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Abstract

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is a useful tool for rapid identification of microorganisms. Unfortunately, its direct application to positive blood culture is still lacking standardized procedures. In this study, we evaluated an easy- and rapid-to-

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	Samples n.	Genus	%	Species	%	Genus	%	Sp
Gram-positive bacteria	121	79	65.3	74	61.2	108	89.3	10
		MALDI-TOF MS identification results						
Gram-positive cocci	116	79	68.1	74	63.8	107	92.2	10
		3 h				**5 h**		
Enterococci	12	10	83.3	10	83.3	11	91.7	10
Enterococcus faecalis	10	9	90.0	9	90.0	10	100.0	10
	Samples n.	**Genus**	**%**	**Species**	**%**	**Genus**	**%**	**Sp**
Enterococcus faecium	1	0	0.0	0	0.0	1	100.0	0
Enterococcus gallinarum	1	1	100.0	1	100.0	0	0.0	0
Micrococcus luteus	1	1	100.0	1	100.0	1	100.0	1
Staphylococci	99	66	66.7	63	63.6	92	92.9	89
Staphylococcus aureus	14	12	85.7	12	85.7	14	100.0	14
Coagulase-Negative Staphylococci	85	54	63.5	51	60.0	78	91.8	75
Staphylococcus capitis	3	2	66.7	2	66.7	3	100.0	3
Staphylococcus cohnii	1	0	0.0	0	0.0	0	0.0	0
Staphylococcus epidermidis	47	35	74.5	35	74.5	44	93.6	43
Staphylococcus haemolyticus	11	5	45.5	4	36.4	10	90.9	9
Staphylococcus hominis	20	11	55.0	10	50.0	19	95.0	19
Staphylococcus simulans	1	1	100.0	0	0.0	1	100.0	0
Staphylococcus warneri	2	0	0.0	0	0.0	1	50.0	1
Streptococci	4	2	0.0	0	0.0	3	75.0	2
Streptococcus anginosus	1	0	0.0	0	0.0	0	0.0	0
Streptococcus bovis	1	0	0.0	0	0.0	1	100.0	1

		MALDI-TOF MS identification results						
		3 h				5 h		
Streptococcus dysagalactiae	1	1	100.0	0	0.0	1	100.0	0
Streptococcus pneumoniae	**Samples n.**	**Genus**	**%**	**Species**	**%**	**Genus**	**%**	**Sp**
Gram-positive rods	5	0	0.0	0	0.0	1	20.0	0
Brevibacterium casei	2	0	0.0	0	0.0	0	0.0	0
Corynebacterium striatum	2	0	0.0	0	0.0	1	50.0	0
Propionibacterium acnes	1	0	0.0	0	0.0	0	0.0	0
Gram-negative bacteria	32	25	78.1	24	75.0	29	90.6	28
Enterobacteriaceae	29	23	79.3	23	79.3	27	93.1	27
Enterobacter cloacae/asburiae	4	4	100.0	4	100.0	4	100.0	4
Escherichia coli	10	8	80.0	8	80.0	10	100.0	10
Klebsiella oxytoca	2	2	100.0	2	100.0	2	100.0	2
Klebsiella pneumoniae	9	6	66.7	6	66.7	8	88.9	8
Morganella morganii	1	1	100.0	1	100.0	1	100.0	1
Proteus mirabilis	3	2	66.7	2	66.7	2	66.7	2
Non-fermenting Gram-negative bacteria	3	2	66.7	1	33.3	2	66.7	1
Acinetobacter lwoffi	1	0	0.0	0	0.0	0	0.0	0
Aeromonas hydrophila	1	1	100.0	0	0.0	1	100.0	0
Stenotrophomonas maltophilia	1	1	100.0	1	100.0	1	100.0	1
Yeasts	9	0	0.0	0	0.0	0	0.0	0

		MALDI-TOF MS identification results						
		3 h				5 h		
Candida albicans	4	0	0.0	0	0.0	0	0.0	0
Candida parapsilosis	**Samples n.**	0 **Genus**	0.0 **%**	0 **Species**	0.0 **%**	0 **Genus**	0.0 **%**	0 **Sp**
Torulopsis glabrata	3	0	0.0	0	0.0	0	0.0	0
Total	162	104	64.2	98	60.5	137	84.6	13

Table 2

Positivity time of blood culture and MALDI-TOF MS correct identification

	MALDI-TOF MS correct identification at genus level (%)			
Incubation time	**3 h**		**5 h**	
Blood culture positivity time	**<24 h**	**>24 h**	**<24 h**	**>24 h**
Microorganisms	67.4	45.8	86.5	70.8
Bacteria	69.0	55.0	88.5	85.0
Gram-positive cocci	64.6	56.3	89.2	87.5
Gram-negative bacteria	81.8	100.0	86.4	100.0
Relationship between positivity time of blood culture (cut-off 24 h) and MALDI-TOF MS correct identification of pathogens at genus level at 3 and 5 h-incubation time				

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Discussion

In this study we evaluated the performance of MALDI-TOF MS for direct identification of microorganisms from positive blood cultures. MALDI-TOF MS direct identification for Gram-positive cocci and Gram-negative bacteria achieved good results with concordance with conventional methods near to 90% at genus and >85% at species level in 5 h-incubation time. Considering only Gram-positive cocci, as already described in previous studies, *Staphylococcus aureus* had the best identification results, whereas the less good results were

obtained with Streptococci's group, although the isolates number was low. In addition, we observed good results for CoNS after only 5 h of incubation with no misidentification as *Staphylococcus aureus* and an identification rate at 5 h of 88.2% at species level. This could be a critical point in the interpretation of bloodstream infection etiology, as it could allow for a rapid discrimination between true pathogens and possible contaminants. As reported in literature and showed by our results, the use of formic acid significantly improves identification performance for Gram-positive bacteria and has to be considered mandatory [4, 8]. Considering Gram-negative bacteria, *Enterobacteriaceae* had the highest identification rate, whereas unsatisfactory results were obtained for non-fermenting Gram-negative bacteria as well as for Gram-positive rods and yeast with no identification at species level even at 5 h-incubation time. However, as regards the Gram-positive rods, this should be considered in terms of clinical relevance, as Gram-positive rods are often considered contaminants. These unsatisfactory findings are probably due to microorganism intrinsic low growth rate. For this reason, it could be hypothesized that low blood culture positivity time (i.e., <24 h) could correlate with better results in identification due to a higher microorganism load and/or an intrinsic microorganism shorter time of growing. However, even if in terms of percentage, this hypothesis seems to be correct, with the exception of Gram-negative Bacteria, the correlation resulted not statistically significant.

At moment, for direct identification of microorganisms from positive blood culture bottles, several in-house protocols and some commercial kits have shown good results, however many of them are laborious, time consuming, or expensive. On the basis of literature data and considering our laboratory workflow, we have chosen and evaluated the most suitable, rapid, and cost-saving method for direct MALDI-TOF MS pathogen identification from positive blood culture samples. In particular, we tested the identification performance of VITEK MS platform combined with rapid subculture method on solid medium. Few studies have focused on this combination as most of them are based on another commercial platform, Bruker MALDI Biotyper System or protocols such as serum-separator, lysis-filtration, spin-lysis, and others. [2, 8, 9, 10, 11, 12]. Comparison of ours with those obtained from other studies could be difficult due to different instrumentation in use, several samples processing, various number of testing replicates and assorted MALDI-TOF MS results analysis, or interpretation rules. According to Martinez et al., data obtained with the commercial Bruker Sepsityper kit (Bruker Daltonics), on Bruker MALDI Biotyper System, performances of our protocol at 5 h were encouraging and even higher considering Staphylococci, Streptococci, and *Enterobacteriaceae* (89.9 vs. 87.9%; 50.0 vs. 42.9%; 93.1 vs. 91.4%) with low processing time (few seconds vs. ≥5 min), labor, and costs [10]. Monteiro et al., in their proposed

protocol, used the same identification platform VITEK MS, but a centrifugation-based method for fast and direct bacteria identification. Their results were better for Gram-negative bacteria (99.0 vs. 87.5% at 5 h) but not for Gram-positive bacteria (82.3 vs. 84.3% at 5 h; CoNS 77.3 vs. 88.2% at 5 h); moreover, >42 min with several processing steps were required [12]. In comparison to the study by Vereroken et al. applying the same rapid protocol based on 5 h subculture on solid media used in the present study, but different identification platform (VITEK MS *vs.* Bruker MALDI Biotyper System), we achieved for monomicrobial culture better results on Gram-positive bacteria in general (84.3 vs. 82.2%) and, in particular, for Staphylococci and *Staphylococcus aureus* (89.9 vs. 85.6%; 100.0 vs. 95.5%) [15]. In a similar study, using a short 4 h-incubation method on solid medium combined with Bruker MALDI Biotyper System, Kohlmann et al. applied some variations: single replicate testing, chocolate agar as solid medium, and two ways of interpretation of results, with manufacturer and modified cut-off values. In comparison to Kohlmann et al. findings, adopting manufacturer recommended cut-off values, our method was superior, or at least equal, even at 3 h-incubation time (61.2 vs. 33.3% Gram-positive bacteria; 63.8 vs. 37.3% Gram-positive cocci; 0.0 vs. 0.0% Gram-positive rods; 0.0 vs. 0.0% yeasts) with the exception of Gram-negative bacteria (75.0 vs. 96.4%; 79.3 vs. 97.3% *Enterobacteriaceae*) [7]. The correct identification gap between the study by Kohlmann et al. with modified cut-off values and our results at 5 h-incubation time was reduced, although our performances were still competitive (84.3 vs. 60.8% Gram-positive bacteria; 87.9 vs. 67.5% Gram-positive cocci; 89.9 vs. 66.5% Staphylococci; 0.0 vs. 0.0% yeasts) with same exceptions, as for examples, Enterococci, Streptococci, Gram-positive rods, and Gram-negative bacteria in general [7]. This comparison suggests that even with 1 h less of incubation time and a single replicate testing, a modification on manufacturer's criteria for successful identification, could improve results and a similar adjustment could improve also our performances. Nevertheless, our protocol is especially designed for routine clinical microbiology laboratory. Even if an evaluation of patients' outcome has to be performed, as supported by Idelevich et al. results, we suggest its application after 5 h of incubation time in particular for Gram-positive cocci and Gram-negative bacteria as a good compromise between identification rapidity and accuracy [4]. In our experience, this method is not applicable for rapid identification of yeasts. For these reasons, we suggest the application of this protocol only on the bases of Gram staining observation in order to limit possible diagnostics errors, time wasting, or useless labor.

Some factors could have limited the relevance of our results, in particular the small specimen number, the limited variety of species, and the exclusion of polymicrobial samples. Further studies should include more isolates and protocol

evaluation in the presence of polymicrobial infections. Moreover, in the future, in order to improve our protocol's performances for fastidious bacteria and *Haemophilus* spp., it should be recommendable to incubate samples on chocolate agar as suggested by Kohlmann et al. [7].

Conventional methods for microorganism identification and susceptibility testing in bloodstream infections provide a complete microbiological report useful for the therapeutic management of the patient after about 24–48 h [6, 13]. Even if conventional methods are considered the gold standard, several microbiology laboratories have incorporated in their routine workflow MALDI-TOF MS rapid protocols in order to accelerate microorganism identification and timely shift from empirical to targeted antimicrobial therapy [12]. The proposed method is easy to integrate in the clinical laboratory routine, not requiring additional time- or labor-consuming sample preparation steps, and leads to adequate identification results available to the clinician within the same day of blood culture positivity. The commercial platform VITEK MS combined with a rapid solid subculture method directly from positive blood culture samples after Gram staining observation could represent a relevant implementation in the diagnostic workflow of blood stream infections. In fact, Gram staining remains a key information even in the presence of a powerful instrument such as MALDI-TOF MS. Although a larger number of samples and species needs to be analyzed, these preliminary results could be considered for a timely therapeutic choice based on the epidemiological data about antimicrobial susceptibility in the hospital and in each ward. Thus, MALDI-TOF MS rapid identification represents a very powerful diagnostic tool for promptly and pathogen-driven antimicrobial therapy. Further studies, beside considering higher number of specimen, should also evaluate MALDI-TOF MS-based approach in relationship to patient's outcome.

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Compliance with Ethical Standards

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

Ethical Approval "All procedures performed in studies involving human participants where in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."