 48 h after blood culture bottle positivity but the extraction and the MALDI-TOF MS identification could be delayed. If these latter steps were delayed, the samples were frozen. Therefore, for practical reasons, some samples were frozen at −20 °C after preparation with the Sepsityper kit in each hospital prior to formic acid/acetonitrile extraction and MALDI-TOF MS identification subsequently performed for all samples in H1. If Gram staining already suggested the presence of multiple microorganisms, the Sepsityper sample processing was realized immediately. When only subculture highlighted the presence of several microorganisms, the processing prior to formic acid/acetonitrile extraction was delayed nevertheless performed within 48 h after blood culture positivity on the bottle stored at room temperature.

Routine identification methods

In each hospital, a Gram staining was performed immediately upon removal of the positive blood culture from the incubator. Then blood inoculation was carried out on a Columbia agar plate with 5% sheep blood (COL; Becton Dickinson, Franklin Lanes, NJ, USA). The following day, subcultures were observed for colony growth and each distinct colony was identified with MALDI-TOF MS.

Patients' files

Patients' files were consulted to identify whether recovered strains were to be considered as pathogens or as a contamination according to the US Centers for Disease Control and Prevention/National Healthcare Safety Network definitions of bloodstream infection events [22].

Ethics statement

Testing was performed in accordance with the ethical standards of the Cliniques universitaires Saint-Luc, in accordance with the ethical standards of the national research committee, and in accordance with the 1964 Helsinki declaration and its later amendments. Information from microbiological and clinical files was anonymously analyzed and did not require patient's informed consent.

Statistical analysis

A chi-squared test was performed to assess the performances of the Sepsityper module for the identification of bi-microbial samples containing two pathogens versus two contaminants. Statistical significance was considered with a P value < 0.05.

Results

Polymicrobial blood culture bottles

A total of 143 polymicrobial blood culture bottles were collected. Using conventional culture, 121, 16, 5, and 1 bottles contained respectively 2, 3, 4, and 5 different microorganisms.

The MBT Sepsityper IVD module allowed concordant identification of two microorganisms in the same bottle in 49/143 cases (34.3%). For 78 bottles (54.5%), the module only identified one concordant microorganism and in 16 bottles (11.2%), the module failed to retrieve any microorganism detected through routine culture.

The different combinations of microorganisms in the 121 bi-microbial blood culture bottles are summarized in Fig. 2. Among those, the module correctly detected the two microorganisms in 40 bottles (33.1%). In 66 bottles (54.5%), the module only identified 1 concordant microorganism and no concordant identification occurred in 15 bottles (12.4%) (Fig. 3). Among these non-concordant identifications, the Sepsityper module led to failures of identification for 11

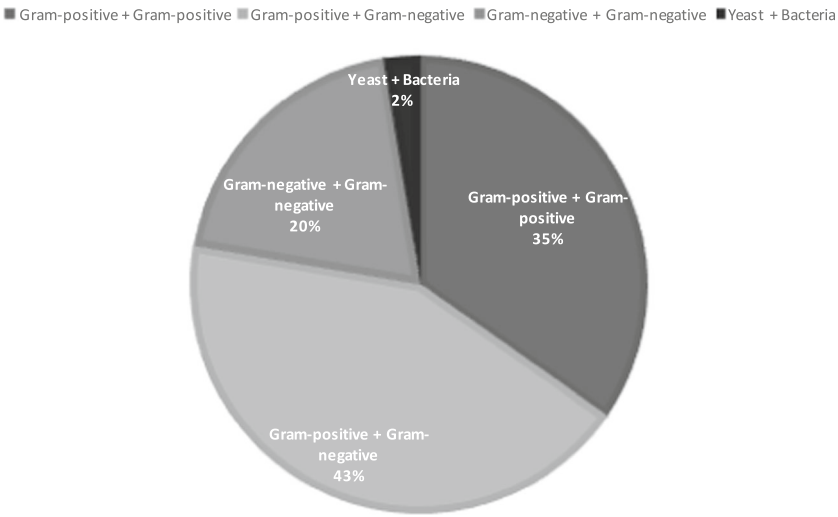

Fig. 2 Combinations of microorganisms in the 121 bi-microbial blood culture bottles according to routine identification

bottles and to discordant results for the four other bottles. Considering the 242 microorganisms recovered by routine from the 121 bi-microbial samples, there were 137 Gram-positive bacteria, 102 Gram-negative bacteria, and three yeasts. Using the Sepsityper module, identification was successful for 146/242 strains (60.3%) of which 82/137 (59.9%) Gram-positive bacteria, 63/102 (61.8%) Gram-negative bacteria, and 1 yeast (Table 1). No Sepsityper identification was obtained for 82 microorganisms. Eventually, 14 strains were recovered with the Sepsityper approach but not recovered with conventional routine identification (Table 2).

After patients' files analysis, among the 121 bi-microbial samples, the two microorganisms were both pathogens in 56.2% (68/121) of the samples and were both contaminants in 28.1% (34/121). The Sepsityper module allowed complete concordance in 36.8% (25/68) of the pathogenic samples and in 29.4% (10/34) of the contaminated samples. In the remaining 19 bottles containing both a pathogen and a contaminant, the module correctly identified the two microorganisms in 5/19 cases, 0 microorganism in 2/19 cases and only 1 microorganism in 12/19 cases. Among these 12 latter cases, the module correctly identified the pathogen in 10 cases while in the other two cases, only the contaminant was identified.

Among the 22 polymicrobial samples containing more than two different microorganisms, two correct identifications occurred in 10 cases (45.5%). Only one microorganism was identified in 11 cases (50%) and no identification occurred in one case (4.5%). Two additional microorganisms were identified by the Sepsityper module but not recovered by conventional identification method.

Monomicrobial blood culture bottles

Identification results obtained with the Sepsityper IVD module of the 50 monomicrobial blood culture bottles were in concordance with the routine identification results. The

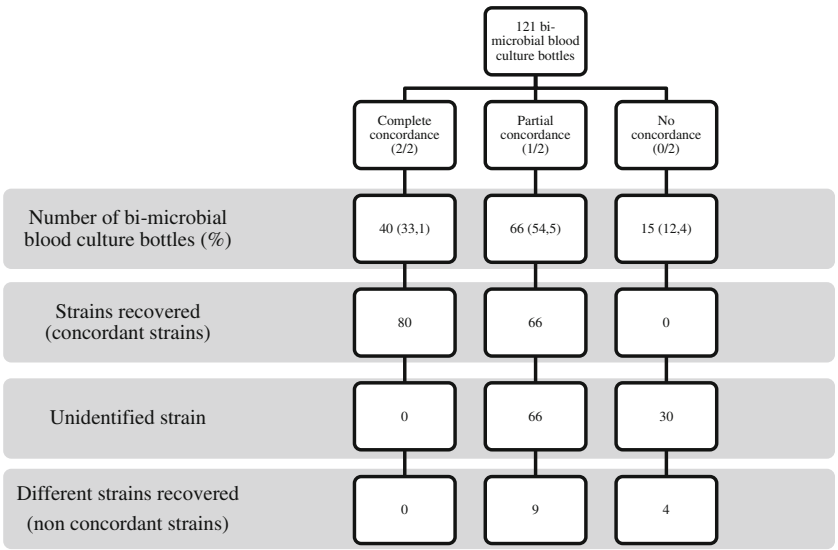

Fig. 3 Number of bi-microbial blood culture bottles showing complete concordance, partial concordance, and no concordance with routine. Number of strains recovered and different strains identified by the MBT Sepsityper module

Table 1 Comparison of identification results between the routine and the MBT Sepsityper approach of all bi-microbial blood culture bottles. *ID* identification, *MBT* MALDI Biotyper

	Routine ID (*n*)	MBT Sepsityper ID (*n*)	MBT Sepsityper ID performances (%)
Gram-positive bacteria	137	82	59.9
Arthrobacter cumminsii	1	0	0.0
Bacillus cereus	1	0	0.0
Clostridium	3	2	66.7
Enterococcus	30	22	73.3
Lactococcus lactis	1	0	0.0
Listeria monocytogenes	1	1	100.0
Paenibacillus	1	1	100.0
Peptoniphilus harei	1	1	100.0
Staphylococcus	58	31	53.4
Staphylococcus aureus	7	4	57.1
Coagulase-negative staphylococcus	51	27	52.9
Viridans streptococcus	38	22	57.9
β-haemolytic streptococcus	2	2	100.0
Gram-negative bacteria	102	63	61.8
Acinetobacter	7	1	14.3
Actinomyces	3	0	0.0
Bacteroides	4	1	25.0
Citrobacter	8	3	37.5
Enterobacter	10	6	60.0
Escherichia coli	33	30	90.9
Haemophilus influenzae	1	0	0.0
Klebsiella	17	13	76.5
Morganella morganii	1	1	100.0
Neisseria	2	0	0.0
Prevotella heparinolytica	1	1	100.0
Proteus	4	2	50.0
Pseudomonas aeruginosa	8	4	50.0
Rhizobium radiobacter	1	0	0.0
Stenotrophomonas maltophilia	1	0	0.0
Veillonella parvula	1	1	100.0
Yeasts	3	1	33.3
Candida	3	1	33.3
Total	242	146	60.3

module correctly identified 40 microorganisms and failed to identify the remaining 10 due to a poor confidence score. Under any occasion, the module gave an erroneous identification result neither suggested the presence of more than one microorganism from the 40 monomicrobial identified blood culture bottles.

Discussion

Polymicrobial bloodstream infections are a growing threat in clinical practice and intra-abdominal sepsis considered as the main source of PBSI, appeared to have significantly worse initial antimicrobial coverage [6, 8, 23, 24]. PBSI are also associated with a worse clinical situation including higher severity of sepsis and a longer duration of infection [7, 15, 25] but also with an increased mortality, a longer length of hospital stay and higher healthcare costs [6, 8, 9, 11, 12, 14, 15, 26–29]. MALDI-TOF MS is now well implemented in many clinical laboratories for the identification of bacteria and yeasts from fresh colonies grown on solid culture media. However, even though MALDI-TOF MS is associated with more rapid microbial identification of blood cultures and strengthens antimicrobial treatment specificity, rapid identification of polymicrobial blood culture bottles remains challenging [16, 30]. A timeframe from 18 to 48 h may be required

Table 2 Discordant identifications among the 121 bi-microbial samples

	Routine ID		MBT Sepsityper ID		
			Concordant ID	Discordant ID	
	Strain 1	Strain 2		Strain 1	Strain 2
1 concordant ID	*Streptococcus anginosus* group	*Bacteroides fragilis*	*Streptococcus anginosus* group	*Clostridium aldenense*	
	Bacteroides fragilis	*Actinomyces turicensis*	*Bacteroides fragilis*	*Clostridium citroniae*	
	Escherichia coli	*Enterobacter aerogenes*	*Escherichia coli*	*Klebsiella variicola*	
	Streptococcus agalactiae	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Streptococcus porcinus*	
	Escherichia coli	*Clostridium tertium*	*Escherichia coli*	*Clostridium perfringens*	
	Streptococcus anginosus group	*Bacteroides ovatus*	*Streptococcus anginosus* group	*Clostridium clostridioforme*	
	Escherichia coli	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus haemolyticus*	
	Staphylococcus epidermidis	*Citrobacter koseri*	*Staphylococcus epidermidis*	*Escherichia coli*	
	Paenibacillus	*Neisseria subflava*	*Paenibacillus*	*Neisseria macacae*	
0 concordant ID	*Staphylococcus haemolyticus* *Bacteroides fragilis*	*Enterococcus faecalis* *Bacteroides thetaiotamicron*		*Enterobacter cloacae* *Eggerthella lenta*	*Streptococcus mitis* group
	Streptococcus sanguinis group	*Neisseria flavescens*		*Streptococcus mitis* group	
	Staphylococcus epidermidis	*Staphylococcus pettenkoferi*		*Staphylococcus capitis*	

from initial blood culture positivity towards complete bacterial identification.

We herein report the performances of MALDI-TOF MS and the MBT Sepsityper Compass IVD module to give a strong indication that a sample contains a mixture of organisms by identifying two of them. Using the manufacturer's criteria, 34.3% of all polymicrobial blood culture bottles were completely identified by the module. There is no statistically significant difference between the Sepsityper's ability to fully identified bi-microbial samples containing two pathogens (36.8%) and bi-microbial samples containing two contaminants (29.4%) ($P = 0.46$). Among the 19 bi-microbial blood culture vials containing a pathogen and a contaminant, only five vials were fully identified by the Sepsityper module. In the 12 bi-microbial vials for which only one microorganism was identified by the Sepsityper module, it is interesting to note that it was the pathogen in 10 cases. This high rate of detected pathogens supports the hypothesis that bacterial load may play a role in the detection of the microorganisms by the Sepsityper module: the relative amount of the pathogen would presumably be higher than the contaminant, and therefore the pathogen would be more easily detected. Using several types of bacterial mixtures in various ratios, Hariu et al. also challenged the detection of polymicrobial blood culture bottles through spiking bottles with different combinations of strains and demonstrated that MALDI-TOF MS direct identification was able to detect combined bacteria at ratios ranging from 1:3 to 1:1 [31]. The Sepsityper module clearly has a capital gain when a single morphology is seen on the Gram staining and therefore microscopic observation cannot predict the presence of more than one microorganism.

Among the 121 bi-microbial samples containing 242 microorganisms identified by conventional methods, the Sepsityper module correctly identified 146 microorganisms (60.3%). We noticed that in 14 cases discordant identifications occurred. A previous study also described discrepant results through direct identification of bacteria in polymicrobial blood culture bottles [32]. La Scola et al. performed direct MALDI-TOF MS on 584 positive blood culture bottles among which 22 were polymicrobial and observed erroneous identifications for three samples among which two were polymicrobial. In our study, a closer analysis of the clinical context linked with the microorganisms exclusively identified with the Sepsityper module leads us to consider most of them as additionally recovered strains rather than discordant strains. For instance, five unrecovered pathogens were strict anaerobes. The high sensitivity to oxygen and the relatively slow growth of strict anaerobes might explain failure to isolate them on solid culture media [33]. For the three uncultured Enterobacteriaceae, the clinical context suggesting an intra-abdominal origin of the bacteremia allowed us to consider them as additional pathogens. Also, in accordance with the clinical context, *Streptococcus porcinus* may be considered as a pathogen whereas the two uncultured viridans streptococci and the *Staphylococcus capitis* could easily be linked as third contaminants to the prior cultured two

contaminants. In the last two cases, discrepant results could not be explained.

Among the three polymicrobial samples containing a yeast, only one *Candida glabrata* could be detected together with another bacteria. A larger number of yeast-including PBSI would be necessary to confirm the poor detection power of yeast with the evaluated module. Nevertheless, this observation could already be explained by the fact that the yeast growth might be inhibited by the bacterial development in blood culture vials as demonstrated in previous studies [34, 35].

On the basis of the Sepsityper results obtained from the 50 monomicrobial blood cultures, we can confirm the excellent specificity of the module concerning the detection of polymicrobial cultures. For the record, at any occasion, multiple strains were detected in the monomicrobial bottles.

Ultimately, we aimed at evaluating to what extent we can validate a polymicrobial identification result obtain through the use of the Sepsityper module. It is nonetheless important to point out that other approaches for the rapid identification of polymicrobial blood culture exist. A suggestive polymicrobial Gram staining of the positive blood culture can initiate the subcultivation onto selective media for Gram-positive and Gram-negative microorganisms followed by MALDI-TOF MS identification after a short incubation.

Molecular identification methods also have the potential to be rapid and reliable for the early detection of multiple microorganisms directly from a positive blood culture bottle [36]. The very short hands-on processing time is also a great advantage of this approach. However, if identification performances are very high (> 90%) in monomicrobial blood cultures, these performances seem to be considerably lower in case of PBSI [37, 38]. Additionally, these molecular panels only include a limited number of pathogens and are quite expensive.

The main limitation of this study is the timeframe within which the Sepsityper sample processing was performed. First, blood culture bottles detected positive overnight remained incubated till routine management the following morning. Moreover, when Gram staining was not suggestive of a polymicrobial sample, only the 24-h subculture allowed us to detect polymicrobial blood culture samples. Under these conditions, the potentially increased bacterial load may have artificially improved the detection of the microorganisms by the Sepsityper module. Ultimately, some samples were frozen at − 20 °C after preparation with the Sepsityper kit in each hospital prior to formic acid/acetonitrile extraction and MALDI-TOF MS identification subsequently performed for all samples in the laboratory of H1. Even though approved by the manufacturer, this freezing process may have affected the viability of some microorganisms and consequently impacted on the rate of accurate results.

To conclude, the reduction of the turnaround time for the identification of microorganisms present in blood cultures is essential to adjust empiric antibiotherapy. Polymicrobial identification results obtained with the MBT Sepsityper Compass IVD module can be considered as valuable information for the therapeutic management of the septic patient. The performances of this new module might presumably be linked to the relative abundance of each microorganism. Importantly, conventional culture methods are still required to complete the results and to carry on susceptibility testing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Testing was performed in accordance with the ethical standards of the Cliniques universitaires Saint-Luc, in accordance with the ethical standards of the national research committee, and in accordance with the 1964 Helsinki declaration and its later amendments.

Informed consent Information from microbiological and clinical files was anonymously analyzed and did not require patient's informed consent.

References

1. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV (2003) Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol 41(8): 3655–3660
2. Bearman GM, Wenzel RP (2005) Bacteremias: a leading cause of death. Arch Med Res 36(6):646–659. https://doi.org/10.1016/j.arcmed.2005.02.005
3. Goto M, Al-Hasan MN (2013) Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect 19(6):501–509. https://doi.org/10.1111/1469-0691.12195
4. Lenz R, Leal JR, Church DL, Gregson DB, Ross T, Laupland KB (2012) The distinct category of healthcare associated bloodstream infections. BMC Infect Dis 12:85. https://doi.org/10.1186/1471-2334-12-85
5. Luzzaro F, Ortisi G, Larosa M, Drago M, Brigante G, Gesu G (2011) Prevalence and epidemiology of microbial pathogens causing bloodstream infections: results of the OASIS multicenter study. Diagn Microbiol Infect Dis 69(4):363–369. https://doi.org/10.1016/j.diagmicrobio.2010.10.016
6. Royo-Cebrecos C, Gudiol C, Ardanuy C, Pomares H, Calvo M, Carratala J (2017) A fresh look at polymicrobial bloodstream infection in cancer patients. PLoS One 12(10):e0185768. https://doi.org/10.1371/journal.pone.0185768
7. Bouza E, Burillo A, Munoz P, Guinea J, Marin M, Rodriguez-Creixems M (2013) Mixed bloodstream infections involving bacteria and Candida spp. J Antimicrob Chemother 68(8):1881–1888. https://doi.org/10.1093/jac/dkt099
8. Lin JN, Lai CH, Chen YH, Chang LL, Lu PL, Tsai SS, Lin HL, Lin HH (2010) Characteristics and outcomes of polymicrobial bloodstream infections in the emergency department: a matched case-control study. Acad Emerg Med 17(10):1072–1079. https://doi.org/10.1111/j.1553-2712.2010.00871.x
9. Pammi M, Zhong D, Johnson Y, Revell P, Versalovic J (2014) Polymicrobial bloodstream infections in the neonatal intensive care unit are associated with increased mortality: a case-control study. BMC Infect Dis 14:390. https://doi.org/10.1186/1471-2334-14-390

10. Rolston KV, Bodey GP, Safdar A (2007) Polymicrobial infection in patients with cancer: an underappreciated and underreported entity. Clin Infect Dis 45(2):228–233. https://doi.org/10.1086/518873
11. Tsai MH, Chu SM, Hsu JF, Lien R, Huang HR, Chiang MC, Fu RH, Lee CW, Huang YC (2014) Polymicrobial bloodstream infection in neonates: microbiology, clinical characteristics, and risk factors. PLoS One 9(1):e83082. https://doi.org/10.1371/journal.pone.0083082
12. Kim SH, Yoon YK, Kim MJ, Sohn JW (2013) Risk factors for and clinical implications of mixed Candida/bacterial bloodstream infections. Clin Microbiol Infect 19(1):62–68. https://doi.org/10.1111/j.1469-0691.2012.03906.x
13. Lagnf AM, Zasowski EJ, Claeys KC, Casapao AM, Rybak MJ (2016) Comparison of clinical outcomes and risk factors in polymicrobial versus monomicrobial enterococcal bloodstream infections. Am J Infect Control 44(8):917–921. https://doi.org/10.1016/j.ajic.2016.02.017
14. Park SY, Park KH, Bang KM, Chong YP, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS (2012) Clinical significance and outcome of polymicrobial Staphylococcus aureus bacteremia. J Inf Secur 65(2):119–127. https://doi.org/10.1016/j.jinf.2012.02.015
15. Sutter D, Stagliano D, Braun L, Williams F, Arnold J, Ottolini M, Epstein J (2008) Polymicrobial bloodstream infection in pediatric patients: risk factors, microbiology, and antimicrobial management. Pediatr Infect Dis J 27(5):400–405. https://doi.org/10.1097/INF.0b013e31816591be
16. Dixon P, Davies P, Hollingworth W, Stoddart M, MacGowan A (2015) A systematic review of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry compared to routine microbiological methods for the time taken to identify microbial organisms from positive blood cultures. Eur J Clin Microbiol Infect Dis 34(5):863–876. https://doi.org/10.1007/s10096-015-2322-0
17. Idelevich EA, Schule I, Grunastel B, Wullenweber J, Peters G, Becker K (2014) Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. Clin Microbiol Infect 20(10):1001–1006. https://doi.org/10.1111/1469-0691.12640
18. Jakovljev A, Bergh K (2015) Development of a rapid and simplified protocol for direct bacterial identification from positive blood cultures by using matrix assisted laser desorption ionization time-of- flight mass spectrometry. BMC Microbiol 15:258. https://doi.org/10.1186/s12866-015-0594-2
19. Kohlmann R, Hoffmann A, Geis G, Gatermann S (2015) MALDI-TOF mass spectrometry following short incubation on a solid medium is a valuable tool for rapid pathogen identification from positive blood cultures. Int J Med Microbiol 305(4–5):469–479. https://doi.org/10.1016/j.ijmm.2015.04.004
20. Morgenthaler NG, Kostrzewa M (2015) Rapid identification of pathogens in positive blood culture of patients with sepsis: review and meta-analysis of the performance of the sepsityper kit. Int J Microbiol 2015:827416. https://doi.org/10.1155/2015/827416
21. Verroken A, Defourny L, Lechgar L, Magnette A, Delmee M, Glupczynski Y (2015) Reducing time to identification of positive blood cultures with MALDI-TOF MS analysis after a 5-h subculture. Eur J Clin Microbiol Infect Dis 34(2):405–413. https://doi.org/10.1007/s10096-014-2242-4
22. Centers for Disease Control and Prevention. Atlanta. National Healthcare Safety Network CDC/NHSN bloodstream infection event (central line-associated infection and non-central line-associated bloodstream infection) (Update January 2016) http://www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABScurrent.pdf
23. Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, Dodek P, Wood G, Kumar A, Simon D, Peters C, Ahsan M, Chateau D, Cooperative Antimicrobial Therapy of Septic Shock Database Research G (2009) Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 136(5):1237–1248. https://doi.org/10.1378/chest.09-0087
24. Pulimood S, Ganesan L, Alangaden G, Chandrasekar P (2002) Polymicrobial candidemia. Diagn Microbiol Infect Dis 44(4):353–357
25. Valles J, Rello J, Ochagavia A, Garnacho J, Alcala MA (2003) Community-acquired bloodstream infection in critically ill adult patients: impact of shock and inappropriate antibiotic therapy on survival. Chest 123(5):1615–1624
26. Beekmann SE, Diekema DJ, Chapin KC, Doern GV (2003) Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. J Clin Microbiol 41(7):3119–3125
27. Norgaard M, Larsson H, Pedersen G, Schonheyder HC, Sorensen HT (2006) Haematological malignancies–a predictor of a poor outcome in patients with bacteraemia. J Inf Secur 53(3):190–198. https://doi.org/10.1016/j.jinf.2005.10.024
28. Sancho S, Artero A, Zaragoza R, Camarena JJ, Gonzalez R, Nogueira JM (2012) Impact of nosocomial polymicrobial bloodstream infections on the outcome in critically ill patients. Eur J Clin Microbiol Infect Dis 31(8):1791–1796. https://doi.org/10.1007/s10096-011-1503-8
29. Yamaga S, Shime N (2018) Association between appropriate empiric antimicrobial therapy and mortality from bloodstream infections in the intensive care unit. J Infect Chemother 24(4):267–271. https://doi.org/10.1016/j.jiac.2017.11.011
30. Kok J, Thomas LC, Olma T, Chen SC, Iredell JR (2011) Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper and time of flight mass spectrometry. PLoS One 6(8):e23285. https://doi.org/10.1371/journal.pone.0023285
31. Hariu M, Watanabe Y, Oikawa N, Seki M (2017) Usefulness of matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify pathogens, including polymicrobial samples, directly from blood culture broths. Infect Drug Resist 10:115–120. https://doi.org/10.2147/IDR.S132931
32. La Scola B, Raoult D (2009) Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One 4(11):e8041. https://doi.org/10.1371/journal.pone.0008041
33. Gajdacs M, Spengler G, Urban E (2017) Identification and antimicrobial susceptibility testing of anaerobic bacteria: Rubik's cube of clinical microbiology? Antibiotics (Basel) 6(4). https://doi.org/10.3390/antibiotics6040025
34. Cateau E, Cognee AS, Tran TC, Vallade E, Garcia M, Belaz S, Kauffmann-Lacroix C, Rodier MH (2012) Impact of yeast-bacteria coinfection on the detection of Candida sp. in an automated blood culture system. Diagn Microbiol Infect Dis 72(4):328–331. https://doi.org/10.1016/j.diagmicrobio.2011.12.012
35. Hockey LJ, Fujita NK, Gibson TR, Rotrosen D, Montgomerie JZ, Edwards JE Jr (1982) Detection of fungemia obscured by concomitant bacteremia: in vitro and in vivo studies. J Clin Microbiol 16(6):1080–1085
36. Timbrook T, Boger MS, Steed LL, Hurst JM (2015) Unanticipated multiplex PCR-based identification of polymicrobial blood culture resulting in earlier isolation, determination of susceptibilities, and optimization of clinical care. J Clin Microbiol 53(7):2371–2373. https://doi.org/10.1128/JCM.00058-15
37. Altun O, Almuhayawi M, Ullberg M, Ozenci V (2013) Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol 51(12):4130–4136. https://doi.org/10.1128/JCM.01835-13
38. Southern TR, VanSchooneveld TC, Bannister DL, Brown TL, Crismon AS, Buss SN, Iwen PC, Fey PD (2015) Implementation and performance of the BioFire FilmArray (R) Blood Culture Identification panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. Diagn Microbiol Infect Dis 81(2):96–101. https://doi.org/10.1016/j.diagmicrobio.2014.11.004