International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 11, Issue 2, pp: (95-100), Month: October 2023 - March 2024, Available at: www.researchpublish.com

Bacterial Culture in Clinical Microbiology

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DOI: https://doi.org/10.5281/zenodo.10435398

Published Date: 27-December-2023

Abstract: **Bacterial culture is a fundamental technique in clinical microbiology that involves the growth and isolation of bacteria from patient samples. This technique has evolved significantly over the years, with improvements in both the methods of isolation and identification of bacteria. In this essay, we will discuss the current and past strategies for bacterial culture in clinical microbiology. In the past, the primary method for bacterial culture involved plating patient samples onto solid growth media, such as blood agar or MacConkey agar, and incubating the plates at specific temperatures. This technique allowed for the identification and isolation of bacteria based on their colony morphology and biochemical properties. However, this method had its limitations, as it was time-consuming and required a significant amount of expertise to interpret the results accurately.**

Keywords: **Bacteria culture, Microbiology lab, new analysis.**

1. INTRODUCTION

Bacterial culture is an essential diagnostic technique in clinical microbiology, allowing precise identification of pathogenic bacteria and determination of antibiotic susceptibility. Over the years, various strategies have been employed to enhance the efficiency and accuracy of bacterial culture.[1] This essay aims to explore both current and past strategies used in clinical microbiology at the master's level. By analyzing these strategies, we can gain insights into the evolution of bacterial culture methods and their impact on clinical diagnostics.[2]

Bacterial culture was the first method developed to study the human microbiota [20], employing an artificial medium that allows bacteria to grow and be isolated.

Today, new culture media mimic the natural environment of bacteria by incorporating different elements into culture medium to cultivate previously uncultivated bacteria.

Louis Pasteur was the first to culture bacteria in a liquid culture medium in a reproducible manner. He invented a culture medium containing "yeast soup," ashes, sugar, and ammonium salts in 1860. His goal was to develop a fermentation medium that would show that each fermentation (alcoholic, acetic, lactic, etc.) was associated with the development of a specific microorganism. Because of the presence of these various elements in the medium, he was able to observe that some of these components could promote or inhibit the growth of certain bacteria, as well as allow the emergence of certain bacteria over others [21].

The use of a minimal medium prevents the growth of certain bacteria that require specific elements to survive. To increase bacterial multiplication, growth factors are sometimes added to culture media. Growth factors are elements that bacteria cannot synthesize from available nutrients in the environment [22]. Growth factors are required in small quantities in the culture medium, and their requirement is justified by the bacterium's lack of or blockage of a metabolic pathway.

To isolate a specific bacterial species or genus, a selective culture medium is used. The goal of this type of medium is to eliminate unwanted microbial flora after a number of inhibitors are added to the culture medium. The selective medium is made up of a basic medium that can be supplemented with antibiotics, chemicals, dyes, antiseptics, sodium salts, or phages [23].

Recent studies have also demonstrated the value of faster resistance detection by laboratories, which must be paired with more extralaboratory intervention. Rapid resistance detection has been shown to improve patient outcomes, including lower mortality, shorter hospital stays, lower rates of superinfection and adverse drug reactions, and lower costs.[24]

Although detecting bacteria and their resistance mechanisms directly from blood specimens remains a difficult goal, it has been accomplished on growing blood cultures (BCs), which typically become positive after 12 to 16 hours of incubation.6 Many systems for rapid bacterial identification from positive BCs have been developed, as have rapid automated antimicrobial susceptibility tests (ASTs). [25]

Microbial culture, in particular, has high specificity but is a relatively insensitive method [26].

Multiple reports have shown that polymerase chain reaction (PCR) has higher sensitivity than traditional methods. The Gramm stain, blood agar, and Sabouraud agar are commonly used staining and culturing media, but others are available depending on the targeted microorganism [27]. Culturing can take several days to produce a diagnosis; bacterial cultures can take 2-4 days, and fungal cultures can take 2-10 days [28]. Traditional viral cultures can take days to weeks to complete, depending on the virus, but modern culturing methods can reduce the time to 24 hours . Staining of corneal scrapings, on the other hand, can provide immediate identification of the causative agent.

Gramm staining can identify bacteria and fungi correctly 60-75% of the time and 35-90% of the time, respectively. Similarly, cultures identify 59% of bacteria and 45% of fungi. These tests produce relatively low sensitivities with significant variation, leaving room for diagnostic improvement.[30]

For both bacteria and fungi, polymerase chain reaction demonstrated higher positivity rates and sensitivity than culture and stains [29].

The ability to test for multi-drug resistance is one advantage of PCR. As demonstrated with MRSA, one advantage of multiplex PCR is the ability to test for multiple antibiotic genes with a single sample, guiding specific antimicrobial treatment while reducing the risk of drug resistance. Interestingly, another way PCR can assess antimicrobial sensitivity is through quantitative analysis of viral load after treatment.[31]

Aerobic culture refers to the ability of most bacteria to grow in the presence of oxygen. However, for optimal growth, the conditions should be tailored to the target bacterium. In the presence of oxygen, species found in atmospheric conditions, such as on the skin's surface or in the upper respiratory tract, will typically grow well. Species found in low oxygen environments, such as deep wounds or abscesses or the deep ocean, will typically grow best in the absence of oxygen - anaerobic culture. Some organisms, known as obligate anaerobes, cannot grow in the presence of oxygen at all. Fusobacterium and Bacteroides are two examples.[32] Similarly, obligate aerobes are those that cannot grow in the absence of oxygen. Gramme negative Pseudomonas aeruginosa and Mycobacterium tuberculosis, the causative agent of tuberculosis, are two examples for culture purposes.. However, studies indicate that both can engage in anaerobic respiration under certain conditions. Facultative anaerobes are bacteria that can grow in either aerobic or anaerobic conditions, switching from aerobic respiration to fermentation or anaerobic respiration if oxygen is unavailable. Gram-positive staphylococci, Escherichia coli (E. coli), Salmonella, and Listeria spp. are some examples.[33]

1.1 Current Strategies

Advancements in technology have revolutionized bacterial culture techniques in clinical microbiology. One of the notable current strategies is the use of automated culture systems, such as the BD BACTEC™ system. These systems utilize fluorescent sensors to detect bacterial growth in blood cultures. The real-time monitoring of growth enables early detection of bacterial pathogens and reduces the time to result. This technology has substantially improved the speed and accuracy of bacterial culture, facilitating prompt diagnosis and initiation of appropriate antimicrobial therapy.[3]

Another current strategy is the implementation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for bacterial identification. This technique allows rapid and accurate identification of microorganisms by analyzing their protein profiles. MALDI-TOF MS has proven to be a valuable tool in clinical microbiology, providing quick and reliable identification compared to traditional methods like biochemical testing or DNA sequencing. Its ability to identify bacteria directly from culture plates has significantly enhanced the efficiency of bacterial culture.[4]

Furthermore, molecular techniques, such as polymerase chain reaction (PCR), have been incorporated into bacterial culture protocols. PCR-based methods allow for the rapid detection and identification of bacteria at the molecular level. These techniques can target specific bacterial genes or regions to identify pathogens with high specificity and sensitivity.

Moreover, quantitative PCR enables the determination of bacterial load, aiding in the monitoring of infections and assessing treatment responses.[5]

1.2 Past Strategies

1.2.1 Historical Perspective

The earliest strategies for bacterial culture involved the use of basic nutrient agar and broth media, which allowed for the growth of a wide range of bacteria.[6] The primary objective was to create a favorable environment to support bacterial growth and multiplication. These initial culture methods were relatively simple and did not involve the extensive use of selective or differential media. However, they provided a foundation for the development of more advanced strategies.[7]

1.3 Evolution of Culture Media

Culture media have undergone significant transformation over time, giving rise to selective and differential media that facilitate the isolation and characterization of specific bacteria. Selective media are designed to favor the growth of certain organisms while inhibiting others. For example, MacConkey agar, a selective medium, is used to isolate gram-negative bacteria.[8] It contains bile salts and crystal violet, which prevent the growth of gram-positive bacteria. Differential media, on the other hand, allow the differentiation of various bacterial species based on their metabolic properties. Blood agar is a commonly used differential medium that distinguishes pathogenic bacteria based on their ability to lyse red blood cells.[9]

2. METHODOLOGY

2.1 Techniques for Bacterial Culture

Various techniques have been developed to improve the efficiency and accuracy of bacterial culture. One such technique is streak plating, which involves spreading a bacterial sample across a solid agar surface in a series of streaks.[10] This technique allows for the isolation of colonies arising from individual bacterial cells. Another widely used technique is the pour plate method, which involves mixing bacterial samples with warm liquid agar and pouring the mixture into Petri dishes. This method allows for the growth of bacteria both on the surface and within the agar.[11]

2.2 Microscopy

Microscopy is important in biological and medical sciences, contributing to significant progress in understanding living systems and diseases. Such advancements are made possible by their optical resolution property, which allows for the determination of structures with extremely small sizes. Improvements in this area aim to improve optical resolution and, as a result, the quality of images viewed through a microscope [34]

This method in microbiology allows the health professional to describe the pathogen's morphology, count the number of microorganisms present per field of view, and determine the type of motility of the bacteria. In some cases, staining techniques are required to visualize the shape and bacterial arrangement. Gramme stain, for example, is used to detect the presence of coconuts that can be joined together, resembling grape bunches in Staphylococcus spp. [35]

Gramm staining is a tintorial method used to classify pathogens based on their size, shape, cell structure, and colour. Bacteria are classified as Gram-positive or Gram-negative using this methodology. Gram-positive bacteria are purple, while Gram-negative bacteria are red. [36]

Violet crystal (VC) and safranin are the dyes used. VC is a cationic dye from the triphenylmethane chemical group that is sold in concentrations ranging from 1-2% and is responsible for the characteristic colour of Gram-positive organ isms. Safranin, on the other hand, is a cationic dye that belongs to the triarylpyrazines group. It has a different colour spectrum than the VC, which gives it Gram-negative pigmentation [37].

2.3 Culture media

Culture media are used to identify microorganisms in order to promote an environment conducive to the multiplication and survival of bacteria and fungi by supplying them with essential substances such as carbon, nitrogen, minerals, water, growth factors, and vitamins [37].

2.3.1 Advancements in Bacterial Culture Methodologies

In recent years, several advancements in bacterial culture methodologies have revolutionized clinical microbiology. One notable advancement is the utilization of automated systems for bacterial identification and antimicrobial susceptibility.

These systems, such as VITEK and MALDI-TOF, analyze the characteristics and metabolic properties of bacteria, enabling rapid and accurate identification.[12] Additionally, the introduction of real-time polymerase chain reaction (PCR) has allowed for the rapid detection of bacterial DNA in clinical samples. This technique provides faster results compared to traditional culture methods, particularly in cases where the bacteria are slow-growing or fastidious.[13]

2.4 Instrumentation

The development of sophisticated instruments has greatly contributed to the improvement of bacterial culture strategies. Automated blood culture systems, such as the BACTEC system, have significantly reduced the time required to detect bacteria in blood samples. These systems employ resin or charcoal-containing culture bottles that continuously monitor the growth of bacteria by detecting carbon dioxide production. Additionally, automated streaking instruments, such as the WASPLab, have been introduced to enhance the efficiency and standardization of streak plating. These instruments reduce the risk of contamination and enable the processing of a higher volume of samples in a shorter timeframe.[14]

To perform antimicrobial sensitivity tests, the automated methods employ three commercially available pieces of equipment: VITEK GPI (bioMerieux®), PhoenixTM (BD Biosciences), and Microscan (Dade Behring). These systems allow for faster susceptibility testing, as well as Gramme identification and evaluation of the sensitivity or resistance of each antimicrobial, making them semi-quantitative. They are, however, expensive, do not allow a MIC to be as accurate (with the exception of PhoenixTM, which has the ability to perform serial dilution), and cannot identify VRSA strains [36].

Before the advent of automated systems and molecular techniques, bacterial culture heavily relied on traditional methods. One of the most conventional strategies was the use of solid culture media, such as agar plates. These media provided a nutritive base for bacterial growth and allowed for the isolation of pure cultures. While solid media are still widely used today, they require longer incubation times and are less efficient for large-scale operations compared to automated systems.[15]

Additionally, the development of selective media played a crucial role in past strategies for bacterial culture. Selective media contain specific chemical components that inhibit the growth of unwanted bacteria and favor the growth of target pathogens. Examples include MacConkey agar for selectively isolating Gram-negative bacteria and Mannitol Salt agar for selective culturing of Staphylococcus species. [16] These media have been instrumental in increasing the accuracy of bacterial culture and aiding in the identification of clinically relevant bacteria.[17]

Moreover, the introduction of antimicrobial susceptibility testing (AST) methods significantly impacted bacterial culture strategies in the past.[18] Techniques such as disk diffusion and broth microdilution helped determine the susceptibility or resistance of bacteria to different antibiotics. The results of AST guided clinicians in selecting appropriate antibiotics for treatment, contributing to better patient outcomes. Despite advancements in AST methods, they continue to be an indispensable component of bacterial culture in clinical microbiology.[19]

3. RESULTS

The diagnostic laboratory's biochemical and growth-based approaches arose from the discovery of microorganisms and the need to confirm their causative link to infectious disease. As the need and requirement for quality health care became more common, laboratories improved diagnostic capabilities and increased testing capacity without sacrificing results quality by first utilising miniaturised, multitest kits, followed by computer automation for routine clinical isolate identification.

The introduction of advanced identification instruments into the clinical laboratory has resulted in more timely and accurate identification of microorganisms, resulting in improved diagnosis and a shorter time to appropriate therapy. The practice of clinical microbiology will be transformed as these platforms improve and become more widely available.

4. DISCUSSION

It is likely that a number of new microbiological methods will enhance our capacity to rapidly and accurately identify pathogens in critically unwell patients. However, well-designed studies assessing key clinical outcomes are needed to define their role in improving the management of severe infections.

It is critical for the doctor to understand pathogenesis of infection as well as pathogenesis not only characteristics of an organism, and to be able to communicate with clinical and nursing colleagues on the same level of understanding disease, therapy, and epidemiology.[38]

5. CONCLUSION

In conclusion, the strategies for bacterial culture in clinical microbiology have evolved significantly over time. From the basic nutrient agar and broth media to the utilization of advanced culture media, techniques, and instruments, the field has undergone remarkable progress. The introduction of selective and differential media, as well as automated systems for bacterial identification and antimicrobial susceptibility, has revolutionized diagnostic procedures. Moreover, the advancements in PCR and automated blood culture systems have expedited bacterial detection. These developments have not only enhanced the accuracy and efficiency of diagnosis but also have contributed to better patient outcomes.

The strategies employed in bacterial culture for clinical microbiology have evolved significantly over time. Current methodologies, such as automated culture systems, MALDI-TOF MS, and molecular techniques, have revolutionized the field by improving turnaround time, accuracy, and efficiency. Nevertheless, it is important to acknowledge the contributions of past strategies, including solid culture media, selective media, and antimicrobial susceptibility testing. Together, these advancements have enabled laboratories to provide timely and accurate diagnoses, ultimately improving patient care.

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