e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(3); 1557-1564

Original Research Article

Diagnostic Techniques in Microbiology: A Prospective Observational Study at Darbhanga Medical College & Hospital, Darbhanga, Bihar, India

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Received: 06-01-2026 / Revised: 16-02-2026 / Accepted: 06-03-2026
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Conflict of interest: Nil

Abstract

Background: Diagnostic microbiology has moved from conventional smear, culture and biochemical methods toward integrated platforms that include MALDI-TOF MS, rapid molecular panels, targeted sequencing, metagenomic approaches and accelerated antimicrobial susceptibility testing. In tertiary-care settings, especially in resource-constrained regions, the challenge is to optimize diagnostic yield without compromising turnaround time, quality assurance or interpretive stewardship.

Aim: To evaluate the role and comparative clinical utility of major diagnostic techniques in microbiology among patients investigated for suspected infection at a tertiary care teaching hospital in Bihar, India.

Methods: This prospective observational hospital-based study was designed in the Department of Microbiology, Darbhanga Medical College & Hospital, Darbhanga, Bihar, India, from 15 February 2025 to 15December 2025 and planned to include 85 consecutively enrolled patients. Clinical specimens such as blood, urine, respiratory samples, pus/wound swabs and body fluids were to be processed by direct microscopy, culture-based identification, antimicrobial susceptibility testing and molecular methods where clinically indicated and available. Diagnostic performance, positivity rate, organism spectrum, multidrug resistance frequency and turnaround time were predefined study outcomes.

Results: Because the case-wise dataset was not supplied in the current conversation, numerical findings are not inserted in order to avoid fabrication. Instead, four publication-style tables and two statistical diagrams have been prepared in the companion Excel workbook for direct completion from the real 85-patient dataset. Table 1 summarizes baseline characteristics; Table 2 compares specimen-wise testing pattern and positivity; Table 3 computes sensitivity, specificity, predictive values, accuracy and kappa for each modality versus final composite microbiological status; and Table 4 summarizes isolate distribution, AST coverage and multidrug resistance pattern. Figure 1 displays positivity by specimen and technique, whereas Figure 2 compares turnaround time across diagnostic modalities.

Conclusion: A modern microbiology service in a tertiary-care Indian hospital should integrate conventional microscopy and culture with rapid identification, molecular testing and structured diagnostic stewardship. Final publication-quality conclusions must be based on the real local dataset; however, the present manuscript and workbook together provide a journal-ready framework for rapid completion once validated study data are entered.

Keywords: microbiology diagnostics; culture; MALDI-TOF MS; molecular testing; antimicrobial susceptibility testing; diagnostic stewardship; tertiary care hospital; India.

DOI: 10.25258/ijcpr.18.3.248

Introduction

Diagnostic microbiology remains central to the clinical management of infectious diseases because microbiological confirmation informs pathogen-directed therapy, infection-control action, surveillance and antimicrobial stewardship. In most hospital laboratories, the diagnostic journey still begins with direct microscopy and culture, followed by biochemical identification and antimicrobial susceptibility testing. These methods remain clinically indispensable because they provide organism recovery, permit susceptibility testing and support outbreak investigation;

however, they are also limited by time to positivity, variable sensitivity after prior antibiotic exposure and the need for sustained technical expertise [1-3]. Even in high-income settings, a purely conventional workflow may require two to five days before a definitive report becomes available, whereas critically ill patients often need targeted decisions within hours rather than days [1,2].

Over the last decade, clinical microbiology has therefore entered a period of rapid technological expansion. Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) has transformed organism identification by allowing accurate species-level identification in minutes once growth is available, with important downstream effects on turnaround time and reagent cost [1,4]. Rapid multiplex molecular syndromic panels now permit simultaneous detection of multiple pathogens and selected resistance determinants directly from clinical specimens or positive blood culture bottles, thereby compressing the time to actionable information in sepsis, pneumonia and other acute syndromes [2,5]. More recent platforms using nanopore sequencing, rapid phenotypic AST, automation and metagenomic next-generation sequencing (mNGS) further extend the diagnostic armamentarium, particularly in culture-negative infections, difficult-to-grow organisms and complex polymicrobial disease [6-8].

Yet technological expansion alone does not guarantee better patient care. Diagnostic value depends on pre-test probability, specimen quality, test selection, laboratory workflow, clinical interpretation and communication to the treating team. Studies evaluating rapid diagnostics consistently emphasize that the largest clinical gains occur when the laboratory result is integrated with antimicrobial stewardship or expert clinical review rather than reported in isolation [9,10]. In other words, the relevant question is not only whether a test is fast, but whether it changes management appropriately, shortens exposure to ineffective therapy, reduces unnecessary broad-spectrum prescribing and improves outcomes. This distinction is especially important in low- and middle-income settings, where procurement decisions must balance capital cost, consumables, quality assurance, local pathogen distribution, staffing patterns and test utilization discipline [10,11].

For resource-constrained tertiary hospitals in eastern India, these issues are highly relevant. Such centres manage a wide spectrum of infectious syndromes ranging from bacteremia, urinary tract infection and wound sepsis to respiratory, fungal and healthcare-associated infections. At the same time, laboratories may face uneven access to automation, intermittent reagent availability and high disease burden with substantial antimicrobial resistance pressure. Under these circumstances, a pragmatic comparative evaluation of diagnostic techniques is valuable not merely as a technology inventory but as a service-delivery assessment: which methods contribute most to positivity, which improve timeliness, which support earlier antimicrobial optimization, and where traditional culture remains irreplaceable [3,8,10,11].

Recent literature provides a strong rationale for such evaluations. Reviews of contemporary microbiological identification strategies highlight that conventional methods retain foundational value while MALDI-TOF MS and molecular assays offer major gains in speed [1,4]. Rapid sepsis diagnostics continue to evolve, including targeted sequencing and rapid AST platforms capable of shortening the interval between pathogen detection and therapeutic refinement [2,6,8]. Meta-analytic evidence suggests that rapid diagnostic tests combined with stewardship improve time to optimal therapy and may reduce mortality in bloodstream infection when compared with blood culture alone [9]. At the same time, mNGS and broader sequencing strategies appear particularly useful in selected circumstances such as culture-negative infection, rare pathogens, immunocompromised hosts or mixed infections, although cost, contamination control and interpretive complexity remain barriers to routine deployment [7,11].

Despite this expanding evidence base, there is limited location-specific literature describing how diverse microbiological techniques are used together in everyday practice in a tertiary-care teaching hospital in Bihar. A hospital-based observational study can help map the real distribution of specimens, positivity rates, organism spectrum, resistance burden and reporting timelines across modalities. Such evidence is important for diagnostic stewardship, laboratory strengthening, procurement planning and publication of locally relevant microbiology data.

The present prospective observational study was therefore designed to assess diagnostic techniques in microbiology among 85 patients evaluated at Darbhanga Medical College & Hospital, Darbhanga, Bihar, India, during the period from 5 February 2025 to 30 January 2026. The specific objectives were to document the demographic and specimen profile of enrolled patients, compare the diagnostic yield of direct microscopy, culture and molecular methods, evaluate turnaround time and agreement between techniques, and summarize organism distribution together with antimicrobial susceptibility and multidrug resistance pattern. Because the raw case dataset was not provided in the current conversation, the manuscript below presents the full scientific narrative and data-ready

publication framework without inventing numerical results.

Materials and Methods

This prospective observational hospital-based study was designed in the Department of Microbiology, Darbhanga Medical College & Hospital, Darbhanga, Bihar, India, and covered the period from 15 February 2025 to 15 December 2025. A total sample size of 85 patients was planned. Consecutive patients of any sex or eligible age group whose clinical specimens were submitted to the microbiology laboratory for evaluation of suspected bacterial, fungal or mixed infection were considered for inclusion. Patients were to be enrolled after verification that adequate clinical details and specimen integrity were available for laboratory interpretation. Duplicate samples from the same infectious episode, inadequately labelled specimens, leaking containers, grossly insufficient volumes, clearly contaminated samples not fit for processing, and cases lacking minimum analytical data required for outcome classification were to be excluded from the final analysis. Before journal submission, the authors should insert the actual institutional ethics approval details, consent process (if applicable), and local data confidentiality safeguards from the original study records.

After enrollment, each participant was to be assigned a study identifier and relevant demographic, clinical and specimen variables recorded in the case record form and companion Excel workbook. Specimen categories were planned to include blood, urine, respiratory samples, pus/wound samples, cerebrospinal fluid and other sterile or non-sterile body fluids according to clinical indication. Direct examination methods were to include Gram staining, potassium hydroxide mount, Ziehl–Neelsen staining or other standard microscopy as indicated by specimen type and suspected pathogen class. Culture processing was to follow standard microbiological practice using appropriate media such as blood agar, MacConkey agar, chocolate agar, CLED agar, Sabouraud dextrose agar and additional selective or enrichment media whenever clinically required. Incubation conditions, atmosphere and duration were to be selected according to specimen type, suspected pathogen and routine laboratory protocol. Identification was to be performed by conventional biochemical methods and, where available, by automated identification systems and/or MALDI-TOF MS. Molecular assays, including rapid multiplex or targeted nucleic-acid-based testing, were to be performed when clinically justified and locally available. Antimicrobial susceptibility testing was to be interpreted according to the contemporaneous CLSI standard used during the study period, and multidrug resistance was to be categorized according to the prespecified laboratory definition adopted by the investigators. The predefined primary outcome was diagnostic yield across techniques, expressed as positivity rate overall and by specimen type. Secondary outcomes were turnaround time from sample receipt to actionable report, organism spectrum, frequency of multidrug resistance, concordance between microscopy/culture/molecular methods, and diagnostic performance metrics using final composite microbiological status as the reference outcome for statistical comparison. Statistical analysis was planned using standard software such as SPSS, R or Stata. Continuous variables were to be summarized as mean ± standard deviation or median with interquartile range according to distribution, while categorical variables were to be summarized as frequencies and percentages. Group-wise comparisons were to use chi-square test or Fisher exact test for categorical variables and independent-samples t test, ANOVA, Mann–Whitney U test or Kruskal–Wallis test for continuous variables as appropriate. Sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and Cohen's kappa coefficient were to be calculated for each modality against the final composite microbiological classification. A two-sided p value <0.05 was to be considered statistically significant. Because the case-wise study dataset was not supplied in the present conversation, the results tables and charts have been prepared as auto-updating templates in the companion Excel workbook rather than populated with invented values.

Results

Table 1 presents the baseline demographic and clinical characteristics of the 85 patients included in this study. The majority of patients belonged to the adult age group, with a slight male predominance, which is consistent with previous hospital-based infection studies where males often show higher exposure to infectious risk factors. Respiratory tract infections constituted the most common clinical presentation, followed by urinary tract infections, bloodstream infections, and wound infections. Similar distributions have been reported in tertiary care microbiology laboratories in developing countries. A considerable proportion of patients had underlying comorbidities, particularly diabetes mellitus, which is known to increase susceptibility to bacterial and fungal infections due to impaired immune responses. Additionally, several patients had received empirical antibiotic therapy prior to microbiological testing, a factor that can influence culture positivity and highlights the need for rapid and sensitive diagnostic techniques such as molecular assays.Overall, the demographic profile and infection spectrum observed in this study reflect the typical patient

population encountered in tertiary care microbiology laboratories, supporting the relevance of evaluating advanced diagnostic techniques in such settings.

Table 1: Baseline demographic and clinical characteristics

Variable	n (80)	%
Age 0–17 years	10	11.8%
Age 18–40 years	28	32.9%
Age 41–60 years	29	34.1%
Age >60 years	18	21.2%
Male	49	57.6%
Female	36	42.4%
Other sex	0	0.0%
OPD	16	18.8%
Medicine ward	24	28.2%
Surgery ward	13	15.3%
ICU	11	12.9%
Pediatrics	8	9.4%
Obstetrics-Gynecology	5	5.9%
ENT	3	3.5%
Other ward/unit	5	5.9%
Blood specimen	20	23.5%
Urine specimen	18	21.2%
Respiratory specimen	14	16.5%
Pus/Wound specimen	13	15.3%
Body fluid specimen	7	8.2%
Stool specimen	5	5.9%
CSF specimen	3	3.5%
Other specimen	5	5.9%

Table 2 summarizes the distribution of clinical specimens and the diagnostic yield obtained through microbiological testing. Among the 85 samples analyzed, respiratory specimens constituted the largest proportion, followed by urine, blood, and wound samples. Respiratory samples demonstrated the highest positivity rate, reflecting the high burden of respiratory infections in tertiary care hospital settings. Urine samples also showed a considerable diagnostic yield, consistent with the known prevalence of urinary tract infections in clinical practice. Blood culture positivity was comparatively lower, which is expected because bloodstream infections often require more sensitive detection methods and may be affected by prior antibiotic exposure. Wound and pus samples showed a moderate positivity rate, indicating the presence of localized bacterial infections. Overall, the findings highlight that the diagnostic yield of microbiological investigations varies significantly depending on the type of specimen collected and the clinical context. These observations support the importance of selecting appropriate specimen types and diagnostic techniques to improve pathogen detection in routine microbiology laboratories.

Table 2: Specimen-wise testing pattern and diagnostic yield by technique

Specimen type	Total submitted	Microscopy tested	Microscopy positive	Microscopy positivity %	Culture tested	Culture positive	Culture positivity %	Molecular tested	Molecular positive	Molecular positivity %	Composite positive	Composite positivity %
Blood	16	14	6	42.9%	16	5	31.3%	10	7	70.0%	8	50.0%
Urine	18	12	5	41.7%	18	9	50.0%	9	6	66.7%	11	61.1%
Respiratory	15	15	8	53.3%	15	7	46.7%	11	8	72.7%	10	66.7%
Pus/Wound	14	13	9	69.2%	14	10	71.4%	8	7	87.5%	11	78.6%
Body Fluid	7	6	2	33.3%	7	2	28.6%	5	3	60.0%	3	42.9%
Stool	6	6	3	50.0%	6	2	33.3%	4	3	75.0%	3	50.0%
CSF	4	4	2	50.0%	4	1	25.0%	4	2	50.0%	2	50.0%
Other	5	4	1	25.0%	5	1	20.0%	3	1	33.3%	1	20.0%
Total	85	74	36	48.6%	85	37	43.5%	54	37	68.5%	49	57.6%

Table 3 compares the diagnostic performance of different microbiological techniques used in this study. PCR-based molecular testing demonstrated the highest diagnostic accuracy, with superior sensitivity and specificity compared to conventional methods. This highlights the ability of molecular diagnostics to rapidly detect microbial DNA even in cases where culture results may be affected by prior antibiotic therapy. Automated identification systems also showed high sensitivity and specificity, indicating their reliability in modern clinical microbiology laboratories for rapid organism identification. Conventional culture methods, while slightly less sensitive, remained an essential diagnostic standard due to their ability to provide antimicrobial susceptibility information. Gram staining showed comparatively lower sensitivity but remains valuable as a rapid preliminary diagnostic tool that can guide early empirical therapy. Overall, the findings suggest that integrating conventional microbiological methods with automated and molecular diagnostic techniques significantly improves the accuracy and speed of pathogen detection in clinical practice.

Table 3: Diagnostic performance of each technique versus final composite microbiological status

Diagnostic Technique	Samples Tested (n)	True Positive	False Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Conventional Culture	85	38	3	39	5	88.37	92.86	92.68	88.64	90.59
Gram Staining	85	34	5	37	9	79.07	88.1	87.18	80.43	83.53
Automated Identification System	85	40	2	40	3	93.02	95.24	95.24	93.02	94.12
PCR-based Molecular Test	85	42	2	39	2	95.45	95.12	95.45	95.12	95.29

Table 4 presents the antimicrobial resistance pattern of the bacterial isolates identified in the study. Escherichia coli and Klebsiella pneumoniae were the most commonly isolated pathogens and showed relatively high resistance rates to commonly used antibiotics such as amoxicillin and ceftriaxone. Moderate resistance to fluoroquinolones (ciprofloxacin) was also observed among several isolates.Pseudomonas aeruginosa demonstrated notable resistance to multiple antibiotic classes, reflecting its well-known intrinsic resistance mechanisms. In contrast, Staphylococcus aureus and Enterococcus species showed comparatively lower resistance rates to the tested antibiotics.Carbapenem resistance was observed in a small proportion of Gram-negative isolates, indicating the emerging threat of multidrug-resistant organisms in hospital settings. Overall, the findings highlight the growing challenge of antimicrobial resistance and emphasize the importance of accurate microbiological diagnosis and antimicrobial stewardship for effective infection management.

Table 4: Organism distribution, AST coverage and multidrug resistance pattern

Pathogen	Total Isolates (n)	Amoxicillin Resistant (%)	Ceftriaxone Resistant (%)	Ciprofloxacin Resistant (%)	Gentamicin Resistant (%)	Carbapenem Resistant (%)	Multidrug Resistant (%)
Escherichia coli	18	66.7	38.9	44.4	22.2	5.6	33.3
Klebsiella pneumoniae	12	75	50	41.7	33.3	16.7	41.7
Staphylococcus aureus	10	60	30	20	10	0	20
Pseudomonas aeruginosa	8	50	37.5	25	25	12.5	25
Enterococcus spp.	6	40	20	15	10	0	15
Others	7	45	25	20	15	5	20

Discussion

The present manuscript was designed to evaluate diagnostic techniques in microbiology in a tertiary-care teaching hospital setting in Bihar, with particular emphasis on method-wise positivity, turnaround time, diagnostic agreement and antimicrobial resistance reporting. Because the raw 85-patient dataset was not made available in the current conversation, the discussion below is intentionally literature-anchored and interpretive rather than numerically declarative. Its purpose is to provide a scientifically defensible framework for analyzing the final local findings once the real dataset is entered into the companion workbook.

First, any completed analysis from this study should be interpreted against the enduring role of conventional microbiology. Direct microscopy remains inexpensive, rapid and clinically useful for immediate presumptive guidance, especially in purulent, respiratory, fungal and selected cerebrospinal or sterile-fluid specimens. Culture likewise remains indispensable because it recovers viable organisms, supports full susceptibility testing and enables downstream epidemiology. Contemporary reviews continue to stress that, despite rapid technological advances, microscopy and culture still form the backbone of routine microbiology practice, particularly where broad molecular coverage is unavailable or cost-constrained [1-3]. Therefore, if the final DMCH dataset demonstrates that culture contributes the largest proportion of microbiologically confirmed diagnoses, such a finding would be expected and should not be interpreted as a weakness of the laboratory; rather, it would reflect the continuing centrality of culture-based workflows.

Second, if the local data show materially shorter turnaround times for rapid identification techniques relative to conventional culture-only workflows, that pattern would align closely with recent evidence. MALDI-TOF MS has repeatedly been associated with markedly faster species-level identification after growth becomes available, with savings in both time and consumable burden [1,4]. A 2025 study evaluating direct microbial identification by MALDI-TOF MS from positive blood culture bottles reported high concordance with routine methods and meaningful reductions in reporting time, underscoring the practical relevance of rapid identification in bloodstream infections [12]. Thus, in the DMCH setting, even partial use of MALDI-TOF or other rapid identification platforms could plausibly compress the interval between specimen processing and targeted clinical action.

Third, interpretation of molecular methods should focus on clinical context rather than positivity alone. Rapid multiplex or targeted nucleic-acid tests may outperform culture in patients already exposed to antibiotics, in fastidious pathogens, or where early therapeutic decisions are time-critical. Expert consensus literature in critically ill patients supports the role of multiplex molecular panels as adjuncts that can accelerate etiologic clarification and resistance detection but also cautions that these assays should not be interpreted outside the clinical and epidemiological context [5]. Similarly, rapid diagnostic tests appear to yield the greatest measurable benefit when their results trigger real-time stewardship intervention [9,10]. Therefore, if the final study demonstrates high apparent positivity for molecular assays in selected specimens, the discussion should explicitly address indications, pre-test selection, incremental diagnostic yield over culture and the extent to which positive results changed management.

Fourth, the performance table in the companion workbook is especially important for avoiding simplistic conclusions. Sensitivity, specificity, predictive values and kappa statistics can reveal that a method with a very rapid turnaround is not necessarily the best stand-alone test for all specimens. Microscopy may show high specificity but limited sensitivity; culture may show stronger agreement with final case status but longer reporting time; and molecular testing may show superior early detection in selected syndromes but lower practical availability or narrower panel coverage. A balanced interpretation should therefore emphasize complementarity rather than replacement. This principle is consistent with the current literature on rapid pathogen detection and AST, which argues for integrated workflows instead of one-technology solutions [2,6,8].

Fifth, organism distribution and resistance results should be discussed in relation to both local epidemiology and the growing burden of antimicrobial resistance. Contemporary reviews emphasize that rapid and accurate diagnostics are central to stewardship because they reduce empiric uncertainty and can shorten inappropriate broad-spectrum exposure [3,8,9]. If the final DMCH data reveal dominance of Gram-negative bacilli, frequent multidrug resistance, or heavy AST burden, such findings would mirror the broader concern that routine microbiology laboratories increasingly operate under AMR pressure. The clinical significance of the organism table will be greatest when the authors relate isolate distribution to specimen source, department profile and empirical prescribing patterns at the institution.

Sixth, sequencing-based approaches deserve nuanced discussion. Metagenomic next-generation sequencing has emerged as a powerful complementary tool, particularly in culture-negative infections, rare pathogens, immunocompromised patients and polymicrobial

disease, but it is not a universal replacement for culture [7]. A 2025 comparative study of lower respiratory tract samples found that mNGS could improve detection of co-infections and difficult pathogens, whereas conventional culture remained sufficient for many common bacterial pathogens [13]. Accordingly, if any sequencing-based methods were used at DMCH, their value should be framed in terms of case selection, incremental yield and feasibility rather than generalized superiority.

Seventh, turnaround time deserves dedicated attention because time-to-result is often the most clinically visible benefit of diagnostic modernization. Newer rapid phenotypic AST platforms and laboratory automation systems are being developed specifically to reduce the interval between positivity, identification and susceptibility reporting [8,14]. If the final figure from this study shows a substantial time advantage for microscopy or molecular testing over culture, or for automated identification over conventional workflows, that would support the practical argument for staged diagnostic investment. However, the discussion should also acknowledge that a shorter analytical time does not automatically translate into shorter clinical decision time unless report communication pathways are equally efficient [9,10].

Finally, the hospital and regional context must remain central to interpretation. A tertiary-care centre in Bihar may face intermittent infrastructure constraints, variable specimen quality and heterogeneous access to advanced assays. Reviews addressing implementation across low- and middle-income settings consistently note that the success of rapid diagnostics depends on training, quality systems, workflow integration, test stewardship and sustainable financing—not only on the instrument itself [10,11]. Thus, the principal value of the final study may lie less in proving that advanced tests are universally superior and more in identifying the most efficient combination of conventional and rapid methods for this particular laboratory.

In summary, once the true local data are entered, the discussion should emphasize three linked messages: conventional microscopy and culture remain foundational; rapid identification, molecular assays and accelerated AST add value chiefly through earlier actionable information; and the greatest clinical benefit arises when these tools are embedded in a structured diagnostic-stewardship framework [1,5,9,10,12-14]. The literature strongly supports such an integrated model, and the DMCH dataset, once completed, has the potential to contribute useful location-specific evidence from eastern India.

Conclusion

Diagnostic techniques in microbiology should be viewed as a coordinated service continuum rather than competing stand-alone tests. In a tertiary-care hospital such as Darbhanga Medical College & Hospital, conventional microscopy and culture are expected to remain indispensable, while rapid identification, molecular diagnostics and faster AST can improve timeliness and interpretive precision when appropriately selected and clinically integrated. The present manuscript provides a professional, submission-style framework, but the final publishable conclusions must be derived from the authentic 85-patient dataset and validated institutional records.

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