COMPARATIVE EFFECTIVENESS OF MOLECULAR AND BACTERIOLOGICAL METHODS FOR DIAGNOSING SHIGELLOSIS

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Abstract. Shigellosis remains a significant cause of acute diarrheal disease worldwide, particularly in developing countries. Early and accurate diagnosis is crucial for timely treatment and control of outbreaks. Traditional bacteriological culture methods have long been considered the gold standard; however, molecular diagnostic techniques, including polymerase chain reaction (PCR), offer enhanced sensitivity, specificity, and rapidity. This review explores the comparative effectiveness of molecular and bacteriological approaches in diagnosing shigellosis, highlighting their respective advantages, limitations, and roles in clinical and public health settings.

Keywords: Shigellosis, diagnosis, PCR, bacteriological culture, sensitivity, specificity

Introduction. Shigellosis, caused by bacteria of the genus Shigella, is responsible for an estimated 164.7 million cases and over 600,000 deaths annually, with the greatest burden in low-income regions [1]. Accurate diagnosis is critical not only for patient management but also for surveillance and outbreak control. The diagnostic tools used for shigellosis include classical

bacteriological culture and modern molecular techniques, which differ significantly in their diagnostic performance.

Bacteriological Culture Methods. Bacteriological culture remains the **classical diagnostic approach** for detecting *Shigella* in stool specimens. This method involves the cultivation of viable bacteria on selective or differential media, followed by identification through morphological, biochemical, and serological testing [2].

Procedure and Diagnostic Basis. In routine practice, stool samples are cultured on Xylose Lysine Deoxycholate (XLD)**,** Salmonella-Shigella (SS) agar, or Hektoen Enteric (HE) agar. After 24–48 hours of incubation at 37°C, suspicious colonies are subcultured and subjected to biochemical identification (e.g., TSI, SIM, urease tests) and serotyping using specific antisera [3]. According to Pavlov D. L. et al., identification based on conventional culture methods remains the reference standard for confirmation of shigellosis in many public health laboratories, due to its specificity and provision of antimicrobial susceptibility information [3].

Advantages of Culture Methods

- Phenotypic antimicrobial susceptibility testing (AST) can be directly performed on cultured isolates, which is critical in light of rising antibiotic resistance [7].
- Strain typing and outbreak tracing are possible using cultured isolates for further genotyping or pulsed-field gel electrophoresis (PFGE).

Limitations of Bacteriological Methods.Despite being a diagnostic mainstay, bacteriological culture has notable limitations:

- Low sensitivity: Detection rates range from 30–60%, particularly in patients with low bacterial loads, delayed sample transport, or prior antibiotic therapy [3].

- Time-consuming: Requires up to 72 hours from sample collection to confirmation.
- Viability-dependent**:** Cannot detect dead or non-culturable *Shigella*, limiting its utility in subacute or chronic carriers.

As a result, culture alone may lead to underdiagnosis, especially in outbreak investigations or among asymptomatic carriers.

Molecular Diagnostic Techniques. Molecular Diagnostic Techniques (Expanded). Molecular diagnostic methods, particularly polymerase chain reaction (PCR) and its variants, have revolutionized the detection of enteric pathogens, offering high sensitivity, rapidity, and the ability to detect non-culturable bacteria [4].

Genetic Targets and Primer Design. PCR assays typically target the invasion plasmid antigen H gene (ipaH)**,** present in multiple copies in all *Shigella* species and enteroinvasive *Escherichia coli (EIEC)*, increasing assay sensitivity [4]. Other targets include **ial**, **virA**, and **set1A/B**, which may help distinguish between *Shigella* serotypes or assess virulence potential.

Types of Molecular Methods

- Conventional PCR: Cost-effective and widely used in research labs.
- Real-time PCR (qPCR)**:** Offers quantification and higher sensitivity with reduced contamination risk.
- Multiplex PCR**:** Simultaneously detects multiple pathogens, reducing time and reagent use [5].
- LAMP (Loop-mediated isothermal amplification)**:** An alternative to PCR suitable for field conditions due to its simplicity and lack of thermocycling requirement [5].

Advantages of Molecular Diagnostics

- High sensitivity (up to 95–100%) even with low bacterial loads.

- Rapid turnaround time**:** Results within 4–6 hours.
- Applicable to non-viable organisms**:** Useful for patients who started antibiotics before sample collection.
- Reduced reliance on expert microbiological interpretation**,** improving standardization.

Limitations and Challenges

- Cannot assess antimicrobial susceptibility, requiring culture as a complementary test.
- False positives: Due to detection of DNA from dead bacteria, leading to potential overdiagnosis.
- High cost and infrastructure needs: Real-time PCR and multiplex systems require trained personnel, quality reagents, and maintenance [6].

Conclusion. Molecular diagnostics, especially PCR, significantly outperform bacteriological culture in terms of sensitivity and turnaround time for the detection of Shigella. However, culture remains essential for antimicrobial resistance profiling and is more accessible in low-resource settings. Integrating both methods into diagnostic workflows can optimize shigellosis detection and treatment, particularly in endemic regions.

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