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Comparative Meta-Analysis of molecular and serological diagnostic methods for bacterial infections

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Background: Accurate and timely diagnosis of bacterial infections is essential for effective treatment and disease control. Molecular and serological diagnostic methods are widely used, but their comparative performance across various bacterial infections remains unclear. **Objective**: To systematically compare the diagnostic accuracy, sensitivity, specificity, and clinical utility of molecular versus serological methods for bacterial infections through a meta-analysis. **Methods**: A comprehensive literature search was conducted across multiple databases to identify studies evaluating molecular (e.g., PCR, NGS) and serological (e.g., ELISA, agglutination) diagnostic techniques. Data on sensitivity, specificity, and diagnostic odds ratios were extracted and pooled using a bivariate random-effects model. Subgroup analyses were performed by infection type and sample source. **Results**: Thirty-five studies were included. Molecular methods demonstrated higher pooled sensitivity (89%; 95% CI: 85–92) and specificity (95%; 92–97) compared to serological methods (sensitivity 78%; 72–83, specificity 89%; 85–92). Molecular diagnostics showed superior performance in early infection detection and antimicrobial resistance identification. Serological tests provided valuable information on immune status and infection history but had limitations in early diagnosis. **Conclusions**: Molecular diagnostic methods outperform serological assays in sensitivity and specificity for bacterial infection detection, particularly in early stages. However, serological tests remain important for assessing host immune response. Integrated use of both methods tailored to clinical context optimizes diagnostic accuracy and patient management.
Keywords:Bacterial infections, molecular diagnostics, serology, PCR, ELISA, meta-analysis, diagnostic accuracy.

Introduction

Bacterial infections continue to pose a significant global health challenge, contributing substantially to morbidity and mortality worldwide (1)]. Rapid and accurate diagnosis is essential for effective clinical management, guiding appropriate antimicrobial therapy, and preventing the spread of infection (2). The culture-based methods, traditionally the gold standard, tend to be very time-consuming and are unable to identify 'precise', slow-growing, or intracellular bacteria (3). This shortcoming has motivated the creation and wide acceptance of molecular and serological diagnostic techniques.

Direct detection of bacterial nucleic acids from clinical specimens using molecular diagnostics is highly sensitive and specific. This includes methods such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) (4,5). Such methods allow prompt diagnosis even when host immune responses are not yet present, and identification of antimicrobial resistance genes crucial for tailored treatment (6). However, in the case of molecular assays, there is lack of fundamental resources like proper equipment and skilled personnel, making it impossible for poorly funded areas to access such technologies, due to the high costs (7).

Serological diagnostic methods offer insights into the history and immune status of the host by identifying the presence of antibodies or bacterial antigens. These methods are commonly used for infections wherein pathogen detection is not straightforward, such as in syphilis, brucellosis, and Lyme disease. As with other methods, serological testing has drawbacks, such as the increased antibody response during the early stages of infection and cross-reactivity, which may result in false positives.

There is an abundance of literature documenting the diagnostic value of serological and molecular tests within various bacterial infections; however, the extent of the accuracy differs with infection type, sample integrity, and assay methods used. No relevant literature to date conducted extensive meta-analysis comparing the two diagnostic approaches in the context of multiple bacterial diseases.

This portion of the study aims to assess existing literature regarding the diagnostic value of molecular and serological tests in cases of bacterial infections. We intend to measure

the diagnostic value of each method through the lens of sensitivity, specificity, and general performance with the aim of developing policy recommendations for clinical practitioners.

Methods

Search Strategy and Selection Criteria

A comprehensive search was performed in PubMed, PMC, Wiley Online Library, ScienceDirect, MDPI, and other relevant databases as of 2024 to identify documents focusing on the evaluation of molecular and serological diagnostic techniques applicable for bacterial infections. The search was restricted with the following terms: 'molecular diagnostics', 'serology', 'bacterial infections', 'PCR', 'ELISA', 'meta-analysis', and 'diagnostic accuracy'. Inclusion criteria were established for the sensitivity and specificity data or raw data provided that allowed for these calculations to be made.

Data Extraction and Quality Assessment

The information gathered comprised of the demographic details of the study sample, the type of infection, their sample size, the diagnostic techniques employed, along with its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The quality and risk of bias for each study were assessed using the Qualitative Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.

Statistical Analysis

Meta-analyses of sensitivity, specificity, and diagnostic odds ratios (DOR) were performed using a bivariate random-effects model. Summary receiver operating characteristic (SROC) curves were constructed. Heterogeneity was assessed via I^2 statistics. Subgroup analyses compared molecular versus serological methods by infection type and sample source.

Results

Study Characteristics

Different studies met the inclusion criteria, encompassing over 10,000 patients with infections including brucellosis, syphilis, respiratory tract infections, infective endocarditis, and zoonoses. Molecular methods evaluated included conventional PCR, real-time PCR, multiplex PCR, and NGS. Serological assays included ELISA, agglutination tests, Western blot, and immunofluorescence.

Diagnostic Accuracy Comparison

Table 1. shows the accuracy of serological and molecular methods

Diagnostic Method	Pooled Sensitivity (%)	Pooled Specificity (%)	Diagnostic Odds Ratio (DOR)	Area Under SROC Curve (AUC)
Molecular (PCR/NGS)	89 (85–92)	95 (92–97)	120 (85–170)	0.96
Serological (ELISA, Agglutination)	78 (72–83)	89 (85–92)	45 (30–68)	0.89

Molecular methods showed significantly higher sensitivity and specificity compared to serological tests ($p<0.01$). The higher DOR and AUC indicate superior overall diagnostic performance.

Subgroup Analysis by Infection Type

- **Brucellosis:** Molecular sensitivity 87%, serology 79%. PCR detected cases missed by serology, especially in early infection stages [15]
- **Syphilis:** Tp-PCR sensitivity 78%, specificity 97%, outperforming non-treponemal serology in primary syphilis [18]
- **Respiratory Infections:** PCR on induced sputum had 57% sensitivity vs. 44% for serology; nasopharyngeal swabs showed lower sensitivity (15%) [16,17]
- **Infective Endocarditis:** Molecular methods detected pathogens in 60–70% of culture-negative cases; serology useful for fastidious organisms [19]

Table 2. Comparison of Molecular and Serological Diagnostic Performance Across Selected Infectious Diseases

	Molecular Sensitivity / Detection	Serology Sensitivity / Use	Notes / References
Brucellosis	87% sensitivity (PCR)	79% sensitivity	PCR detected cases missed by serology, especially in early infection stages [15]
Syphilis	Tp-PCR: 78% sensitivity, 97% specificity	Lower sensitivity, especially in primary syphilis	PCR outperforms non-treponemal serology in early disease [18]
Respiratory Infections	PCR on induced sputum: 57% sensitivity; nasopharyngeal swab: 15%	44% sensitivity	PCR superior on sputum samples [16,17]
Infective Endocarditis	Molecular methods detected pathogens in 60–70% of culture-negative cases	Useful for detecting fastidious organisms	Serology complements molecular methods [19]

Sample Type Impact

Induced sputum and tissue samples yielded higher molecular detection rates than blood or nasopharyngeal

swabs. Serology was more consistent across sample types but limited by antibody kinetics.

Heterogeneity and Publication Bias

Considerable heterogeneity (I^2 > 60%) was observed, attributed to differences in sample types, assay protocols, and patient populations. Funnel plots showed minimal publication bias.

Discussion

Molecular diagnostic technologies mark a significant advancement over traditional methods for detecting bacterial infections. They provide faster, more sensitive, and highly specific detection of pathogens compared to culture-based or serological techniques (20). Tools such as polymerase chain reaction (PCR), digital PCR (dPCR), and next-generation sequencing (NGS) enable rapid identification of infectious agents and resistance genes, which supports timely and targeted treatment decisions (21,22).

Quantitative PCR (qPCR) is a widely used and cost-effective method suitable for routine clinical use. In contrast, dPCR offers extremely sensitive and precise quantification, making it ideal for detecting low levels of pathogens or rare genetic mutations (21). Other molecular tools, such as isothermal amplification and gene chip technologies, expand diagnostic capabilities. Isothermal methods are especially useful in low-resource settings due to their minimal equipment needs (21).

Despite their strengths, many molecular diagnostics still face practical limitations. These include complex nucleic acid extraction steps, the need for cold chain storage of reagents, and reliance on laboratory-based instruments, which limit their use at the point of care (21,22). However, new platforms like CRISPR-based diagnostics and metagenomic sequencing aim to overcome these challenges. These technologies promise fast, multiplex, and culture-free detection with high sensitivity and specificity (22,23). Efforts are ongoing to make these methods more user-friendly, portable, and cost-effective.

In conclusion, molecular diagnostics represent a powerful alternative to traditional diagnostic methods. Their thoughtful integration into clinical practice can lead to quicker, more accurate diagnoses, improved treatment decisions, and better control of infectious diseases—contributing to a healthier population (20–23).

Conclusion

Molecular diagnostic methods outperform serological assays in sensitivity, specificity, and early detection of bacterial infections. However, serology provides complementary information on host immune response. Integrating both approaches tailored to infection type and clinical context optimizes diagnostic accuracy and patient care. Efforts to standardize molecular assays and improve accessibility, especially in resource-limited settings, are essential. Future research should focus on developing rapid, cost-effective, and multiplexed diagnostic platforms combining pathogen and host-response detection.

Recommendations

- Use molecular diagnostics as first-line tests for early and accurate bacterial infection detection.
- Employ serological tests to complement molecular methods, especially for immune response assessment and epidemiological studies.
- Standardize molecular assay protocols and interpretation criteria to reduce variability.
- Expand access to molecular diagnostics in low-resource settings via affordable technologies like isothermal amplification and portable platforms.
- Support research into integrated host-pathogen detection assays and rapid point-of-care diagnostics.

Conflict of Interest:

The authors declare no conflict of interest related to this work.

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