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AN EVALUATION OF DIRECT IDENTIFICATION OF PATHOGENS FROM BLOOD CULTURES BY MALDI-TOF MASS SPECTROMETRY

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Key words: bloodstream infection, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS).

Summary
Rapid identification of the infecting organism may aid in choosing appropriate antimicrobial therapy. We used MALDI-TOF mass spectrometry to identify bacteria directly from the positive blood culture samples (n=21). 85,71 percent of these results was identified using of MALDI-TOF mass spectrometry. Identification time of bacteria directly from the blood culture takes more than 1 hour for 27,8 percent results.

Introduction

Accurate and fast diagnostic methods are necessary in order to be able to initiate correct treatment for bloodstream infection. Adequate empirical antibiotic therapy reduces the mortality rates in these patients (1,2). Blood culture growing in automated systems allow bacteria to multiply, and usually takes a minimum of at least one day prior to the positive automated system signal about the levels of bacterial growth, and then a blood smear by Gram staining takes a few minutes, however, the mere microscopy results often do not provide sufficient information for the doctor to begin proper antibiotic therapy.

Recently, clinical microbiology laboratory of Klaipeda university hospital started to use sensitive, rapid and high-throughput identification method, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). It has been introduced in bacterial taxonomy and successfully applied to a number of taxa (3-9,11). MALDI-TOF mass spectrometry has the ability to measure peptides and other compounds in the presence of salts and to analyze complex peptide mixtures, making it an ideal method for measuring non-purified extracts and intact bacterial cells. Different experimental factors, including sample preparation, matrix solutions and organic solvents, affect the quality and reproducibility of bacterial MALDI-TOF mass spectrometry fingerprints (11-16). With a special set of MALDI Sepsityper is possible to identify bacteria directly from the positive blood culture (17-25). Most reports describe the blood culture must be grown in automated BACTEC system vials (26,27). MALDI-TOF mass spectrometry has a high sensitivity and specificity (32).

Work objective: we investigated the direct identification of pathogens from the positive blood cultures using of MALDI-TOF mass spectrometry.

Materials and methods

The blood culture 8-10 ml was collected in standart BD BACTEC Plus Aerobic/F and Plus Anaerobic/F vials for adults and 1-3 ml was collected in BD BACTEC Peds Plus/F vial for newborns. The automated system BD BACTEC Fx gives a signal if there is bacterial growth in each bottle.It is necessary to separate bacteria from the liquid culture media prior to detection by MALDI-TOF mass spectrometry. Removal of red blood cells and serum separation by centrifugation, and there after neutralization of bacteria using ethanol, precipitation and extraction, using formic acid and acetonitrile, must be done.Identification of microorganisms by MALDI-TOF mass spectrometry was rated by scores: range from 2,300 till 3,000 – highly probable species identification; 2,000 ... 2,299 – secure genus identification, probable species identification; 1,700 ... 1,999 – probable genus identification; 0,000 ... 1,699 – not reliable identification.

Results

In all 494 blood culture samples, registered in clinical microbiology laboratory of Klaipeda university hospital from 2016 January 2nd till 2016 June 1st, 431 had negative results and 63 (12,75%) had bacterial growth. 24 (38,1%) blood culture samples had staphylococcal growth. MALDI Sepsityper was applied to 12 (50%) of this growth and 9

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(75%) was identified: 25 percent – *Staphylococcus aureus*; 8,33 percent – *Staphylococcus epidermidis*; 25 percent – *Staphylococcus hominis*; 8.333 percent – *Staphylococcus haemolyticus*; 8.333 percent – *Micrococcus luteus*. 55,55 percent of staphylococcal growth was rated as secure genus identification, probable species identification and 44,44 percent of staphylococcal growth was rated as probable genus identification using of MALDI-TOF mass spectrometry.

See Table 1 for details.

All growing blood culture samples had only 11,11% of *E. coli* growth. MALDI Sepsityper was applied to 9 (85,71%) blood culture samples of this growth and all was identified. 66,66 percent of *E. coli* was rated as highly probable species identification, 11,11 percent – as secure genus identification, probable species identification and 22,22 percent – as probable genus identification using of MALDI-TOF mass spectrometry. See Table 2 for details.

In this study using of MALDI-TOF mass spectrometry as highly probable species identification we had only for *E. coli* growth (33,33%), as secure genus identification, probable species identification – less for *E. coli* (5,55%) and more – for staphylococcal (27,77%) growth, as probable genus identification – less for *E. coli* (11,11%) and more – for staphylococcal (22,22%) growth (r=0,616, p=0,006). Of all identified (n=18) blood culture samples 50 percent had *E. coli* growth, 27,8 percent had plasma negative staphylococcal growth and 16,7 percent had *Staphylococcus aureus* growth. The most growing BACTEC vials – Aerobic (61,1%).

Time taken to identify staphylococcal and *E.coli* growth, of fixed automated system BD BACTEC Fx signal time to MALDI-TOF mass spectrometry identification time, is mostly more than 3 hours (44,4%). 27,8 percent takes more than 1 hour, 22,2 percent – more than 2 hour, and less, 5,6 percent takes more than 4 hours. See Table 3 for details.

Table 1.

Table 1

Bacteria / vial	MALDI-TOF MS score
S. aureus / Aerobic	2,235
S. aureus / Aerobic	2,266
S. aureus / Anaerobic	2,206
S. haemolyticus / Aerobic	2,104
S. hominis / Aerobic	1,836
S. hominis / Anaerobic	1,939
S. epidermidis / Aerobic	1,879
Micrococcus luteus / Aerobic	1,814
S. hominis / Peds	2,175

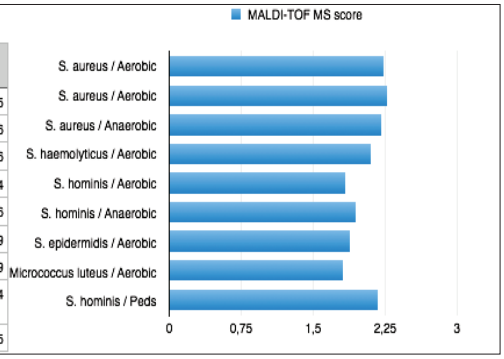


Table 2.

Table 2

Bacteria / vial	MALDI-TOF MS score
E. coli / Aerobic	2,123
E. coli / Aerobic	2,311
E. coli / Anaerobic	2,325
E. coli / Anaerobic	1,924
E. coli / Aerobic	1,919
E. coli / Aerobic	2,313
E. coli / Anaerobic	2,402
E. coli / Aerobic	2,377
E. coli / Anaerobic	2,495

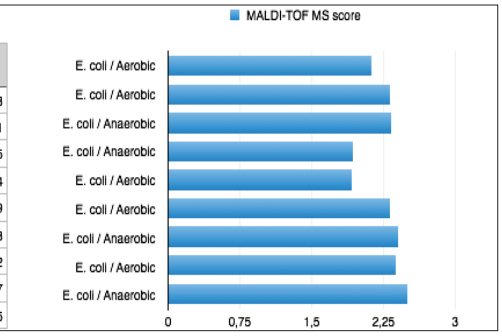
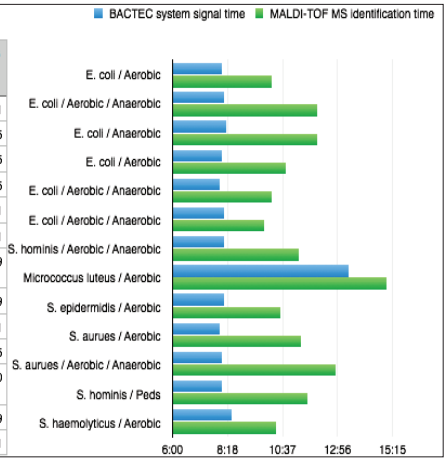


Table 3.

Table 3

Bacteria / vial	BACTEC system signal time	MALDI-TOF MS identification time
E. coli / Aerobic	8:05	10:11
E. coli / Aerobic / Anaerobic	8:10	12:05
E. coli / Anaerobic	8:15	12:05
E. coli / Aerobic	8:05	10:45
E. coli / Aerobic / Anaerobic	8:00	10:11
E. coli / Aerobic / Anaerobic	8:10	9:51
S. hominis / Aerobic / Anaerobic	8:10	11:19
Micrococcus luteus / Aerobic	13:25	14:59
S. epidermidis / Aerobic	8:10	10:31
S. aurues / Aerobic	8:00	11:25
S. aurues / Aerobic / Anaerobic	8:05	12:50
S. hominis / Peds	8:05	11:39
S. haemolyticus / Aerobic	8:30	10:21



Discussion

Any way traditional pathogen identification from blood culture takes more than 24 hours and so is not able to guide early choice of antimicrobial therapy in sepsis patients.

Identification of microorganisms from positive blood culture using of MALDI Sepsityper processing kit requires approximately 20 minutes and it does independent of species (6,10,13,18,20,25,26,32,33). Batch processing added approximately 1 min per blood culture analyzed.Our estimated time to identify bacteria from the positive blood culture, of fixed automated system BD BACTEC Fx signal time to MALDI-TOF mass spectrometry identification time, is not an objective, it includes the blood smear preparation time by Gram staining,

as well as organizational reasons of most microbiological laboratories to perform the method in batches. But in comparison with other studies our estimated time correspond to with foreign laboratories (10).Some studies even describe MALDI-TOF mass spectrometry advantages compared to other direct methods to identify the bacteria from the blood, for example, using polymerase chain reaction and the latter method is limited by the high cost, not every species of microorganisms is determined in one study, in addition, it is enough difficult to realize in practic (28-31).

Conclusion

In conclusion, the exact bacteria identification from the positive blood culture using of MALDI-TOF mass spectrometry and presentation to the doctor allows to start adequate empirical antibiotic therapy in less time.

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TIESIOGINĖS BAKTERIJŲ IDENTIFIKACIJOS IŠ KRAUJO PASĖLIO ĮVERTINIMAS PANAUDOJANT MALDI-TOF MASIŲ SPEKTROMETRIJĄ

L. Savickaitė, J. Kopeykinienė

Raktažodžiai: kraujo infekcijos, MALDI-TOF masių spektrometrija (MALDI-TOF MS).

Santrauka

Greitas infekcijos nustatymas organizme gali padėti pasirinkti tinkamą antimikrobinį gydymą. Tikslūs ir greiti diagnostikos metodai yra ypač aktualūs nustatant kraujo infekcijos sukėlėjus, o paskirta adekvati antibiotikų terapija sumažina mirtingumo rodiklius. Todėl panaudojant MALDI-TOF masių spektrometriją bandėme nustatyti bakterijas tiesiogiai iš teigiamų kraujo pasėlio mėginių (n = 21). Iš jų 85,71 proc. buvo identifikuoti panaudojant MALDI-TOF masių spektrometriją. Bakterijų identifikavimo laikas tiesiogiai iš kraujo kultūros užima daugiau nei 1 valandą 27,8 proc. rezultatų, daugiau nei 2 val. – 22,2 proc. rezultatų, daugiausiai rezultatų, daugiau nei 3 val. – 44,4 proc. ir mažiausiai rezultatų, daugiau nei 4 val. – 5,6 proc.

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