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Identification of rod-shaped Gram-negative bacilli of *Enterobacteriaceae* family by MALDI-TOF mass spectrometry

Identyfikacja pałeczek Gram-ujemnych z rodziny *Enterobacteriaceae* przy użyciu spektrometrii masowej MALDI-TOF

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Key words

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Słowa kluczowe

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Summary

Introduction. Matrix assisted laser desorption ionization-time of flight mass spectrometry is a new rapid method for identification of microorganisms isolated from clinical specimens.

Aim. The aim of the study was to compare the results of identification *Enterobacteriaceae* strains obtained by using MALDI Biotyper and Vitek 2.

Material and methods. Identification of 313 *Enterobacteriaceae* isolates obtained from clinical specimens was performed simultaneously by using MALDI Biotyper and Vitek 2.

Results. In the case of 84.98% of the strains (n = 266) the same effectiveness of identification to the species level was obtained using both analyzers. 13.42% strains (n = 42) have been equally identified only to the genus level by both analyzers. Only in the case of 4 strains (1.28%) the results were divergent.

Conclusions. MALDI TOF-MS technology provides an excellent tool for identification of bacteria from clinical specimens. It allows obtaining reliable results within time unattainable for biochemical methods of microorganisms identification.

Streszczenie

Wstęp. Spektrometria masowa z użyciem desorpcji/jonizacji laserowej wspomaganej matrycą z analizatorem czasu przelotu (ang. *matrix assisted laser ionization-time of flight mass spectrometry* – MALDI-TOF MS) jest nową metodą szybkiej identyfikacji drobnoustrojów wyhodowanych z materiałów klinicznych.

Cel pracy. Celem pracy było porównanie wyników identyfikacji szczepów rodziny *Enterobacteriaceae* uzyskanych za pomocą aparatu MALDI Biotyper oraz Vitek 2.

Materiał i metody. Przeprowadzono jednoczesną identyfikację 313 pałeczek Gram-ujemnych z rodziny *Enterobacteriaceae* za pomocą dwóch analizatorów – MALDI Biotyper oraz Vitek 2.

Wyniki. Wyniki identyfikacji do gatunku uzyskane w aparacie MALDI Biotyper oraz Vitek 2 były zgodne dla 84,98% (n = 266) szczepów. W przypadku 13,42% (n = 42) szczepów zgodność wyników w obu systemach dotyczyła identyfikacji pałeczek Gram-ujemnych do rodzaju. Wyniki rozbieżne uzyskano w przypadku 4 szczepów (1,28%).

Wnioski. Technologia MALDI-TOF MS stanowi dobre narzędzie do identyfikacji bakterii pochodzących z materiałów klinicznych, zapewniając uzyskanie wiarygodnych wyników w czasie nieosiągalnym dla biochemicznych metod identyfikacji drobnoustrojów.

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INTRODUCTION

Matrix assisted laser ionization-time of flight mass spectrometry MALDI-TOF MS is a new method used in microbiological diagnostics, which allows shortening the time of identification of pathogen from the culture obtained from the moment of its growth. The principle of MALDI-TOF MS is based on the analysis of a unique protein profile, specific for a particular species, to allow diagnosis. This technique requires a small number of cells of microorganisms, it is possible to start analysis at the time of obtaining visible growth on the medium, which is usually possible within 16-24 hours after seed-

ing material. Currently, there are cases reported of the use of MALDI-TOF MS in rapid microbiological diagnostic testing, wherein identification of species was performed with cultures incubated for approximately 4-6 hours (1).

Sample analysis using MALDI-TOF MS starts by connecting the test culture with a matrix solution on a surface of specially-designed slides. Depending on the manufacturer, up to 96 samples can be placed on one slide. After the application of the test strain, usually using a disposable plastic loop, less frequently, a toothpick or a pipette tip, and adding the matrix, the slide is left to dry and then to be placed in the analyzer. A single sample preparation and control time does not take more than a minute. A number of processes take place in the analyzer. First, absorption of laser energy by the matrix, which is then converted into heat energy. Under the influence of the heat generated a small amount of the matrix is abruptly heated and then undergoes desorption (evaporation) together with the test material. At the same time, under the influence of the laser, the remainder of the matrix is ionized, and the protons resulting in the process combine with the test substance. The resulting charged particles are suppressed in the electromagnetic field. The time of flight (TOF) of particles to the detector is dependent on the mass-to-charge ratio (m/z). Thus, particles with a smaller m/z reach the detector sensor more quickly than those with higher molecular mass and charge. In this way, a mass spectrum is obtained, which is then compared with the standard mass spectra contained in the database, as developed on the basis of reference strains. Databases containing reference spectra are developed by apparatus manufacturers and are being continuously expanded. Some systems allow you to manually add some standard spectra (2).

AIM

The purpose of the study was to compare the results of the identification of *Enterobacteriaceae* strains obtained by MALDI Biotyper apparatus (Bruker Daltonics) and a microbiological analyzer Vitek 2 (bioMérieux).

MATERIAL AND METHODS

The study involved 313 strains of Gram-negative *Enterobacteriaceae* cultured from clinical specimens derived from patients of the Infant Jesus Teaching Hospital in Warsaw.

Bacterial cultures were handled for 18-24 hours on the following bases: Columbia Agar with 5% sheep erythrocytes and MacConkey. Identification of microorganisms was performed simultaneously on two devices: a microbiological analyzer Vitek 2 (bioMérieux), based on the biochemical characteristics of bacteria (identification cards of the ID-GN), and using the MALDI-TOF MALDI Biotyper (Bruker Daltonics), based on mass spectrometry (MS) with time-of-flight measurement. Alpha-cyano-4-hydroxycinnamic acid (Bruker Daltonics), dissolved in a mixture composed of: water, trifluoroacetic acid, acetonitrile, ratios of 19:1:20 (all HPLC grade reagents (Sigma)), was used as matrix for the MS analyzer.

RESULTS

A detailed overview of the results of the identification of 313 Gram-negative *Enterobacteriaceae* is shown in table 1. The results were compared in three categories: 1 – identification identical to the species, 2 – identification identical to the type, 3 – identification different than the type, 4 – lack of identification or low differentiation (identification not acceptable).

Table 1. Comparison of the results of the identification of Gram-negative *Enterobacteriaceae* using the MALDI Biotyper and a microbiological analyzer Vitek 2.

MALDI Biotyper Apparatus	Microbiological analyzer Vitek 2	Number of strains tested
Citrobacter braakii	*Citrobacter freundii*	3
C. freundii	*C. braakii*	3
C. freundii	*C. freundii*	26
C. koseri	*C. freundii*	1
C. koseri	*C. koseri*	8
C. murliniae	*C. freundii*	3
C. youngae	*C. freundii*	3
Enterobacter asburiae	*Enterobacter cloacae complex*	7
E. cloacae	*E. gergoviae*	1
E. cloacae	*E. cloacae*	152
E. cloacae	*Klebsiella oxytoca*	1
E. cloacae	*Pantoea agglomerans*	1
E. cloacae	*Raoultella ornithinolytica*	1
E. kobei	*Enterobacter cloacae complex*	5
E. ludwigii	*E. cloacae complex*	3
Escherichia coli	*E. coli*	8
Klebsiella oxytoca	*K. oxytoca*	24
K. pneumoniae	*E. cloacae*	1
K. pneumoniae	*K. pneumonia*	6
Morganella morganii	*M. morganii*	2
Proteus penneri	*Proteus vulgaris group/ Proteus penneri*	1
Raoultella terrigena	*Low discrimination*	1
Salmonella sp.	*Salmonella sp.*	9
Serratia liquefaciens	*S. liquefaciens group*	1
S. marcescens	*S. marcescens*	40
S. ureilytica	*S. marcescens*	2
Total		313

Comparative analysis of the results obtained in the MALDI Biotyper and Vitek 2 showed that 85% (n = 266) of strains were identified as identical to the species level by both systems. In the case of 42 (13.4%) strains, the same result of identification was obtained only for the type. Divergent results were found in 4 strains (1.3%), where 3 strains were

identified by the MALDI Biotyper as *Enterobacter cloacae* and by the Vitek 2 analyzer as *Klebsiella oxytoca*, *Pantoea agglomerans* and *Raoultella ornithinolytica*. The fourth strain was identified by the MALDI Biotyper as *Klebsiella pneumoniae* and by the Vitek 2 as *Enterobacter cloaecae*. One of the strains analyzed was not identified on the basis of biochemical characteristics in the Vitek 2 apparatus, and it was recognized as *Raoultella terrigena* on the basis of mass spectrometry. Both analyzers were not able to determine the species of 9 strains of *Salmonella.*

In the case of 15 (4.8%) strains classified as *Enterobacter cloacae* complex, on the basis of biochemical characteristics, using mass spectrometry, 3 species were differentiated: *E. asburiae* (n = 7), *E. kobei* (n = 3), *E. ludwigii* (n = 3).

DISCUSSION

Many new drugs, such as corticosteroids, anti-proliferative drugs or calcineurin inhibitors, induce iatrogenic immunosuppression that predisposes patients to the development of invasive, rapidly progressing infection, not only caused by pathogenic but also opportunistic microorganisms. Common infections in immunoincompetent patients run a subclinical course that does not give explicit subclinical symptoms, and diagnosis is based solely on the results of laboratory tests and microbiological tests. Given that the effectiveness of therapy largely depends on the speed of the introduction of appropriate antimicrobial therapy, the time to identify the etiological agent of infection plays a key role in the treatment process. In this context, searching takes place for new methods to enable fast microbiological diagnosis. Such methods include, inter alia, MALDI-TOF MS, which can significantly reduce the time to identify pathogens. Possible simultaneous analysis of up to 95 strains in the technique of MALDI-TOF MS also reduces the execution time of many tests and facilitates the organization of laboratory work. The results presented in the work show high similarity of identification of Gram-negative *Enterobacteraceae* using MALDI-TOF MS, as compared to the recommended biochemical diagnostics performed by the microbiological analyzer Vitek 2 (85% of the strains identified as the same species). This is consistent with results obtained by other authors, describes highly effective identification of Gram-negative and Gram-positive aerobic bacteria and fungi (3-6).

It has been demonstrated that MALDI-TOF MS is an effective and rapid method for identifying the obligatory anaerobic bacteria causing infections in humans, which is of particular importance because of the diagnostic problems encountered in the traditional methods of identification of anaerobic bacteria (7).

Numerous publications describe the possibility of using MALDI-TOF MS in the diagnosis of pathogens incubated on bases only for 4 to 6 hours. Despite such a short period of incubation, results in most cases are consistent with the identification of bacteria in MALDI-TOF MS obtained after overnight incubation (8, 9). The data demonstrates high efficiency of the identification using MALDI-TOF MS during times unattainable through conventional methods and give hope for the future use of MALDI-TOF in rapid microbiological diagnosis, especially in the case of bacteremia and septic conditions.

CONCLUSIONS

1. Materials from patients were dominated by rods (*E. cloacae* (n = 152), *S. marcescens* (n = 40), *C. freundii* (n = 26), *K. oxytoca* (n = 24), *Salmonella* sp. (n = 9), *C. koseri* (n = 8), *E. coli* (n = 8), *K. pneumoniae* (n = 6), *M. morganii* (n = 2) that have been identified identically in both systems, whereas in the case of four strains (1.3%) different identification results have been obtained.
2. The MALDI-TOF MS technology is a useful tool for the identification of bacteria isolated from clinical specimens, providing reliable results during times unattainable for biochemical methods. This is particularly important in serious clinical conditions of bacteremia and septic conditions.

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