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MASS SPECTROMETRIC IDENTIFICATION OF BLOODSTREAM INFECTION AGENTS: EXPERIENCE IN PEDIATRIC PRACTICE

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Abstract:

Rapid diagnostics of bloodstream infections is a pressing issue in modern pediatrics. The paper presents data from a comparative analysis of two methods for identifying microorganisms in the blood of children with suspected bloodstream infections: routine microbiological testing and MAldI-TOF mass spectrometry. The results obtained using the mass spectrometric (MAldI-TOF) method for identifying microorganisms in blood cultures from children with bloodstream infections containing one pathogen are highly consistent with the results of classical microbiological testing (Cohen's kappa 0.97, $p < 0.001$). MAldI-TOF mass spectrometric identification of bloodstream infection pathogens can be recommended as an additional diagnostic method aimed at reducing analysis time.

Keywords: bloodstream infections; bacteria; microscopic fungi; diagnostics; MAldI-TOF mass spectrometry.

INTRODUCTION

Infections associated with the circulatory system, or bloodstream infections, are a pressing problem in modern medicine. They are recorded in 15% of patients in intensive care units and are fatal in 12–25% of cases [1, 2]. In pediatric practice, hospital mortality from the most severe manifestation of bloodstream infections, sepsis, averages 10.3% [3]. The effectiveness of bloodstream infection treatment is largely determined by timely and accurate diagnosis. It is necessary to focus on the necessary condition for its development - the presence of live bacteria (bacteremia) and/or fungi (fungemia) in the blood. Bacteremia and fungemia are conditions that not only are the pathogenetic basis of bloodstream infections, but are also used as the most important criteria for diagnosis. Microbiological diagnosis of bloodstream infections must answer two main questions: are microorganisms present in the blood and, if present, what species they belong to.

MATERIALS AND METHODS

Historically, the first and most common method is based on classical microbiological cultivation. If performed correctly, it has sufficient sensitivity and specificity, but has an important drawback - the duration of execution (from 48 to 96 hours). The second approach is associated with the detection of genetic markers of a particular type of pathogen in the blood - specific nucleotide sequences. The advantage of genetic diagnostics is the speed of obtaining the result. But this method is not without a fundamental drawback. A positive result indicates not the presence of a living pathogen in the blood, but the presence of nucleic acids, which can be derivatives of dead microbes, which leads to false positive diagnoses [2]. This work is devoted to the assessment of the third methodological approach to the diagnosis of bloodstream infections, based on a combination of microbiological preparation of a blood sample (cultivation in an incubator) and mass spectrometric identification of the pathogen by its proteomic profile. Mass spectrometry was performed using the time-of-flight technology for recording mass spectra obtained using matrix-assisted laser desorption/ionization of sample proteins – MALDI-TOF (from the English Matrix-Assisted Laser Desorption/Ionization, Time-Of-Flight).

The objects of the study were blood samples obtained from children from the hospitals of the Scientific Center for Children's Health and the Research Institute of Emergency Children's Surgery and Traumatology.

To increase reliability, each sample was tested in triplets. Spectra were recorded automatically (detection mode – MBT-FC), the spectrum range was 2–20 kD. 240 spectra were obtained from each sample. The degree of identification reliability was assessed by the obtained Score values. Cases with Score < 1.7 were considered unreliable and were not counted as cases of successful determination of the taxonomic affiliation of the isolate [4].

RESULTS AND DISCUSSION

Using classical microbiological research, 85 blood cultures were tested, from which 103 isolates were isolated. The spectrum of identified microorganisms included 19 species of bacteria and 2 species of yeast-like fungi (Candida albicans (3 isolates), Candida parapsilosis (13 isolates)). Among the bacteria, 7 gram-positive species were found: Staphylococcus

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aureus (3 isolates), Staphylococcus epidermidis (13 isolates), Staphylococcus haemolyticus (8 isolates), Staphylococcus hominis (6 isolates), enterococcus faecalis (5 isolates), enterococcus faecium (1 isolate), and Streptococcus vestibularis (1 isolate). The 12 identified Gram-negative species were Acinetobacter baumannii (8 isolates), Klebsiella pneumoniae (19 isolates), Pseudomonas aeruginosa (9 isolates), Pseudomonas putida (1 isolate), Stenotrophomonas maltophilia (5 isolates), enterobacter aerogenes (1 isolate), enterobacter cloacae (2 isolates), enterobacter kobei (1 isolate), escherichia coli (1 isolate), Chryseobacterium indologenes (1 isolate), Serratia marcescens (1 isolate), and Neisseria meningitidis (1 isolate).

In 72 cases, the blood cultures contained only one microorganism species and were monocultures. A total of 16 bacterial and 2 fungal species were detected in the monocultures. An association of microorganisms was identified in 13 samples. Therefore, it was essential to divide all the results into 2 groups for the analysis of the results. The 1st group included the results obtained in the study of blood cultures containing one microorganism species. The results obtained for monocultures by the MALDI-TOF method corresponded to the classical identification in 70 (97.2%) cases out of 72 (see Table 1). Two discrepancies between the identification methods concerned gram-positive bacteria: in one case, S. haemolyticus was identified by the MALDI-TOF method as Staphylococcus warneri, in the other case, S. vestibularis was erroneously identified as S. salivarius. All positive mass spectroscopy results were characterized by a high degree of reliability: the Score values for bacteria ranged from 1.91 to 2.39, for Candida spp. – from 2.08 to 2.27. The degree of agreement between the results obtained by the two methods was very high – the Cohen's kappa value was 0.97 ($p < 0.001$).

The results of the study of polymicrobial blood cultures are summarized in Table 2. In 6 (46.2%) samples out of 13 polymicrobial blood cultures, not a single species was identified by the MALDI-TOF method with a reliable Score level. For the remaining 7 (53.8%) blood cultures, the determination of the species with a reliable Score was recorded only for one of the pathogens included in the association. Coincidence of the results of MALDI-TOF mass spectrometry and classical microbiological research was observed only for 7 (22.6%) species out of 31 species of polymicrobial cultures. The degree of agreement between the results of the two methods was statistically unreliable (Cohen's kappa was 0.70; $p > 0.05$). Our data indicate two levels of agreement between the results of microbiological and mass spectrometric diagnostics. The first level concerned monohemocultures. For gram-negative bacteria and fungi, the identification results coincided 100%. Monohemocultures with gram-positive pathogens demonstrated incomplete (91.3%), but fairly high coincidence (Cohen's kappa 0.89), which is consistent with previously obtained data in adults and newborns [3]. In general, the correspondence of the identification results of microbes from monohemocultures was high and statistically proven.

Unfortunately, the results of the mass spectrometric study of polymicrobial blood cultures were less optimistic. Statistical processing of the data confirmed the impossibility of full identification of pathogens present in the association. Only in 53.8% of cases one of the present pathogens was identified by the MALDI-TOF method. The literature also contains more positive results of MALDI-TOF diagnostics of polymicrobial bloodstream infections. For example, in the work of T. Gray et al. [2], where 26 cases of polymicrobial bloodstream infections were analyzed, one of the pathogens with a sufficient Score level was identified in 96.2%. In 30.8% of cases, the software indicated a high probability of the presence of other microbes.

The use of this method may be justified as an additional study that allows for a reduction in identification time by 24–48 hours, and therefore, accelerates the use of adequate antimicrobial drugs, taking into account the natural (species) resistance of the pathogen. It should be remembered that every hour of delay in prescribing adequate therapy for bloodstream infections reduces the patient's survival by 8% [4]. Therefore, it is necessary to use any opportunity to accelerate the establishment of a microbiological diagnosis.

CONCLUSION

Thus, the results obtained using the mass spectrometric (MALDI-TOF) method for identifying microorganisms in blood cultures from children with bloodstream infections containing one pathogenic microbe have a high degree of correspondence with the data of classical microbiological research. MALDI-TOF mass spectrometric identification of pathogens of bloodstream infections can be recommended as an additional diagnostic method aimed at reducing the analysis time.

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