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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Molecular Identification Techniques of Bacteria: A Review

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A B S T R A C T

Molecular identification methods have transformed bacterial identification by providing quick and precise detection of specific species. PCR-based identification methods such as HRMA and qPCR have many clinical and environmental microbiology applications, but limitations like the requirement for specific primers and false negatives due to inhibitors in the sample. DNA sequencing techniques such as Sanger sequencing, NGS, and third-generation sequencing has many applications, for instance identifying bacterial species and monitoring the spread of genes encoding antibiotic resistance. However, limitations include the need for specialized equipment, reagents, and potential errors in data interpretation. MALDI-TOF MS offers rapid and accurate identification of microorganisms in clinical, food, and environmental samples. The FISH offers specific and sensitive detection of low abundance microorganisms or genes, while the WGS provides comprehensive genomic information, detecting genes encoding antibiotic resistance, virulence factors and genetic diversity, despite being more expensive and requiring specialized bioinformatic analysis.

Keywords: Bacteria, molecular identification, PCR, DNA sequencing, MALDI-TOF MS, FISH, WGS.

1. Introduction

Bacterial identification is a crucial aspect of microbiology and clinical diagnosis. Correct treatment of bacterial illnesses and a reduction in the transmission of infectious diseases can result from accurate bacterial identification. Traditional identification methods of bacteria such as culture-based techniques have limitations in accuracy and speed, which can result in delayed or ineffective treatments. In contrast, the molecular techniques provide faster and accurate results in bacterial identification. Molecular techniques involve the use of genetic material to identify bacteria. PCR or polymerase chain reaction is one of these techniques. By using DNA sequencing or hybridization methods to identify bacteria, PCR amplifies specific regions of their DNA (Järvinen *et al*., 2009). Another method, DNA sequencing can directly identify bacterial species by comparing the DNA sequence to a reference database (Janda and Abbott, 2007; Lasken and McLean, 2014). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is another technique that can identify bacteria rapidly by analyzing the mass and charge of bacterial proteins (Hrabák *et al*., 2013). FISH or Fluorescence in situ hybridization uses DNA probes that fluorescently labeled to hybridize with specific regions of bacterial DNA for identification (Wagner and Loy, 2002). Also, Whole Genome Sequencing (WGS), involves the sequencing of the entire bacterial genome which enables a deeper investigation of bacterial species and genetic features (Maguvu and Bezuidenhout, 2021). Here, we give an overview of these various methods including their applications, advantages and limitations.

2. Polymerase Chain Reaction (PCR)

In order to amplify DNA segments the Polymerase Chain Reaction (PCR), a potent molecular biology method, is used. PCR's three sequential stages: denaturation, annealing and extension are crucial to its efficacy. Denaturation requires heating the DNA template to a specific temperature, commonly around 95°C, and thus separating the two strands of DNA. Annealing step includes cooling the template to a lower temperature, generally around 55-60°C, and facilitates the primers' hybridization to their corresponding sequences on the DNA template. Ultimately, extension entails an increase in temperature to around 72°C, allowing the Taq polymerase enzyme to fabricate new DNA strands by elongating the primers. This sequence of operations produces an amplification of a specific DNA region that has multiple applications, such as the identification of bacteria (Lorenz, 2012).

A. Applications in bacterial identification

The PCR technique, a revolutionary tool in bacterial identification, has expedited and improved the identification of specific bacterial species. It achieves this feat by amplifying exceptionally conserved regions of bacterial DNA, such as the 16S ribosomal RNA gene, which provides a firm foundation for bacterial species identification. As the 16S ribosomal RNA gene is present in all bacteria and demonstrates high conservation, it makes an ideal target for PCR-based bacterial identification (Patel *et al*., 2017).

Identification of bacteria by PCR can be done by using multiple techniques such as Sanger sequencing, HRMA or high-resolution melting analysis and quantitative PCR (qPCR). Sanger sequencing entails sequencing the amplified DNA region to determine the bacterial species, according to Furutani *et*

al. (2022). HRMA, as per Dehbashi *et al*. (2020), is an analytical technique that scrutinizes the melting curve of the amplified DNA region to differentiate between various bacterial species. qPCR, as elaborated by Kralik and Ricchi (2017), entails measuring the amount of DNA amplified during PCR using fluorescent probes, providing an opportunity to determine bacterial load and identify bacterial species.

PCR-based bacterial identification is pertinent to clinical and environmental microbiology. In clinical microbiology, PCR can be employed to detect bacterial pathogens in clinical samples such as blood, urine, and respiratory secretions, permitting the swift diagnosis of bacterial infections (Calderaro *et al.*, 2022). In environmental microbiology, PCR can detect bacterial contaminants in food and water, allowing for a rapid and sensitive detection of potential health risks (Aw and Rose, 2012).

B. Advantages and limitations

The employment of PCR-based bacterial identification technique provides numerous advantages over conventional culture-based methods. These advantages include rapid turnaround time, heightened sensitivity and specificity, and the capability to detect viable but non-culturable bacteria (Schoonbroodt *et al*., 2023). PCR technique's ability to detect bacterial DNA in even minuscule quantities makes it a potent instrument for detecting bacterial infections during the early stages. The PCR-based bacterial identification process also demonstrates a remarkable degree of specificity, allowing for precise and accurate identification of bacterial species (Toze, 1999).

Apart from its manifold benefits, PCR-based bacterial identification technique is not without limitations. For example, the PCR process can be adversely affected by inhibitors present in the sample, resulting in misleading negative results. Moreover, PCR requires the utilization of specific primers that target the desired DNA region, which limits the range of bacterial species that can be detected (Yang and Rothman, 2004). Finally, PCR-based bacterial identification is a relatively expensive technique, requiring specialized equipment and reagents.

3. DNA Sequencing

DNA sequencing is an intricate molecular technique that determines the precise arrangement of nucleotides in a DNA molecule. Frederick Sanger created the first DNA sequencing method in the 1970s. Since then, the field of DNA sequencing has experienced noteworthy advancements. Sanger sequencing, NGS or next-generation sequencing, and third-generation sequencing are techniques used for DNA sequencing (Dewey *et al.*, 2012). Sanger sequencing also called chain termination sequencing, employs a primer, DNA polymerase, and dideoxynucleotides to sequence DNA. By terminating the elongation of the DNA strand with dideoxynucleotides, fragments of distinct lengths are formed which can be separated by size and analyzed to determine the DNA sequence (Sanger *et al*., 1977).

NGS is a more recent sequencing technique that allows for the parallel sequencing of millions of DNA fragments, creating a massive amount of data. NGS techniques include Illumina sequencing, Ion Torrent sequencing and PacBio sequencing, which differ in their sequencing chemistry and the length of DNA fragments that can be sequenced (Goodwin *et al*., 2016). Third-generation sequencing is another newfangled technology, that enables long-read sequencing of DNA molecules. This method involves techniques, such as Oxford Nanopore sequencing and PacBio sequencing, which employ nanopores or single-molecule real-time sequencing to read the DNA sequence directly (Athanasopoulou *et al*., 2022).

A. Applications in bacterial identification

The enthralling realm of DNA sequencing finds various applications in the sphere of bacterial identification. A pivotal role of DNA sequencing is in the realm of identifying bacterial species. This is executed through the comparison of the sequence of a specific gene or cluster of genes to a colossal database of already known bacterial sequences, giving rise to sequence-based identification. In the clinical and environmental domains, this methodology is extensively used to identify bacteria (Reller *et al*., 2007).

Aside from identification of bacterial species, the DNA sequencing is critical for understanding the genetic diversity and evolution of bacterial communities. Such vital information can help researchers better understand the epidemiology of bacterial infections and consequently, monitor the spread of antibiotic resistance genes (Didelot *et al*., 2012). Further, DNA sequencing is a potent tool in detecting and identifying bacterial pathogens in clinical samples. This is highly imperative for diagnosing infectious diseases since it allows for swift and accurate identification of the causative agent (Janda and Abbott, 2007). In certain situations, DNA sequencing has even been employed to identify the strain of bacteria accountable for an outbreak or epidemic (Gilchrist *et al*., 2015), adding another dimension to the already intriguing field of DNA sequencing.

B. Advantages and limitations

The ability of the DNA sequencing to identify bacterial species with high degree of precision and accuracy at the species level is remarkable advantage. This has a paramount significance in the domain of the clinical and the public health settings in that accuracy of the bacterial species identification can furnish essential information regarding the treatment decisions and infection control measures. Furthermore, the DNA sequencing has an added advantage of identifying genetic markers linked with the virulence or resistance to antibiotics which can pave the way for the developing of new therapies and monitoring the diffusion of the genes coding antibiotic resistance (Didelot *et al*., 2012).

Despite the enormous benefits of the DNA sequencing, it also has limitations. As an example of these limitations is cost and intricacy of the technology. The cost of DNA sequencing has decreased significantly over the last few decades but it remains somewhat expensive when compared to the other molecular methods like as Polymerase Chain Reaction (Goodwin *et al*., 2016). Another significant limitation is the time required for analysis. While novel sequencing technologies such as Next Generation Sequencing and Third Generation Sequencing have made sequencing faster, the procedures of

sample preparation and data analysis can still be time consuming (Goodwin *et al*., 2016). Furthermore the interpretation of sequencing data demands specialized training and expertise, which may not be universally available (Didelot *et al*., 2012). Also, there is a concern of contamination as DNA sequencing highly sensitive to even the slightest contamination. Even minute contamination can lead to errors in sequencing data, which can hamper the accuracy of the results. This represents particular importance in the clinical and environmental samples in which multiple bacterial species may coexist (Salter *et al*., 2014).

4. Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS has revolutionized the field of microbial identification with its powerful analytical method. This technique employs the ionization of microbial cells by a matrix solution, which is then subjected to mass spectrometric analysis, resulting in the generation of a mass spectrum. The distinctive mass-to-charge (m/z) ratios obtained from the mass spectrum of the microbial cells are subsequently cross-referenced against a reference database of known microbial species to determine the presence of the microorganism in the sample (Singhal *et al*., 2015).

A. Applications in bacterial identification

MALDI-TOF MS in the identification of bacterial pathogens, have been at the forefront of contemporary research. This method has come to be critical tool for rapid and precise identification of clinical pathogenic bacteria in different specimens such as blood cultures, urine and the respiratory samples (Huang *et al*., 2013). Also, MALDI-TOF MS is increasingly becoming a prevalent method for identifying bacterial pathogens in food samples like genera: Campylobacter, Cronobacter, Listeria, Salmonella and Vibrio (Pavlovic *et al*., 2013).

Furthermore, the contribution of MALDI-TOF MS to the field of environmental microbiology cannot be overstated. Bacterial species in environmental samples such as water and soil, can be identified by this technique. This can be critical for environmental monitoring and assessment, highlighting its critical role in the sphere of environmental research (Ashfaq *et al*., 2022).

B. **Advantages and limitations**

MALDI-TOF MS technique is a highly advantageous tool for the identification of microorganisms in clinical settings. The technique is significantly faster than traditional culture-based methods and providing accurate results in minutes. This rapid turnaround time is crucial in clinical settings, in that prompt identification and treatment are crucial to patient outcomes (Rychert, 2019). In addition, another significant advantage of the MALDI-TOF MS technique is its high degree of accuracy with studies showing high sensitivity and specificity in the identification of microbial species. This level of precision is critical in therapeutic settings since inaccurate identification can result in improper therapy and poor patient outcomes (Haider *et al*., 2023). Moreover, MALDI-TOF MS technique is an easy method that is with simple equipment and reagents that do not require specialized training, making it accessible to a wide range of users and laboratories. The technique is also versatile and capable of identifying a broad range of microorganisms including bacteria, fungi and viruses (Rychert, 2019).

However, there are some restrictions to MALDI-TOF MS technique when it comes to the identification of bacterial pathogens. The demand for up-to-date database for correct identification is one of the most critical restrictions. If the bacterial species that present in the sample do not included in the database, it can lead to inaccurate or no identification of the bacteria. This misidentification will affect the accuracy of the results. Thus, having an up-to-date database is essential to for correct detection of bacteria and to fully understand the potential limitations of this technique (Singhal *et al*., 2015).

5. Fluorescence In Situ Hybridization (FISH)

FISH is a molecular biology technique that uses fluorescently labeled nucleic acid probes to target specific sequences of DNA or RNA in situ, meaning in their original location within a sample. The probes hybridize to their complementary sequences in the target DNA or RNA, in that allowing the detection of specific microorganisms or genes of interest. FISH has been widely used in bacterial identification as it allows for the direct visualization of microbial cells and their location within the sample (Huber *et al*., 2018).

A. Applications in bacterial identification

The FISH has numerous applications in bacterial identification, including the identification of specific bacterial species or groups, the detection of bacteria that resist antibiotics and the analysis of microbial community structures (Gu *et al*., 2022). One of the common applications of FISH in bacterial identification is the detection of pathogens in clinical samples such as samples obtained from blood and urine source. Bacterial species like S. pneumoniae and H. influenzae can be identified by FISH (Peters *et al*., 2006; Wu *et al*., 2010). FISH has also been used in identification of the bacteria that are resistant to antibiotics in clinical and environmental samples. FISH, for instance, can be used to detect E. coli that has ability to resist ampicillin (Lee *et al*., 2019). In case of the environmental samples, FISH can be used to detect Bacteria that degrade simazine in soil (Martin *et al*., 2008).

B. Advantages and limitations

One of the main advantages of FISH is its specificity and sensitivity. FISH probes can be designed to target specific bacterial species or genes of interest, this leads to detection of low abundance microorganisms or genes. FISH is also a rapid technique, in which results typically available within hours (Gu *et al*., 2022).

However, FISH has some limitations. For instance, FISH may not be able to identify minor genetic abnormalities, balanced rearrangements, or the particular genes involved in chromosomal abnormalities. Additionally, the availability of probes used in FISH may be restricted (Gozzetti and Le Beau, 2000).

6. Whole Genome Sequencing (WGS)

The WGS is a technique that been used to determines the complete DNA sequence of an organism. The technique produces large amounts of data in a relatively short time by high-throughput sequencing. WGS, recently, is widely used in microbiology in order to identify and characterize bacteria. The process of WGS consists of DNA extraction, library preparation, sequencing and bioinformatics analysis (Kwong *et al*., 2015).

A. Applications in bacterial identification

WGS has revolutionized the field of bacterial identification and characterization. One of important applications of this method is that It facilitates the detection of genes encode foe antimicrobial resistance, virulence factors, and other traits that are essential for understanding the pathogenicity of bacteria (Surleac *et al*., 2020). WGS has the potential to facilitate tracking and monitoring of the dissemination of infectious diseases, including instances of foodborne illness or hospital-acquired infections, through genome-wide comparison of diverse bacterial isolates (Ashton *et al*., 2016). Furthermore, WGS can be used for surveillance of bacterial pathogens, allowing for rapid detection and response to new or emerging strains (Grad and Lipsitch, 2014).

B. Advantages and limitations

The utilization of WGS provides numerous benefits compared to traditional techniques that used for the identification of bacteria. One of the main advantages is its capability to achieve higher resolution and sensitivity. Additionally, WGS is capable of detecting multiple pathogens simultaneously (Kwong *et al*., 2015). WGS can also provide information on the evolutionary history of bacterial populations. This leads to identification of potential sources of infection and the tracking of transmission pathways (Grad and Lipsitch, 2014). However, some restrictions to the technique are present. WGS generates vast amounts of data which can be challenging to analyze and interpret, and the cost of the equipment and analysis can be high (Surleac *et al*., 2020). Moreover, it should be emphasized that the utilization of this method necessitates DNA samples of exceptional quality, a task that may pose difficulties when working with certain clinical specimens (Ashton *et al*., 2016).

7. Conclusion

In conclusion, molecular identification techniques have revolutionized the field of bacterial identification that lead to rapid and accurate detection of specific bacterial species. PCR is a widely used technique in identification of bacterial species through the ability to amplify specific regions of DNA like 16S rRNA gene. This method inclusive of several variants like Sanger sequencing, HRMA and qPCR, provides an array of utilities in clinical and environmental microbiology. It is capable of offering quick turnaround times, heightened specificity and sensitivity, and the ability to detect viable but non-culturable bacteria. However, PCR method is limited by the necessity for specific primers which may not be available for some organisms. False negative outcomes are also possible due to inhibitors present in the sample. DNA sequencing, including Sanger sequencing, NGS, and third-generation sequencing, is another molecular identification technique that has applications in bacterial identification, genetic diversity and evolution. Sequence-based identification using DNA sequencing is commonly used to identify bacterial species and track the spread of antibiotic resistance genes. The process of DNA sequencing, while presenting notable advantages over traditional identification methods, also exhibits certain limitations. Notably, it demands specialized equipment and reagents, as well as stringent protocols for accurate analysis and interpretation of the generated data.

MALDI-TOF MS offers rapid, accurate and easy identification of microorganisms from different sources like clinical, food, and environmental samples. Although it requires an updated database for accurate identification, it remains a versatile and easy technique with a high degree of sensitivity and specificity. FISH, on the other hand, offers specific and sensitive detection of low abundance microorganisms or genes, which enables quick detection of antibiotic-resistant microorganism or the analysis of microbial community structures. However, it may not identify minor genetic abnormalities or chromosomal abnormalities and probe availability may be restricted. WGS is a powerful and comprehensive tool that can provide information on the entire genome of the microorganism, therefore, allowing the detection of genes coding antibiotic resistance, virulence factors and genetic diversity. Despite the fact that it is more expensive and requires specialized bioinformatics analysis, WGS is becoming increasingly accessible and is revolutionizing the field of bacterial identification and outbreak investigation.

References

Ashfaq, M. Y., Da'na, D. A. and Al-Ghouti, M. A. (2022). Application of MALDI-TOF MS for identification of environmental bacteria: A review. *Journal of Environmental Management*, 305, 114359.

Ashton, P. M., Nair, S., Peters, T. M., Bale, J. A., Powell, D. G., Painset, A., Tewolde, R., Schaefer, U., Jenkins, C., Dallman, T. J., de Pinna, E. M. and Grant, K. A. (2016). Identification of Salmonella for public health surveillance using whole genome sequencing. *PeerJ*, 4, e1752.

Athanasopoulou, K., Boti, M. A., Adamopoulos, P. G., Skourou, P. C. and Scorilas, A. (2022). Third-Generation Sequencing: The Spearhead towards the Radical Transformation of Modern Genomics. *Life*, 12(1), 30.

Aw, T. G. and Rose, J. B. (2012). Detection of pathogens in water: from phylochips to qPCR to pyrosequencing. *Current Opinion in Biotechnology*, 23(3), 422–430.

Calderaro, A., Buttrini, M., Farina, B., Montecchini, S., De Conto, F. and Chezzi, C. (2022). Respiratory Tract Infections and Laboratory Diagnostic Methods: A Review with A Focus on Syndromic Panel-Based Assays. *Microorganisms*, 10(9), 1856.

Dehbashi, S., Tahmasebi, H., Sedighi, P., Davarian, F. and Arabestani, M. R. (2020). Development of high-resolution melting curve analysis in rapid detection of vanA gene, *Enterococcus faecalis*, and *Enterococcus faecium* from clinical isolates. *Tropical Medicine and Health*, 48(1), 8.

Dewey, F. E., Pan, S., Wheeler, M. T., Quake, S. R. and Ashley, E. A. (2012). DNA Sequencing: Clinical applications of new DNA sequencing technologies. *Circulation*, 125(7), 931-944.

Didelot, X., Bowden, R., Wilson, D. J., Peto, T. E. A. and Crook, D. W. (2012). Transforming clinical microbiology with bacterial genome sequencing. *Nature Reviews Genetics*, 13(9), 601-612.

Furutani, S., Furutani, N., Kawai, Y., Nakayama, A. and Nagai, H. (2022). Rapid DNA Sequencing Technology Based on the Sanger Method for Bacterial Identification. *Sensors*, 22(6), 2130.

Gilchrist, C.A., Turner, S.D., Riley, M.F., Petri Jr., W.A. and Hewlett, E.L. (2015). Whole-Genome Sequencing in Outbreak Analysis. *Clinical Microbiology Reviews*, 28(3), 541-563.

Goodwin, S., McPherson, J. D. and McCombie, W R. W. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17(6), 333-35.

Gozzetti, A. and Le Beau, M. M. (2000). Fluorescence in situ hybridization: Uses and limitations. *Seminars in Hematology*, 37(4), 320-333.

Grad, Y. H. and Lipsitch, M. (2014). Epidemiologic data and pathogen genome sequences: a powerful synergy for public health. *Genome Biology*, 15, 538.

Gu, J., Wang, H., Zhang, M., Xiong, Y., Yang, L., Ren, B. and Huang, R. (2022). Application of Fluorescence In Situ Hybridization (FISH) in Oral Microbial Detection. *Pathogens*, 11(12), 1450.

Haider, A., Ringer, M., Kotroczó, Z., Mohácsi-Farkas, C. and Kocsis, T. (2023). The Current Level of MALDI-TOF MS Applications in the Detection of Microorganisms: A Short Review of Benefits and Limitations. *Microbiology Research*, 14(1), 80-90.Hrabák, J., Chudáčková, E. and Walková, R. (2013). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clinical microbiology reviews*, 26(1), 103-114.

Huang, A. M., Newton, D., Kunapuli, A., Gandhi, T. N., Washer, L. L., Isip, J., Collins, C. D. and Nagel, J. L. (2013). Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. *Clinical Infectious Diseases*, 57(9), 1237-1245.

Huber, D., Voith von Voithenberg, L. and Kaigala, G. V. (2018). Fluorescence in situ hybridization (FISH): History, limitations and what to expect from micro-scale FISH? *Micro and Nano Engineering*, 1, 15-24.

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, 45(9), 2761-2764.

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, 45(9), 2761-2764.

Järvinen, A. K., Laakso, S., Piiparinen, P., Aittakorpi, A., Lindfors, M., Huopaniemi, L., Piiparinen, H. and Mäki, M. (2009). Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. *BMC microbiology*, 9(1), 161.

Kralik, P. and Ricchi, M. (2017). A Basic Guide to Real Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. *Front. Microbiol*. 8:108.

Kwong, J. C., McCallum, N., Sintchenko, V. and Howden, B. P. (2015). Whole genome sequencing in clinical and public health microbiology. *Pathology*, 47(3), 199-210.

Lasken, R. S. and McLean, J. S. (2014). Recent advances in genomic DNA sequencing of microbial species from single cells. *Nature Reviews Genetics*, 15(9), 577-584.

Lee, W. S., Lee, S., Kang, T., Ryu, C. M. and Jeong, J. (2019). Detection of ampicillin-resistant *E. coli* using novel nanoprobe-combined fluorescence in situ hybridization. *Nanomaterials*, 9(5), 750.

Lorenz, T. C. (2012). Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. *Journal of Visualized Experiments*, (63), 3998.

Maguvu, T. E. and Bezuidenhout, C. C. (2021). Whole genome sequencing based taxonomic classification, and comparative genomic analysis of potentially human pathogenic Enterobacter spp. isolated from chlorinated wastewater in the North West Province, South Africa. *Microorganisms*, 9(9), 1928.

Martin, M., Gibello, A., Lobo, C., Nande, M., Garbi, C., Fajardo, C., Barra-Caracciolo, A., Grenni, P. and Martinez-Inigo, M. J. (2008). Application of fluorescence in situ hybridization technique to detect simazine-degrading bacteria in soil samples. *Chemosphere*, 71(4), 703-710.

Patel, A., Harris, K. A. and Fitzgerald, F. (2017). What is broad-range 16S rDNA PCR?. *Archives of Disease in Childhood-Education and Practice*, 102, 261-264.

Pavlovic, M., Huber, I., Konrad, R. and Busch, U. (2013). Application of MALDI-TOF MS for the Identification of Food Borne Bacteria. *The Open Microbiology Journal*, 7, 135-141.

Peters, R. P. H., van Agtmael, M. A., Simoons-Smit, A. M., Danner, S. A., Vandenbroucke-Grauls, C. M. J. E. and Savelkoul, P. H. M. (2006). Rapid identification of pathogens in blood cultures with a modified fluorescence in situ hybridization assay. *Journal of Clinical Microbiology*, 44(11), 4186-4188.

Reller, L. B., Weinstein, M. P. and Petti, C. A. (2007). Detection and Identification of Microorganisms by Gene Amplification and Sequencing. *Clinical Infectious Diseases*, 44(8), 1108-1114.

Rychert J. (2019). Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms. *J Infectiology*, 2(4): 1-5.

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner, P., Parkhill, J., Loman, N. J. and Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, 12(1), 87.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, 74(12), 5463-5467.

Schoonbroodt, S., Ichanté, J-L., Boffé, S., Devos, N., Devaster, J-M., Taddei, L., Rondini, S., Arora, A. K., Pascal, T. and Malvaux, L. (2023). Real-time PCR has advantages over culture-based methods in identifying major airway bacterial pathogens in chronic obstructive pulmonary disease: Results from three clinical studies in Europe and North America. *Front. Microbiol*. 13:1098133.

Singhal, N., Kumar, M., Kanaujia, P. K. and Virdi, J. S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, 6, 791.

Surleac, M., Czobor Barbu, I., Paraschiv, S., Popa, L. I., Gheorghe, I., Marutescu, L., Popa, M., Sarbu, I., Talapan, D., Nita, M., Iancu, A. V., Arbune, M., Manole, A., Nicolescu, S., Sandulescu, O., Streinu-Cercel, A., Otelea, D. and Chifiriuc, M. C. (2020). Whole genome sequencing snapshot of multi-drug resistant *Klebsiella pneumoniae* strains from hospitals and receiving wastewater treatment plants in Southern Romania. *PLoS One*, 15(1), e0228079.

Toze, S. (1999). PCR and the detection of microbial pathogens in water and wastewater. *Water Research*, 33(17), 3545-3556.

Wagner, M. and Loy, A. (2002). Bacterial community composition and function in sewage treatment systems. *Current opinion in biotechnology*, 13(3), 218-227.

Wu, Q., Li, Y., Wang, M., Pan, X. P. and Tang, Y. F. (2010). Fluorescence in situ hybridization rapidly detects three different pathogenic bacteria in urinary tract infection samples. *Journal of Microbiological Methods*, 83(2), 175-178.

Yang, S. and Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *The Lancet Infectious Diseases*, 4(6), 337-348.