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ADVANCES IN THE IDENTIFICATION TECHNIQUES FOR PATHOGENIC MICROORGANISMS

Lívia Caroline Alexandre de Araújo[1]; Rafael Artur de Queiroz Cavalcanti de Sá[2]; Sivoneide Maria da Silva[2]; Francisco Henrique da Silva[2]; Cíntia Justino Rabelo[3]; Mariana Gomes Vidal Sampaio[3];

[1] Centro de Ciências Agrárias, Universidade Federal da Paraíba – CCA/UFPB
[2] Centro de Ciências da Saúde, Universidade Federal de Pernambuco – CCS/UFPE
[3] Centro Universitário Católica de Quixáda - UNICATÓLICA

ABSTRACT

Objectives: The research objective was to present the main techniques for identifying pathogenic microorganisms and the application of new technologies for the clinical diagnosis of infectious diseases. **Methods**: It was made a search for free and recent journals available online in the databases of Pubmed (National Library of Medicine and the National Institutes), Lilacs (Latin American and Caribbean Literature in Health Sciences), and Scielo (Scientific Electronic Library Online), based on keywords related to the proposed theme. **Results**: From the researched literature, it was possible to verify that conventional techniques, despite their limitations, are still widely used for the identification and microbial characterization. However, in the last decades, molecular methods have been widely inserted in the laboratory routine seeking to increase the capacity to detect infectious agents with high sensitivity, specificity, speed, and low cost. Among the various techniques, amplification of DNA sequences is highlighted by the polymerase chain reaction (PCR), the sequencing of the 16S rRNA gene, and other variations of PCR. In addition to these, new technologies have been developed, such as new generation sequencing (NGS) and Matrix-assisted Laser Desorption/ionization-time-of-flight Mass Spectrometry (MALDI-TOF MS). **Conclusion**: The development of new technologies that allow rapid, sensitive, reproducible, and low-cost microbial identification, it is of great relevance for clinical microbiology, and consequently, for public health.

Keyword: Bacteria. Genotyping techniques. Microbiological techniques.

***Correspondence to Author:**
Lívia Caroline Alexandre de Araújo
Centro de Ciências Agrárias, Universidade Federal da Paraíba – CCA/UFPB

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INTRODUCTION

The detection, identification, and characterization of pathogenic microorganisms are crucial for the successful treatment of infectious diseases. For this reason, clinical microbiological diagnostic laboratories have a large arsenal of methods, culture-dependent or independent, to investigate the etiology of microbial infections [1].

Traditionally, the techniques of identification and differentiation of bacterial strains are culture-dependent, based on the phenotypic characteristics of the samples. However, these conventional techniques are often limited by their low capacity to differentiate between strains of a given species and also due to the low reproducibility of the tests [2]. Besides, some microorganisms that cause infections require specific growth conditions and are difficult to reproduce in the laboratory, preventing their identification [3].

Faced with this problem, new techniques for the identification and classification of microorganisms are emerging in an attempt to streamline results, reduce costs, and reach more detailed identifications than conventional methods [4]. Among the various techniques, the molecular ones that are being rapidly developed stand out, contributing significantly to a major advance in clinical microbiology [5].

In general, the molecular techniques for characterizing microorganisms bases on the analysis of proteins and mainly nucleic acids [RNA or DNA], with different levels of resolution for the detection of bacterial isolates [6]. Among these, we highlight the ones based on the amplification of DNA sequences by the polymerase chain reaction [PCR] [7], sequencing of the 16S rRNA gene [8,9], and other techniques based on PCR variations [10,11].

With advances in the study of techniques based on gene sequencing, new known strategies have emerged as new generation sequencing [NGS] [12], enabling the development of whole-genome sequencing [WGS]. The WGS has recently become a highly accessible tool for bacterial genotyping. The analysis of the total bacterial genome not only provides information on bacterial typing and evolutionary strains but also revolutionized our approach to understanding antimicrobial resistance and outbreak investigations [13].

Recently, new technologies based on protein profiles have also been developed, such as the Matrix-assisted Laser Desorption/ionization-time-of-flight Mass Spectrometry [MALDI-TOF MS], which has been used as a simple, reliable, and fast technique [14, 15, 16]. Several studies have demonstrated the effectiveness of this technique in the identification and characterization of microorganisms of clinical origin [14, 17, 18]. Given this context, the present study aims to conduct a literature review on the main techniques for identifying pathogenic microorganisms and their technological advances.

METHODS

The present study is a systematic review of the advances in techniques for identifying pathogenic microorganisms. Given this theme, there made a search for free journals available online in the databases of Pubmed [National Library of Medicine and the National Institutes], Lilacs [Latin American and Caribbean Literature in Health Sciences], and Scielo [Scientific Electronic Library Online], by using the following Keywords: microorganism identification, phenotypic methods, molecular techniques, 16S rRNA gene, MALDI-TOFF.

The criteria for inclusion of journals in the research were: relevant and related studies; complete articles published in English, Portuguese, or Spanish; published from 2010 to 2020. As exclusion criteria, articles that did not meet the proposed theme or outside the selected period.

RESULTS

The identification and characterization of pathogenic microorganisms are extremely important steps for the accurate diagnosis and implementation of appropriate antimicrobial

therapy, particularly in severe infections such as sepsis [19]. Therefore, the used techniques must present characteristics such as universal identification capacity, to have a low operating cost, and present a fast, accurate, and reliable result.

Currently, bacterial identification techniques are based on phenotypic characteristics and are used as the first differentiation between samples [20]. These methods include colony morphological analysis, Gram staining, microscopic examination, and various biochemical and physiological tests, whether manual, automated, or semi-automated [21]. Despite being widely used, these conventional techniques are often limited by their low capacity to differentiate species and cost, in addition to being time-consuming and laborious [2].

With the appearance of Molecular Biology, there developed several techniques, contributing significantly to a great advance in the knowledge of microbial diversity. Among these techniques, those based on the amplification of DNA sequences by polymerase chain reaction [PCR] stand out [22]. PCR is a technique that allows the *in vitro* amplification of small amounts of DNA or RNA sequences, obtaining millions of copies of these specific sequences [23].

Due to its ability to detect infectious agents with high sensitivity and specificity [24], without the need to find viable microorganisms in the biological sample, PCR has become a valuable and very reliable diagnostic tool for the diagnosis and monitoring of diseases. Technological advances have allowed variations of this technique to emerge, such as real-time PCR [RT-PCR]. RT-PCR has some advantages over the conventional one, such as high sensitivity and accuracy, and the ability to monitor DNA amplification in real-time, without the need for other post-PCR techniques [25].

Another molecular technique widely used is the comparative analysis of nucleotide sequences of the 16S rRNA gene, which has been considered as the “gold standard” for bacterial identification, and taxonomic and phylogenetic studies [26]. These genes related to ribosomal RNAs are used for phylogenetic determinations, as they have all the characteristics that define a molecular marker, such as a] they are universally distributed and have the same function in all organisms; b] they originated from common ancestral genes, so they are homologous; c] have very conserved regions and other more variable ones, where the rate of nucleotide variation can be statistically correlated with the distance between genera and species [27]. The advantage of using this technique is the availability of a large number of sequences of this gene deposited in databases such as Gen-Bank, RDP, EMBL, enabling the comparison of new sequences obtained with the sequences present in these bases, facilitating correct microbial identification [28].

Still, on the analysis of nucleic acid sequences, it is worth emphasizing its importance for the differential diagnosis, for the study of the genetic relationships between bacterial isolates, and also for the detection of genetic mutations related to antimicrobial resistance. The extensive use of this method in clinical microbiology has also stimulated its improvement, such as the development of new generation sequencing [NGS] [12]. The NGS allows the sequencing of the total genome of several pathogens at once, either from bacterial isolates from different patients or several species present in an individual's materials [metagenomics] [12].

Recently, new technologies based on protein profiles of microorganisms have been developed, such as Matrix-assisted Laser Desorption/ionization-time-of-flight Mass Spectrometry [MALDI-TOF MS], which has been used as a simple, reliable, and fast technique [15, 16]. This technique bases on the analysis of a fingerprint of ribosomal proteins and other basic proteins that are specific to each microbial species [29].

The use of MALDI-TOF is considered a revolutionary technique in the identification of microorganisms. Among its several advantages,

the use of intact cells of microorganisms that can be obtained directly from the culture medium, cost-benefit [$US 2 /sample], short processing and analysis time, and high yield [biological material consumption value is insignificant] stand out [30]. Its effective application requires the use of commercial databases, in addition to specific software for comparing protein spectra. There developed several commercial platforms for these analyzes. However, they are aimed at microorganisms of clinical interest [31].

Several studies have demonstrated the efficiency and high reproducibility of this method. However, most are directed to microorganisms of clinical interest, such as *Escherichia coli* and other members of the *Enterobacteriacea* family [32], *Staphylococcus aureus* [33] e *Streptococcus pneumoniae* [34]. Literature data report that the percentages of bacterial genera correctly identified by this technique can be 97-99% and at species level 85-97%. It was also verified its application directly in blood samples for the rapid identification of pathogens, leading to a reduction in response time, and consequently, bringing benefits to patients [35, 36].

CONCLUSION

The present study provides an overview of the main techniques used to identify microorganisms of clinical origin. From the data obtained by this research, it can be concluded that the development of new technologies that allow rapid, sensitive, reproducible, and low-cost microbial identification is of great relevance for clinical microbiology, and consequently, for public health. Conventional techniques, despite their limitations, are still widely used in clinical laboratories. However, news technologies are being developed and increasingly inserted into the laboratory routine for the diagnosis of infectious diseases.

REFERENCES

[1]. Ramamurthy T, Ghosh A, Pazhani GP, Shinoda S. Current Perspectives on Viable but Non-Culturable [VBNC] Pathogenic Bacteria. Front. Public Heal. 2014, 2, 103.

[2]. Bochner BR. Global phenotypic characterization of bacteria. FEMS Microbiol. Rev. 2009, 33, 191–205.

[3]. Lagier JC, Hugon P, Khelaifia S, Fournies PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Ver. 2015, 28, 237–264.

[4]. Cliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC et al [2013] Better tests, better care: improved diagnostics for infectious diseases. Clin Infect Dis. 2013, 57[Suppl 3], S139– S170.

[5]. Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. Clin. Microbiol. Rev. 2014, 27, 783–822.

[6]. Palleroni NJ. Pseudomonas classification – a new case history in the taxonomy of Gram-negative bacteria. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology. 1993, 64, 231-251.

[7]. Callaway A, Kostrzewa M, Willershausen B, Schmidt F, Thiede B, Kupper H, Kneist S. Identification of Lactobacilli from deep carious lesions by means of species-specific PCR and MALDI-TOF mass spectrometry. Clin. Lab. 2013, 59, 1373–1379.

[8]. Duskova M, Sedo O, Ksicova K, Zdrahal Z, Karpıskova R. Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. Int. J. Food Microbiol. 2012, 159, 107–114.

[9]. Pendharkar S, Magopane T, Larsson PG, Bruyn G, Gray GE, et al. Identification and characterisation of vaginal lactobacilli from South African women. BMC Infect. Dis. 2013, 13, 43.

[10]. Deggim-Messmer V, Bloemberg GV, Ritter C, Voit A, Homke R, Keller PM, et al. Diagnostic Molecular Mycobacteriology in Regions With Low Tuberculosis Endemicity: Combining Real-time PCR Assays for Detection of Multiple Mycobacterial Pathogens With Line Probe Assays for Identification of Resistance Mutations. EBioMedicine. 2016, 9, 228–237.

[11]. Spanoghe M, Jara MG, Riviere J, Lanterbecq D, Gadenne M, Marique T. Development and application of a quantitative real-time PCR assay for rapid detection of the multifaceted yeast Kazachstania servazzii in food. Food Microbiol. 2017, 62, 133–140.

[12]. Dark MJ. Whole-genome sequencing in bacteriology: state of the art. Infect. Drug Resist. 2013, 6, 115–123.

[13]. Quainoo S, Coolen JPM, Van Hijum SAFT, Huynen MA, Melchers WJG, Van Schaik W, et al. Whole-Genome Sequencing of Bacterial Pathogens: The Future of Nosocomial Outbreak Analysis. Clin. Microbiol. Rev. 2017, 30, 1015–1063.

[14]. De Bruyne K, Slabbinck B, Waegeman W, Vauterin P, De Baets B, Vandamme P. Bacterial species identification from MALDI-TOF mass spectra through data analysis and machine learning. Syst Appl Microbiol. 2011, 34[1], 20-9.

[15]. Martin MS, Santos IC, Carlton DD Jr, Granados P, Hildenbrand ZL, Schug KA. Characterization of bacterial diversity in contaminated groundwater using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Sci Total Environ. 2018, 1, 622-623,1562-1571.

[16]. Starostin KV, Demidov EA, Bryanskaya AV, Efimov VM, Rozanov AS, Peltek SE. Identification of Bacillus strains by MALDI TOF MS using geometric approach. Sci Rep. 2015, 23[5], 16989.

[17]. Lo CI, Fall B, Sambe-Ba B, Diawara S, Gueye MW, Mediannikov O, Sokhna C, et al. MALDI-TOF Mass Spectrometry: A Powerful Tool for Clinical Microbiology at Hôpital Principal de Dakar, Senegal [West Africa]. PLoS One. 2015, 30, 10[12].

[18]. Sharma M, Gautam V, Mahajan M, Rana S, Majumdar M, Ray, P. Direct identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS] from positive blood culture bottles: An opportunity to customize growth conditions for fastidious organisms causing bloodstream infections. Indian J Med Res. 2017, 146[4], 541-544.

[19]. Bergeron MG, Ouellette M. Preventing antibiotic resistance through rapid genotypic identification of bacteria and of their antibiotic resistance genes in the clinical microbiology laboratory. J Clin Microbiol. 1998, 36, 2169–2172.

[20]. Tang YW, Ellis NM, Hopkins MK et al. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. J Clin Microbio. 1998, 36, 3674–3679.

[21]. Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult, D. The Rebirth of Culture in Microbiology through the Example of Culturomics to Study Human Gut Microbiota. Clin. Microbiol. Rev. 2015, 28, 237–264.

[22]. de Boer E, Beumer RR. Methodology for detection and typing of foodborne microorganisms. International Journal of Food Microbiology. 1999, 50, 119-130.

[23]. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Junir, WC. Diagnóstico microbiológico: texto e atlas colorido. 5.ed. Rio deJaneiro: MEDSI, 2001.

[24]. Hofmann MA, Renzullo S, Mader M, Chaignat V, Worwa G, Thuer B. Genetic characterization of Toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. Emerg Infect Dis [serial on the Internet]. 2008

[25]. Sibley CD, Peirano G, Church DL. Molecular methods for pathogen and microbial community detection and characterization: current and potential application in diagnostic microbiology. Infection, genetics and evolution. 2012, 12 [3], 505-21.

[26]. Srinivasan R, Kraoz U, Volegova M, Mackichan J, Kato-maeda M, Miller S, et al. Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. PLoS ONE. 2015, 10[2].

[27]. Clarridge III JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical Microbiology Reviews. 2004, 17 [4], 840-862.

[28]. Coutinho HLC, Oliver VM, Manfio GP, Rosado AS. Evaluating the microbial diversity of soil samples: metodologial innovation. Anais da Academia Brasileira de Ciências. 1999, 71, 3, 491-503, 1999.

[29]. Giebel R, Woden C, Rust SM, Kleinheiz GT, Robbins M. Microbial fingerprinting using matrx-assisted laser desorption ionization timeof flight mas spectrometry [MALDI-TOF MS] Aplications and Challenges. Adv Appl Microbiol. 2010, 71, 149-184.

[30]. Avanzi IR, Gracioso LH, Baltazar MD, Karolski B, Perpetuo EA, do Nascimento CA. Rapid bacteria identification from environmental mining samples using MALDI-TOF MS analysis. Environ Sci Pollut Res Int. 2017, 24[4], 3717-3726.

[31]. Carbonnelle E, Grohs P, Jacquier H, Day N, Tenza S, Dewailly A, et al.. Robustness of two MALDI-TOF mass spectrometry systems for bacterial identification. J Microbiol Methods. 2012, 89[2].

[32]. Yu J, Liu J, Li Y, Yu J, Zhu W, Liu Y, Shen L. Rapid detection of carbapenemase activity of Enterobacteriaceae isolated from positive blood cultures by MALDI-TOF MS. Ann Clin Microbiol Antimicrob. 2018, 18,17[1]:22.

[33]. Zhang T, Ding J, Rao X, Yu J, Chu M, Ren W, et al. Analysis of methicillin-resistant

Staphylococcus aureus major clonal lineages by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry [MALDI-TOF MS]. J Microbiol Methods. 2015, 117,122-7.

[34]. Nakano S, Matsumura Y, Ito Y, Fujisawa T, Chang B, Suga S, et al. Development and evaluation of MALDI-TOF MS-based serotyping for Streptococcus pneumoniae. Eur J Clin Microbiol Infect Dis. 2015, 34[11], 2191-8.

[35]. Foster AG. Rapid Identification of microbes in positive blood cultures by use of the vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system. J. Clin. Microbiol. 2013, 51, 3717–3719.

[36]. Tadros M, Petrich A. Evaluation of MALDI-TOF mass spectrometry and Sepsityper KitTM for the direct identification of organisms from sterile body fluids in a Canadian pediatric hospital. Can. J. Infect. Dis. Med. Microbiol. 2013, 24, 191–194.