

MALDI-TOF Technique Availability for Identification of Septic Agents in Pediatric Practice

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The aim of the investigation was to assess MALDI-TOF mass spectrometry efficiency to identify microorganisms in small blood volumes (1–4 ml) in children with suspected septic conditions by comparing MALDI-TOF mass spectrometry findings with those of a classical microbiological study.

Materials and Methods. The subject of the research was blood of children with suspected septic conditions. For analysis we used blood cultures with microorganism growth recorded by BACTEC 9050. Pathogens isolated from blood culture were identified in two ways — by a classical microbiological study and MALDI-TOF mass spectrometry using α -cyano-4-hydroxycinnamic acid as a matrix. To compare the efficiency of two methods for blood infectious agent identification we applied a standard statistical method determining a concordance coefficient — Cohen's kappa.

Results. The findings of MALDI-TOF mass spectrometry used to identify microorganisms in blood cultures of children with bloodstream infections with one agent were compliant with those of a classical microbial study (Cohen's kappa is 0.96; $p < 0.001$). MALDI-TOF mass spectrometry and classical microbial study findings in the analysis of polymicrobial cultures had low concordance (Cohen's kappa is 0.58; $p > 0.05$).

Conclusion. MALDI-TOF mass spectroscopy identification of bloodstream infection agents can be recommended as a complimentary diagnostic technique aimed at analysis time reduction.

Key words: diagnostics of septic conditions; MALDI-TOF mass spectrometry; microbial identification.

In recent years the incidence of septic conditions (sepsis, severe sepsis and septic shock) in developed countries varies within the range from 240 to 300 cases per 100,000 population annually [1]. Even when treated in intensive care units the lethality rate in sepsis is 17.9%, and in severe sepsis — from 28.6 to 50% [1, 2]. The number of sepsis cases recorded annually is growing: early in the XXI century the increase in the

USA was 1.5% per year [3]. The treatment rates of septic conditions, to a large extent, are due to early and correct diagnostics. The most critical component in diagnosis is microbiological confirmation consisting in detection and identification of viable microbes in blood. Current methods of microbiological diagnostics of septic conditions are based on three methodological concepts [4–6]. The most common method is a con-

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ventional microbiological culture aimed at obtaining pure growth followed by the agent identification based on morphological, biochemical and serological criteria. The technique is highly sensitive and specific, though it has a drawback — a long duration (from 48 to 96 h). The second approach is based on the detection of genetic markers of a particular agent in blood using polymerase chain reaction (PCR). The main advantage of PCR-diagnosis is a quick result. However, the method has a critical disadvantage: positive results cannot serve as evidence of living agent presence in blood; it confirms only the fact that bacterial nucleic acids are present in blood, and they can be the derivatives of dead bacteria. Frequently, it results in false-positive results [6]. The third methodological approach is based on agent identification by its mass spectrometry (MS): ribosomal proteins of an agent are identified according to matrix-assisted laser desorption/ionization of sample proteins — MALDI-TOF (*Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight*) [7]. MS identification of microbes in hemocultures was successfully approved in diagnostics of bacteriemias in adults [8]. Moreover, one of manufacturers of mass spectrometers (Bruker Daltonics, Germany) produces a special MALDI Sepsityper kit designed for blood sample preparation in MS diagnosis of bacteriemias. However, pediatric diagnostics differs fundamentally from that of adults: it cannot apply “adult” diagnostic techniques without their preliminary adaptation, no matter the technique or the interpretation of results it concerns. MS diagnostics of bacteriemias using MALDI Sepsityper kit is limited by impossibility to obtain blood volume recommended by a manufacturing company (minimal volume is 8.0 ml) in some children, e.g. in children with low and extremely low body weight, and in children after blood loss, etc. In neonatology, small blood samples (0.5–1.0 ml) are considered sufficient for definite diagnosis of bacteriemias using routine microbiological methods [9]. To conflict the resolutions we supplemented the blood sample preparation for MS and included a procedure of extra incubation of small blood samples (1.0–4.0 ml) drawn from children and cultured on a special medium. Similar techniques of blood pre-incubation for MS diagnosis of bacteriemias had been applied for adult patients [10], though larger samples had been used.

The aim of the investigation was to assess MALDI-TOF mass spectrometry efficiency to identify microorganisms in small blood volumes (1–4 ml) in children with suspected septic conditions by comparing MALDI-TOF mass spectrometry findings with those of a classical microbiological study.

Materials and Methods. The subject of the research was blood samples of children undergoing treatment in an inpatient department of Scientific Centre of Children Health and Clinical and Research Institute of Emergency Children’s Surgery and Trauma of Moscow Health Department. The inclusion criterion was suspected septic conditions. All blood samples inoculated in hemoculture

bottles were incubated in an analyzer BACTEC 9050 (Becton Dickinson, USA) till bacterial growth was recorded. No bacterial growth in BACTEC incubator was considered an exclusion criterion.

Immediately after the bacterial growth registration the material (4.0 ml) was divided into two parts (2.0 ml each). The first was used for culture in solid media to isolate pure culture of an agent according to classical microbiological techniques. To identify isolates we used microscopic study, inoculation on selective and chromogenic media, as well as immunochemical and biochemical methods including the use of an automatic bacteriological analyzer Vitek 2 (BioMerieux, France). The second part of hemoculture (2 ml) was used for mass spectrometric identification of taxonomic status of an agent performed in accordance with the protocol developed before [11]. The procedure of isolation of bacterial proteins was completed by their (by 0.001 ml) application on target for MALDI-TOF mass spectrometry. α -cyano-4-hydroxycinnamic acid was used as matrix. To improve reliability each sample was tested in triplets. The spectra were taken on MALDI Biotyper MicroFlex (Bruker Daltonics, Germany), the range was 2–20 kDa. Minimum 1000 spectra were obtained from each sample.

Mass spectra were statistically processed using a program Biotyper 3.0 RTC. The degree of identification reliability was assessed by the scores obtained, the scores being comparing with spectra data of reference library Biotyper 3.0. The cases with scores <1.7 were considered as unreliable, and were not taken into account as cases of successful identification of isolate taxonomy [7]. To compare the efficacy of two methods of identification of blood flow infection agents — a classical microbiological study and MALDI-TOF mass spectrometry — we applied a standard statistical method determining a concordance coefficient — Cohen’s kappa, which equals maximum 1 if concordance is complete, and 0 — if concordance between the tests was not more frequently than could be expected in random coincidence. Values >0.75 were considered as sufficient concordance [12].

Results. By means of a classical microbiological study we analyzed 139 hemocultures, and isolated 164 isolates. The range of identified microorganisms included 26 bacterial species and 3 yeast-like fungi (*Candida albicans* — 4 isolates, *Candida parapsilosis* — 14 isolates and *Candida guilliermondii* — 1 isolate). Among bacteria we identified 12 gram-positive species: *Staphylococcus aureus* — 7 isolates, *Staphylococcus capitis* — 1 isolate, *Staphylococcus epidermidis* — 33 isolates, *Staphylococcus haemolyticus* — 17 isolates, *Staphylococcus hominis* — 11 isolates, *Staphylococcus warneri* — 1 isolate, *Enterococcus faecalis* — 9 isolates, *Enterococcus faecium* — 3 isolates, *Streptococcus gordonii* — 1 isolate, *Streptococcus parasanguinis* — 1 isolate, *Streptococcus vestibularis* — 1 isolate, *Streptococcus viridans* — 2 isolates; and 14 gram-

negative species: *Acinetobacter baumannii* — 8 isolates, *Acinetobacter lwoffii* — isolate, *Pseudomonas aeruginosa* — 9 isolates, *Pseudomonas putida* — 1 isolate, *Stenotrophomonas maltophilia* — 7 isolates, *Burkholderia cenocepacia* — 1 isolates, *Klebsiella pneumonia* — 23 isolates, *Klebsiella oxytoca* — 1 isolate, *Escherichia coli* — 1 isolate, *Enterobacter aerogenes* — 1 isolate, *Enterobacter cloacae* — 2 isolates, *Enterobacter kobei* — 1 isolate, *Chryseobacterium indologenes* — 1 isolates and *Serratia marcescens* — 1 isolate.

Hemocultures of 120 cases had only one bacterial type. 21 bacterial types were isolated from monocultures (11 — gram-positive and 10 — gram-negative)

and 4 fungi types (Table 1). From 19 samples we isolated associations containing two or three types of microorganisms. Therefore, to analyze the findings we divided the results into two groups. Group 1 included the findings of hemocultures containing only one type of microorganisms (See Table 1), group 2 had the data of hemocultures with more than one types of microorganisms — polymicrobial cultures (Table 2).

The results obtained for monocultures by MALDI-TOF were consistent with a classical classification in 115 of 120 cases (95.8%) (See Table 1). All discrepancies concerned only gram-positive bacteria. In two cases *S. haemolyticus* was misidentified: by MS it was identified as *S. warneri* or *S. epidermidis*; *S. vestibularis*

Table 1
Results of species identification of microorganisms from hemocultures containing only one type of microorganisms

Sample number	Classical microbiological study		MALDI-TOF mass spectrometry	
	Identified species	Number of cases	Identified species	The number of properly identified cases
1	<i>A. baumannii</i>	3	<i>A. baumannii</i>	3
2	<i>B. cenocepacia</i>	1	<i>B. cenocepacia</i>	1
3	<i>C. albicans</i>	4	<i>C. albicans</i>	4
4	<i>C. guilliermondii</i>	1	<i>C. guilliermondii</i>	1
5	<i>C. indologenes</i>	1	<i>C. indologenes</i>	1
6	<i>C. parapsilosis</i>	12	<i>C. parapsilosis</i>	12
7	<i>E. coli</i>	1	<i>E. coli</i>	1
8	<i>E. faecalis</i>	5	<i>E. faecalis</i>	5
9	<i>E. faecium</i>	2	<i>E. faecium</i>	2
10	<i>E. cloacae</i>	1	<i>E. cloacae</i>	1
11	<i>E. kobei</i>	1	<i>E. kobei</i>	1
12	<i>K. oxytoca</i>	1	<i>K. oxytoca</i>	1
13	<i>K. pneumoniae</i>	17	<i>K. pneumoniae</i>	17
14	<i>P. aeruginosa</i>	7	<i>P. aeruginosa</i>	7
15	<i>P. putida</i>	1	<i>P. putida</i>	1
16	<i>S. aureus</i>	5	<i>S. aureus</i>	5
17	<i>S. capitis</i>	1	<i>S. capitis</i>	1
18	<i>S. epidermidis</i>	28	<i>S. epidermidis</i>	28
19	<i>S. haemolyticus</i>	12	<i>S. haemolyticus</i> (10)* <i>S. epidermidis</i> (1) <i>S. warneri</i> (1)	10
20	<i>S. hominis</i>	5	<i>S. hominis</i>	5
21	<i>S. warneri</i>	1	<i>S. warneri</i>	1
22	<i>S. maltophilia</i>	6	<i>S. maltophilia</i>	6
23	<i>S. parasanguinis</i>	1	<i>S. parasanguinis</i>	1
24	<i>S. vestibularis</i>	1	<i>S. salivarius</i>	0
25	<i>S. viridans</i>	2	<i>S. pneumoniae</i> **	0
Total		120		115

Note: * 10 samples had complete concordance/coincidence of results of bacteriological and mass spectrometry methods, in two cases MALDI-TOF findings were erroneous suggesting the presence of *S. epidermidis* or *S. warneri* in a sample; ** both samples had erroneous results of MALDI-TOF study giving evidence that only *S. pneumoniae* was present in a sample.

was misidentified as *S. salivarius*; in two cases *S. viridans* was misidentified as *Streptococcus pneumoniae*. A concordance coefficient of the findings obtained by two methods was high, Cohen's kappa being 0.96 ($p < 0.001$).

Table 2 shows the research findings of polymicrobial hemocultures. None microorganisms were identified in 7 samples (36.8%) of 19 polymicrobial hemocultures using MALDI-TOF with a reliable Score. In the remaining blood cultures with a mixture of pathogens (63.2%), only one species was identified with a reliable Score. The concordance of the findings of MALDI-TOF mass spectrometry and a classical microbiological study was found only for 27.3% (12 strains) of the total number of isolates ($n=44$) of all polymicrobial cultures. A concordance coefficient of two methods was low, i.e. insignificant (Cohen's kappa was 0.58; $p > 0.05$).

Discussion. The present study is an example of successful application of MALDI-TOF diagnosis of septic conditions in pediatric practice — in cases requiring quick results, but had no possibility to use blood samples exceeding 4.0 ml. The findings give evidence of two levels of concordance of microbiological and MS diagnostics. The first level concerns monohemocultures. For gram-negative bacteria and fungi identification results coincided in 100% cases. Monohemocultures with gram-positive agents demonstrated incomplete (92.6%) though rather high coincidence (Cohen's kappa was 0.89). Thus, we evidenced the possibility to use effectively MS identification of bacteriemia agents for monohemocultures that corresponds to the findings our colleges had previously obtained [13]. In general, the correlation of identification results of microbes from monohemocultures using two methods was high and significant.

The study of polymicrobial hemocultures showed the ineffectiveness of MS technology in bacteriemia diagnostics. Statistical data processing confirmed the infeasibility of adequate identification of agents present in an association using MALDI-TOF mass spectrometry. Only in 27.3% cases the technique detected one of the agents present. It should be noted that in literature there are positive results of MALDI-TOF diagnostics of polymicrobial bacteriemias. For example, Gray et al. [10] studied 26 cases of polymicrobial blood flow infections, and one of the agents with a significant score was identified in 96.2% cases. We think that these findings were related to special selection of hemocultures for analysis: the authors worked only with hemocultures, in which gram-positive bacteria were detected initially by light microscopy (however, other agents in an association could be gram-positive bacteria as well). As previously mentioned,

Table 2

Results of species identification of microorganisms from hemocultures containing more than one type of microorganisms

Blood culture	Classical microbiological study (identified species)	MALDI-TOF mass spectrometry	
		Identified species	True identification/number of species
1	<i>C. parapsilosis</i>	Unidentified	1/3
	<i>S. epidermidis</i>	Unidentified	
	<i>S. hominis</i>	<i>S. hominis</i>	
2	<i>A. baumannii</i>	Unidentified	0/2
	<i>P. aeruginosa</i>	Unidentified	
3	<i>S. aureus</i>	Unidentified	1/2
	<i>S. epidermidis</i>	<i>S. epidermidis</i>	
4	<i>E. faecalis</i>	Unidentified	1/3
	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	
	<i>S. hominis</i>	Unidentified	
5	<i>C. parapsilosis</i>	Unidentified	1/2
	<i>S. epidermidis</i>	<i>S. epidermidis</i>	
6	<i>E. aerogenes</i>	Unidentified	1/2
	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	
7	<i>A. baumannii</i>	Unidentified	1/3
	<i>E. cloacae</i>	<i>E. cloacae</i>	
	<i>S. epidermidis</i>	Unidentified	
8	<i>E. faecium</i>	Unidentified	0/2
	<i>S. haemolyticus</i>	Unidentified	
9	<i>S. haemolyticus</i>	Unidentified	1/2
	<i>S. hominis</i>	<i>S. hominis</i>	
10	<i>A. baumannii</i>	Unidentified	0/2
	<i>K. pneumoniae</i>	Unidentified	
11	<i>A. baumannii</i>	Unidentified	0/3
	<i>E. faecalis</i>	Unidentified	
	<i>K. pneumoniae</i>	Unidentified	
12	<i>A. baumannii</i>	Unidentified	0/3
	<i>K. pneumoniae</i>	Unidentified	
	<i>P. aeruginosa</i>	Unidentified	
13	<i>K. pneumoniae</i>	Unidentified	0/2
	<i>S. marcescens</i>	Unidentified	
14	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	1/2
	<i>S. maltophilia</i>	Unidentified	
15	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	1/2
	<i>E. faecalis</i>	Unidentified	
16	<i>S. hominis</i>	<i>S. hominis</i>	1/2
	<i>A. lwoffii</i>	Unidentified	
17	<i>S. hominis</i>	<i>S. hominis</i>	1/2
	<i>S. aureus</i>	Unidentified	
18	<i>S. hominis</i>	Unidentified	0/3
	<i>S. epidermidis</i>	Unidentified	
	<i>S. gordonii</i>	Unidentified	
19	<i>E. faecalis</i>	<i>E. faecalis</i>	1/2
	<i>S. haemolyticus</i>	Unidentified	
Total	44 isolates		12 isolates

the probability of true MALDI-TOF identification for gram-negative microbes is higher compared to that for gram-positive. There was no special selection of samples with gram-negative bacteria for our experiments.

The primary cause of negative results of identification of microbes from mixed cultures must be related to the imperfection of software of existing mass spectrometers rather to the drawbacks of MALDI-TOF mass spectrometry. The development of the techniques to identify microbes in mixed cultures requires creation of a formidable proteome libraries and improvement of programs for results processing. However, currently, the fact is that a great deal of erroneous results obtained in polymicrobial hemoculture analysis considerably limits the use of MALDI-TOF technique to diagnose septic conditions.

The study results have shown application perspectiveness of mass spectrometry identification of an agent in a monomicrobial hemoculture. However, the use of the method cannot be considered as an absolute alternative to conventional methods of agent identification. The use of mass spectrometry can be reasonable as a sub-study, which enable to reduce identification time by 24–48 h, and, therefore, hasten the administration of adequate antimicrobial agents considering natural (species) resistance of an agent. Early diagnosis of septic conditions is vital since any delay of adequate antibiotic therapy administration reduces survival rate about 8% every hour [14]. Therefore, any opportunity of making a correct microbiological diagnosis should be taken.

Conclusion. The findings of MALDI-TOF mass spectrometry used to identify microorganisms in blood cultures of children with bloodstream infections with one agent were compliant with those of a classical microbial study. MALDI-TOF mass spectrometry identification of agents of blood flow infections can be recommended as a secondary diagnostic method aimed at test time reduction.

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Conflicts of Interest. The authors have no conflicts of interest.

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