



Current Laboratory Techniques for Identification of Enterococcus in Urinary Samples: A Comparative Review of Conventional and Molecular Methods

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KEYWORDS

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ABSTRACT:

Background: Enterococcus species are Gram-positive cocci that have emerged as important opportunistic pathogens, particularly in urinary tract infections (UTIs). Accurate and timely identification of these organisms is essential due to their association with complicated infections and increasing multidrug resistance.

Objective: This review aims to compare conventional and molecular laboratory techniques for the identification of Enterococcus species in urinary samples, highlighting their advantages, limitations, and clinical utility.

Methods: A comprehensive literature review was conducted, focusing on culture-based, biochemical, and molecular methods, including polymerase chain reaction (PCR), multiplex PCR, loop-mediated isothermal amplification (LAMP), and matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI-TOF MS). The performance of each method regarding sensitivity, specificity, turnaround time, and ability to detect resistance and virulence determinants was analysed.

Results: Conventional culture-based methods, including selective media, Gram staining, and biochemical tests, remain widely used due to their simplicity, cost-effectiveness, and provision of viable isolates for antimicrobial susceptibility testing. However, they are labour-intensive, time-consuming (24–72 hours), and may lack specificity for closely related species or multidrug-resistant strains. Molecular techniques provide rapid, sensitive, and specific identification at the species level, detect virulence and resistance genes, and facilitate epidemiological surveillance. Limitations of molecular methods include higher cost, need for specialized equipment, and possible detection of nonviable organisms.

Conclusion: Both conventional and molecular methods have distinct roles in clinical microbiology. A combined diagnostic approach—utilizing culture for viability and antimicrobial susceptibility, alongside molecular assays for rapid species confirmation and resistance profiling—ensures accurate, timely identification of Enterococcus species, informs appropriate antimicrobial therapy, and supports infection control measures. Future research should focus on developing cost-effective, rapid molecular assays suitable for routine clinical implementation to address the rising challenge of enterococcal UTIs.



INTRODUCTION

Enterococci are Gram-positive, facultatively anaerobic cocci that normally colonise the gastrointestinal tract of humans and animals. Over the past few decades, they have emerged as significant opportunistic pathogens, particularly in urinary tract infections (UTIs), bloodstream infections, endocarditis, and intra-abdominal infections [1,2]. Among Enterococcus species, *E. faecalis* and *E. faecium* are most frequently implicated in clinical infections, with *E. faecium* often exhibiting multidrug resistance, complicating treatment options [3,4].

UTIs caused by enterococci constitute a considerable proportion of hospital- and community-acquired infections. They are particularly prevalent in elderly patients, individuals with indwelling catheters, patients with diabetes, and immunocompromised populations [5,6]. Clinical manifestations of enterococcal UTIs range from asymptomatic bacteriuria to severe pyelonephritis, emphasizing the need for accurate and timely pathogen identification [7].

Traditional laboratory methods for identifying enterococci primarily rely on culture-based techniques, including selective media, Gram staining, and biochemical testing [8,9]. These methods provide preliminary detection and species-level differentiation; however, they are time-consuming, often requiring 48–72 hours, and may lack sufficient specificity to distinguish closely related species or detect resistance traits accurately [10].

Molecular diagnostic techniques have revolutionized the detection and identification of Enterococcus species in clinical samples. Polymerase chain reaction (PCR), multiplex PCR, loop-mediated isothermal amplification (LAMP), and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allow rapid, sensitive, and specific detection directly from clinical specimens or cultured isolates [11–14]. These approaches also enable the identification of virulence factors and antimicrobial resistance genes, providing critical information for guiding therapy and infection control measures [15,16].

Given the increasing prevalence of enterococcal UTIs and the growing concern of multidrug resistance, a comprehensive evaluation of both conventional and

molecular laboratory techniques is essential. This review aims to compare the current methods for identifying Enterococcus species in urinary samples, highlighting their advantages, limitations, and clinical utility for accurate diagnosis and management.

Conventional Identification Techniques of Enterococcus

Identification of Enterococcus species in urinary samples has traditionally relied on culture-based and biochemical methods. Despite the emergence of molecular diagnostics, conventional techniques remain the cornerstone of routine clinical microbiology laboratories due to their cost-effectiveness and wide availability [17,18].

1. Culture-Based Methods

Enterococci grow readily on non-selective media such as blood agar and brain-heart infusion agar. Selective and differential media, including bile esculin agar (BEA) and Enterococcosel agar, exploit the ability of enterococci to hydrolyze esculin in the presence of bile salts, producing a characteristic black or brown precipitate around colonies [19,20]. Growth in 6.5% NaCl is another distinguishing feature, separating enterococci from other Gram-positive cocci [21]. Culture-based methods allow initial identification and provide viable isolates for subsequent antimicrobial susceptibility testing.

2. Microscopy and Gram Staining

Microscopic examination of urine sediment or cultured colonies using Gram staining provides preliminary identification. Enterococci appear as Gram-positive cocci, typically in pairs or short chains [22]. Although morphology alone cannot reliably determine the species, it offers rapid initial clues for further testing.

3. Biochemical Testing

Biochemical tests are widely used for species-level identification. Enterococci are catalase-negative and generally produce the pyrrolidonylamidase (PYR) enzyme, detectable using colorimetric assays [23]. Additional tests, including carbohydrate fermentation patterns (mannitol, arabinose, raffinose, sorbitol) and arginine deamination, can differentiate species such as *E. faecalis* and *E. faecium* [24,25]. The Voges-



Proskauer reaction and growth at elevated temperatures (e.g., 45°C) may also aid species discrimination [26].

4. Antimicrobial Susceptibility Patterns

Although primarily used to guide therapy, resistance profiles can assist in presumptive species identification. For example, *E. faecium* frequently exhibits higher intrinsic resistance to ampicillin and low-level resistance to vancomycin compared to *E. faecalis* [27]. However, antimicrobial susceptibility alone is insufficient for definitive identification and must be combined with culture and biochemical methods.

5. Limitations of Conventional Methods

Despite their usefulness, conventional techniques have several limitations. They are labor-intensive, require 24–72 hours for species-level identification, and may fail to differentiate closely related species or detect vancomycin-resistant strains accurately [28,29]. Polymicrobial infections or atypical strains can further complicate identification. These limitations highlight the need for complementary molecular diagnostic approaches for rapid, sensitive, and specific detection of clinically significant enterococci.

Molecular Identification Techniques of Enterococcus

Molecular diagnostic methods have revolutionized the identification of Enterococcus species, providing rapid, sensitive, and specific detection compared to conventional culture-based techniques. These methods enable species-level identification, detection of virulence factors, and antimicrobial resistance genes, which are critical for guiding targeted therapy and infection control [30,31].

1. Polymerase Chain Reaction (PCR)

PCR is widely employed for the detection and identification of Enterococcus species in urinary samples, either directly from clinical specimens or from cultured isolates. Species-specific primers targeting conserved genes, such as the *ddl* gene (D-alanine:D-alanine ligase) and 16S rRNA, allow accurate differentiation of *E. faecalis* and *E. faecium* [32,33]. PCR assays can also detect vancomycin-resistance genes (*vanA*, *vanB*) and other resistance determinants, enabling clinicians to select appropriate antimicrobial therapy [34,35].

2. Real-Time PCR and Multiplex PCR

Real-time PCR (qPCR) combines amplification and detection in a single reaction, providing rapid and quantitative results with high sensitivity. Multiplex PCR allows simultaneous identification of multiple Enterococcus species or resistance genes within a single assay [36,37]. These approaches are particularly valuable for detecting polymicrobial infections and screening high-risk populations for vancomycin-resistant Enterococcus (VRE) colonization.

3. Loop-Mediated Isothermal Amplification (LAMP)

LAMP is an emerging nucleic acid amplification technique that operates at a constant temperature, eliminating the need for thermal cycling equipment. LAMP assays targeting species-specific genes of enterococci have demonstrated high sensitivity and specificity for rapid detection in urinary samples. The simplicity, speed, and minimal equipment requirements make LAMP suitable for low-resource settings [38].

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

MALDI-TOF MS identifies bacteria based on unique protein spectral patterns. It can provide species-level identification of Enterococcus isolates within minutes from cultured colonies, with high accuracy and reproducibility [39,40]. Recent advances have explored the direct detection of enterococci from urine specimens, potentially enabling near real-time diagnosis in clinical laboratories.

5. Advantages and Limitations

Molecular methods offer several advantages over conventional techniques, including rapid turnaround, high specificity, and sensitivity, as well as the ability to detect virulence and resistance genes simultaneously [41]. These methods are particularly important for identifying multidrug-resistant strains and monitoring nosocomial outbreaks.

However, molecular techniques also have limitations. They require specialized equipment, trained personnel, and higher costs compared to culture-based methods. Additionally, while PCR and related techniques can detect DNA from dead organisms, they may not distinguish between viable and nonviable bacteria,



which can be a limitation in certain clinical contexts [42].

6. Integration with Conventional Methods

Combining molecular techniques with conventional culture-based approaches provides an optimal strategy for accurate diagnosis. Initial culture ensures organism viability and allows antimicrobial susceptibility testing, while molecular assays provide rapid species-level identification, resistance profiling, and detection of virulence factors. This integrated approach enhances clinical decision-making, improves patient outcomes, and supports effective infection control [43,44].

Comparative Analysis and Conclusion

The identification of *Enterococcus* species in urinary samples requires a balance between accuracy, speed, and resource availability. Conventional culture-based techniques, including selective media, Gram staining, and biochemical tests, remain fundamental in routine clinical microbiology due to their simplicity, cost-effectiveness, and ability to provide viable isolates for antimicrobial susceptibility testing [17–27]. These methods reliably detect common *Enterococcus* species but are time-consuming, requiring 24–72 hours, and may lack specificity for closely related species or for detecting multidrug-resistant strains [28,29].

Molecular diagnostic methods, such as PCR, multiplex PCR, real-time PCR, LAMP, and MALDI-TOF MS, offer high sensitivity, specificity, and rapid turnaround times [30–40]. They enable species-level identification, detection of virulence determinants, and characterization of antimicrobial resistance genes, which is particularly important for managing vancomycin-resistant *Enterococcus* (VRE) infections [32–35,39,40]. Molecular techniques also facilitate epidemiological surveillance and outbreak monitoring, allowing precise tracking of resistant strains in hospital and community settings [36,37].

Despite these advantages, molecular techniques have limitations, including higher costs, the need for specialized equipment and trained personnel, and potential detection of nonviable organisms, which may complicate clinical interpretation [41,42]. Therefore, combining conventional and molecular methods offers the most comprehensive strategy for accurate and timely identification of enterococci. Conventional

culture provides viable isolates for susceptibility testing, while molecular assays deliver rapid species confirmation, resistance profiling, and virulence detection [43,44].

In conclusion, *Enterococcus* species are increasingly recognized as important urinary pathogens, associated with complicated infections and multidrug resistance [1–4,10,12]. Conventional methods remain essential for routine diagnosis, while molecular methods enhance rapid detection, species-level differentiation, and resistance gene identification. A combined diagnostic approach ensures accurate, timely identification, informs appropriate antimicrobial therapy, improves patient outcomes, and supports infection control measures. Future research should focus on the development of cost-effective, rapid molecular assays suitable for routine implementation in diverse clinical laboratories to address the rising challenge of enterococcal UTIs [31,38,40].

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