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LABORATORY DIAGNOSIS OF STAPHYLOCOCCAL INFECTIONS: TRADITIONAL AND MOLECULAR METHODS

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Introduction

- Clinical importance of staphylococci
- Traditional diagnostic methods
- Microscopy and Gram staining
- Culture, coagulase, catalase, DNase, and mannitol fermentation tests
- Molecular diagnostic methods
- Polymerase chain reaction (PCR)
- Enzyme-linked immunosorbent assay (ELISA)
- Protein A-based assays and rapid diagnostic tests
- Comparison of traditional and molecular diagnostic methods
- Conclusion

Abstract

Staphylococcal infections remain a major concern in clinical practice due to their widespread prevalence, ability to cause severe clinical manifestations, and increasing levels of antimicrobial resistance. *Staphylococcus aureus*, in particular, is responsible for a broad spectrum of community-acquired and hospital-associated infections, ranging from mild skin infections to life-threatening systemic diseases. Accurate and timely laboratory diagnosis is therefore essential for effective patient management and infection control.

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This review provides a comprehensive overview of traditional and molecular diagnostic methods used in the laboratory identification of staphylococcal infections. Conventional diagnostic approaches, including microscopy, culture techniques, and biochemical tests, continue to play a fundamental role in routine microbiology laboratories. However, these methods may be limited by longer turnaround times and reduced sensitivity in certain clinical situations. Advances in molecular diagnostics, especially polymerase chain reaction–based techniques, immunological assays, and rapid diagnostic tests, have significantly improved the speed and accuracy of pathogen detection.

In conclusion, the combined and targeted use of traditional and molecular diagnostic methods represents the most effective strategy for improving diagnostic accuracy, guiding appropriate antimicrobial therapy, and preventing the spread of staphylococcal infections.

Keywords: Staphylococcal infections; *Staphylococcus aureus*; Laboratory diagnosis; Traditional diagnostic methods; Molecular diagnostics; PCR

Introduction

Staphylococci (*Staphylococcus* spp.) are Gram-positive bacteria that exist as part of the normal human flora while also having the potential to cause a wide range of localized and systemic infections (1–3). In particular, *Staphylococcus aureus* is one of the principal causative agents of clinically severe conditions, including skin and soft tissue infections, pneumonia, endocarditis, sepsis, and toxic shock syndrome (3,4). Staphylococcal infections represent a significant proportion of both hospital-acquired and community-acquired infections (5,6,9) and are associated with considerable morbidity and mortality worldwide.

Over recent decades, the rapid emergence of antibiotic resistance among staphylococci has further complicated the management of these infections. The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA)

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and vancomycin-resistant strains has emphasized the critical importance of early and accurate diagnosis. Timely and reliable laboratory identification plays a key role in the selection of appropriate antimicrobial therapy, the control of hospital-acquired infections, and the prevention of epidemiological spread.

Traditional diagnostic methods, including microscopy, culture techniques, and biochemical tests, have long been used in the laboratory diagnosis of staphylococcal infections. Although these methods are generally reliable, they may be time-consuming and may exhibit limited sensitivity in certain clinical settings. In parallel with technological advances, molecular diagnostic approaches— particularly polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), and rapid diagnostic tests—have become increasingly integrated into clinical microbiology laboratories. These methods enable the rapid detection of pathogens with high sensitivity and specificity.

In this review, traditional and molecular diagnostic methods used in the laboratory identification of staphylococcal infections are systematically examined, and the advantages and limitations of each approach are comparatively evaluated. The aim of this article is to provide up-to-date and comprehensive information on available diagnostic strategies to support more effective diagnosis of staphylococcal infections in clinical practice.

Clinical Importance of Staphylococci

Staphylococci are considered among the most important bacterial pathogens in clinical microbiology. While these microorganisms exist as part of the normal human flora, they are capable of causing a broad spectrum of infections when favorable conditions arise. The clinical significance of staphylococcal infections is attributed to their ability to cause both mild localized infections and severe systemic infections associated with a high risk of mortality (4).

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In particular, *Staphylococcus aureus* is one of the leading causative agents of skin and soft tissue infections, surgical site infections, pneumonia, bacteremia, and endocarditis. In addition, various toxins produced by staphylococci may lead to serious clinical conditions such as toxic shock syndrome, food poisoning, and exfoliative dermatitis. These characteristics make staphylococci a major concern not only in infectious diseases but also in public health.

Staphylococcal infections are widely encountered in both hospital-acquired and community-acquired settings. In the hospital environment, prolonged hospitalization, invasive medical procedures, and the use of prosthetic devices and catheters significantly increase the risk of developing staphylococcal infections (6). In community-acquired infections, the increasing prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains has become a serious concern in recent years.

Antibiotic resistance is one of the key factors that further enhances the clinical importance of staphylococci. The presence of strains resistant to methicillin and other beta-lactam antibiotics limits therapeutic options, prolongs hospital stays, and increases treatment-related costs. For this reason, early and accurate diagnosis of staphylococcal infections plays a decisive role in improving clinical outcomes.

The factors outlined above demonstrate that staphylococcal infections remain among the pathogens requiring particular attention in clinical practice. The implementation of reliable laboratory diagnostic methods is essential for the selection of effective treatment strategies and for preventing the spread of these infections.

Traditional Diagnostic Methods in the Laboratory Diagnosis of Staphylococcal Infections

Traditional diagnostic methods have been widely used for many years in the laboratory diagnosis of staphylococcal infections and continue to represent the primary diagnostic approach in many clinical laboratories. These methods are

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mainly based on the evaluation of the morphological, cultural, and biochemical characteristics of the microorganism. When properly applied, traditional diagnostic techniques are reliable and allow differentiation of staphylococci from other Gram-positive bacteria.

Microscopy and Gram Staining

Microscopic examination and Gram staining play a crucial role in the initial identification of staphylococci. When clinical specimens are examined using the Gram staining technique, staphylococci appear as Gram-positive, spherical cocci that are typically arranged in grape-like clusters (1,2). These morphological characteristics assist in distinguishing staphylococci from other Gram-positive cocci, such as streptococci, which are arranged in chains. However, Gram staining alone is insufficient for definitive identification at the species level, and further diagnostic tests are required.

Culture Methods

Culture-based methods are considered one of the gold standard approaches for the diagnosis of staphylococci (1,2,3). Clinical specimens are commonly inoculated onto blood agar and selective media and incubated under appropriate conditions. Staphylococci grow well under aerobic and facultative anaerobic conditions and typically form characteristic colonies within 24–48 hours.

Staphylococcus aureus is distinguished by the formation of golden-yellow colonies, whereas coagulase-negative staphylococci usually produce white or gray colonies. Culture methods are of major diagnostic importance because they allow the isolation of viable microorganisms and enable subsequent identification and antimicrobial susceptibility testing.

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Catalase Test

The catalase test is one of the principal biochemical tests used to differentiate staphylococci from streptococci. This test is based on the ability of the catalase enzyme to decompose hydrogen peroxide into water and oxygen. As staphylococci are catalase-positive, the production of visible oxygen bubbles is observed during the test. This characteristic is particularly useful for the preliminary differentiation of bacteria belonging to the family Micrococcaceae (2,3).

Coagulase Test

The coagulase test is regarded as one of the most important tests for the identification of *Staphylococcus aureus* (11). It is based on the ability of the bacterium to convert fibrinogen in plasma into fibrin. A coagulase-positive result is considered characteristic of *S. aureus* and enables differentiation from coagulase-negative staphylococci. The test can be performed using either the slide or tube method and is widely applied in clinical microbiology laboratories.

DNase and Mannitol Fermentation Tests

The DNase test is used to determine the ability of staphylococci to hydrolyze deoxyribonucleic acid and serves as a supportive tool in the identification of *S. aureus* strains (2,3). Mannitol fermentation testing is performed on selective media containing high salt concentrations and is based on color changes resulting from acid production during mannitol fermentation. *Staphylococcus aureus* is typically recognized as a mannitol-fermenting species, and this property is commonly used to differentiate it from other staphylococcal species.

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Limitations of Traditional Diagnostic Methods

Although traditional diagnostic methods are widely used, they have certain limitations. These approaches are generally time-consuming, and in some cases, obtaining definitive results may take 48–72 hours. In addition, there is a risk of inaccurate identification when rare or atypical strains are encountered. For these reasons, the use of molecular diagnostic methods as complementary tools to traditional techniques has gained increasing importance in recent years.

Microscopy and Gram Staining

Microscopy and Gram staining play a significant role in the initial and rapid assessment phase of the laboratory diagnosis of staphylococcal infections. Examination of preparations prepared from clinical specimens such as pus, blood, sputum, and wound exudates using the Gram staining technique allows determination of whether the causative agent is Gram-positive or Gram-negative and provides important guidance for preliminary differential diagnosis.

During Gram staining, staphylococci appear as Gram-positive bacteria that retain the crystal violet stain and are observed as dark purple structures under the microscope. Morphologically, they are spherical cocci that are typically arranged in irregular, grape-like clusters. This characteristic arrangement represents an important diagnostic feature for distinguishing staphylococci from streptococci, which exhibit a chain-like arrangement.

Microscopic examination may also provide limited information regarding the severity of infection. In particular, the presence of numerous leukocytes and intracellular bacteria may indicate an active infectious process. However, microscopy and Gram staining alone are insufficient for accurate identification of staphylococci at the species level; therefore, the obtained findings must be confirmed by culture and biochemical tests.

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In conclusion, microscopy and Gram staining remain valuable as rapid, inexpensive, and practical methods for the preliminary diagnosis of staphylococcal infections. Nevertheless, due to their limited specificity, these methods are recommended to be used in combination with other traditional and molecular diagnostic techniques.

Molecular Diagnostic Methods

In recent years, the application of molecular diagnostic methods in clinical microbiology has enabled faster and more accurate identification of staphylococcal infections. Although traditional diagnostic techniques are reliable, the time required to obtain results and their limited sensitivity in certain cases have increased the importance of molecular approaches. Because these methods directly target the genetic or antigenic characteristics of bacteria, they are characterized by high specificity and sensitivity (10)

Polymerase Chain Reaction (PCR)

The PCR method is one of the most widely used techniques in the molecular diagnosis of staphylococcal infections (7,10). This method is based on the in vitro amplification of specific genetic regions unique to the bacterium. Using PCR, it is possible to detect genes specific to *Staphylococcus aureus* as well as the *mecA* and *mecC* genes responsible for methicillin resistance.

The main advantage of PCR is its ability to provide highly accurate results within a short period of time. This feature is of particular importance in severe clinical cases and in the timely implementation of infection control measures.

Real-Time PCR and Multiplex PCR

Real-time PCR is an advanced form of conventional PCR that allows simultaneous monitoring of gene amplification during the reaction process. This technique enables faster acquisition of results and reduces the risk of

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contamination. Multiplex PCR, on the other hand, allows the simultaneous detection of multiple genetic targets within a single reaction mixture. This capability provides a significant advantage in the concurrent identification of staphylococcal species and the detection of antibiotic resistance genes.

Immunological Methods (ELISA and Protein A–Based Assays)

Immunological diagnostic methods are based on the detection of antigenic or toxin components of staphylococci. The enzyme-linked immunosorbent assay (ELISA) is commonly used for the identification of staphylococcal toxins and proteins. In particular, the detection of Protein A synthesized by *Staphylococcus aureus* can be achieved using this method. Although ELISA demonstrates high sensitivity, the interpretation of results should be evaluated in conjunction with clinical findings.

Limitations of Molecular Diagnostic Methods

Although molecular diagnostic methods offer high accuracy, they also have certain limitations. One of the main drawbacks is their inability to distinguish between viable and nonviable bacteria. In addition, these methods require expensive equipment and specialized personnel. Therefore, molecular diagnostic techniques are most effective when used as complementary tools rather than as complete replacements for traditional diagnostic methods.

Comparison of Traditional and Molecular Diagnostic Methods

Traditional and molecular diagnostic methods used in the laboratory diagnosis of staphylococcal infections are considered complementary approaches in terms of functionality. Each group of methods has its own advantages and limitations, and the selection of an appropriate diagnostic strategy depends on the clinical context, laboratory resources, and the time required to obtain results.

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Traditional diagnostic methods, including microscopy, culture techniques, and biochemical tests, have demonstrated their reliability in clinical practice for many years (1,3). The main advantages of these methods are their relatively low cost, wide availability across laboratory settings, and their ability to isolate viable bacteria. The isolation of live microorganisms is of particular importance for performing antimicrobial susceptibility testing and guiding appropriate therapy. However, the principal limitations of traditional methods include the longer time required to obtain results and the risk of misidentification, especially in the case of rare or atypical strains.

In contrast, molecular diagnostic methods are characterized by high specificity and sensitivity and allow results to be obtained within a shorter time frame (10). PCR-based techniques, in particular, are highly effective for the early detection of specific pathogens and antibiotic resistance genes.

These methods facilitate rapid clinical decision-making in severe infections and play an important role in infection control and the prevention of disease transmission. Nevertheless, because molecular methods are unable to distinguish between viable and nonviable bacteria, additional clinical and laboratory evaluation is often required for accurate interpretation of results (3,10).

Comparison of these two diagnostic approaches indicates that while traditional methods form the foundation of laboratory diagnosis, molecular techniques serve as essential complementary tools for achieving rapid and precise results in modern clinical practice (1,3). An optimal diagnostic strategy can be achieved through the combined use of traditional and molecular methods. This integrated approach enhances diagnostic accuracy and enables timely and effective selection of treatment strategies.

In conclusion, rather than relying on a single diagnostic method, the integration of traditional and molecular techniques—taking into account clinical and epidemiological conditions—represents the most appropriate approach for the laboratory diagnosis of staphylococcal infections (1,3,10).

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Conclusion

Staphylococcal infections remain one of the major public health concerns due to their widespread occurrence in clinical practice, their potential to cause severe clinical manifestations, and the increasing prevalence of antibiotic resistance. Early and accurate laboratory diagnosis is therefore essential for the effective management of these infections.

Traditional diagnostic methods, including microscopy, culture techniques, and biochemical tests, have long been used as reliable approaches for the identification of staphylococci. Although these methods are practical, accessible, and cost-effective, the time required to obtain results and their limited specificity in certain situations represent important limitations.

Molecular diagnostic methods, on the other hand, are characterized by high sensitivity and specificity and enable more rapid detection of staphylococcal infections. In particular, PCR-based techniques provide significant advantages in species-level identification of pathogens and in the detection of antibiotic resistance genes. Nevertheless, the high cost of these methods and the requirement for specialized equipment may restrict their widespread implementation.

Comparative evaluation indicates that the most effective approach to the laboratory diagnosis of staphylococcal infections is the combined and targeted use of traditional and molecular diagnostic methods. This integrative strategy contributes to improved diagnostic accuracy, timely selection of appropriate treatment strategies, and effective prevention of infection spread.

In conclusion, rational selection of diagnostic methods, taking into account clinical and laboratory conditions, plays a fundamental role in the effective control of staphylococcal infections and in improving patient outcomes.

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References

1. Procop GW, Church DL, Hall GS, Janda WM, Schreckenberger PC, Woods GL. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2017. p. 670–732.
2. Çelebi ŞU. Basic and Clinical Microbiology. Ankara: Güneş Publishing; 1999.
3. Foster T. Staphylococcus. In: Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch; 1996.
4. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339(8):520–532.
5. Duckworth GJ. Diagnosis and management of methicillin-resistant Staphylococcus aureus infection. BMJ. 1993;307(6911):1049–1052.
6. Akyol AD. Staphylococcal infections and control measures in intensive care units. Journal of Intensive Care Nursing. 2006;10(1):26–35.
7. Foster TJ. Antibiotic resistance in Staphylococcus aureus: current status and future prospects. FEMS Microbiol Rev. 2017;41(3):430–449.
8. Avcıoğlu F, Öztürk CE, Şahin İ, Öksüz Ş, Kızılırmak A. Reduced vancomycin susceptibility among methicillin-resistant staphylococci. Duzce Medical Journal. 2020;10(1):81–86.
9. Loewen K, Schreiber Y, Kirlew M, Bocking N, Kelly L. Community-associated methicillin-resistant Staphylococcus aureus infection. Can Fam Physician. 2017;63(7):512–520.
10. Bozdoğan B. Use of molecular diagnostic tests in the detection of antimicrobial resistance. ANKEM Journal. 2012;26(Suppl 2):82–85.
11. Kaçmaz B, Gül S, Öztürk DB, Ecemiş E. Evaluation of coagulase testing in Staphylococcus aureus strains. Turkish Journal of Hygiene and Experimental Biology. 2015;72(2):99–102.