ORIGINAL ARTICLE

Cross-sectional Study: Diagnostic Accuracy of Next-generation Sequencing in a Tertiary Care Intensive Care Unit

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Abstract

Background and aims: Infectious diseases are a major cause of intensive care unit (ICU) mortality, where rapid pathogen identification is crucial. Traditional culture methods are slow and may miss fastidious organisms. Next-generation sequencing (NGS) offers rapid, comprehensive pathogen detection. This study assessed NGS accuracy compared to culture in a tertiary care ICU in India.

Patients and methods: A retrospective observational analysis of 187 ICU patients with suspected infections was conducted with IRB approval. Paired samples from blood, urine, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), and other body fluids underwent NGS and culture testing. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using culture as the reference. Concordance was also assessed.

Results: Next-generation sequencing demonstrated a sensitivity of 75%, specificity of 59.6%, PPV of 62.23%, and NPV of 72.84%. It detected pathogens in 56.68% of cases vs 47.06% by culture, identifying 17 atypical organisms in culture-negative cases. Sensitivity was highest in CSF (100%) and BALF (87.5%), while specificity was highest in pleural fluid (100%) and blood (87.5%). Overall concordance was 57.2%.

Conclusion: Next-generation sequencing has improved pathogen detection, identifying organisms missed by culture. High sensitivity across sample types suggests its value in ICU diagnostics. However, lower specificity, high cost, and standardization challenges limit standalone use.

Clinical significance: Next-generation sequencing facilitates an earlier ICU infection diagnosis, allowing for prompt targeted treatment and potentially reducing antimicrobial resistance. However, false positives and cost remain barriers. Combining NGS with conventional culture techniques could improve diagnostic accuracy and patient outcomes in the right subset of patients.

Keywords: Culture, Infectious diseases, Intensive care unit, Next-generation sequencing, Pathogen detection.

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Introduction

Infectious diseases are a leading cause of mortality globally, especially in low-income nations. In India, they are a major contributor to intensive care unit (ICU) admissions, with a notable prevalence of multidrug-resistant organisms that complicate the treatment of critically ill patients. For ICU patients with infections, the causative agents may include a wide range of common and rare pathogens, spanning viruses, bacteria, fungi, and parasites.[1,2]

The standard of care for identifying these pathogens is the culture-based diagnostic procedure, which is easily accessible and economical for the patient. However, many pathogens are difficult to culture, especially fastidious or slow-growing organisms and it is a time-consuming process.[2–4] The turnover time is at least 3 days for bacteria, 7 days for fungi, and 45 days for mycobacteria.[5] Moreover, the sensitivity of the culture is restricted by the use of empirical antibiotics in clinical practice, leading to a high number of false negative results.[4,6] Inability to detect causative organisms early in the clinical course can hinder precise antimicrobial treatment and lead to inadequate and overuse of broad-spectrum antibiotics, contributing to antimicrobial resistance, and increasing expenditure on healthcare.[7,8]

Next-generation sequencing (NGS) is a promising, unbiased diagnostic tool for detecting a comprehensive spectrum of pathogens in clinical samples, suitable especially for atypical etiologies of complicated infections.[7,9]

It showcases the benefits of rapid, user-friendly analysis and detection independent of culture methods.[6] One of the most significant benefits of NGS is its capability to provide a complete picture of the patient's microbiome of a specific sample, allowing for the detection of coinfections.[10] Despite its various advantages, the clinical application of NGS has lagged behind the research, likely due to high costs, complicated operation, and the lack of uniform standards for experimental procedures.[6,11]

In India, limited research has been conducted to compare NGS with culture-based diagnostic methods for identifying pathogens in various clinical samples. Furthermore, there is a scarcity of studies evaluating the use of NGS to guide organism-specific antibiotic treatments, particularly within the adult population.[12–15] In this study, to provide further evidence for the clinical application of NGS, 187 patients admitted to a tertiary care ICU with infectious

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etiology were analyzed to compare the sensitivity and specificity of NGS and culture in identifying pathogens.

Patients and Methods

We conducted a retrospective, cross-sectional observational pilot study in a tertiary care ICU, with a sample size of 187 patients from March 2024 to August 2024.

Institutional Review Board (IRB) approval was obtained from the Institutional Ethics Committee (Academic) of K.J. Somaiya Medical College & Hospital.

The inclusion criteria were as follows: (1) age ≥ 12 years, (2) visit time from March 2024 to August 2024, and (3) admitted with infectious etiology. The exclusion criteria were as follows: (1) incomplete clinical data, including microbiological data and (2) failure to acquire a sufficient sample for NGS analysis.

All 187 samples were subjected to culture media and NGS in a pairwise manner and were included in the final analysis.

20–30 mL of blood was typically drawn, with 10–15 mL per bottle, and inoculated into aerobic blood culture bottles. Blood samples were collected in pairs from both the central venous catheter (CVC) and a peripheral vein. If a central line was not present, two peripheral venous samples were taken. The bottles were promptly placed in an automated blood culture system for continuous incubation and monitoring. When the system flagged a sample as positive, the specimen was sub-cultured onto suitable solid media for pathogen isolation. Isolate identification and antimicrobial susceptibility testing (AST) were carried out using an automated identification and susceptibility platform, adhering to established protocols.

Cerebrospinal fluid (CSF) and other body fluid samples were cultured on conventional media, including blood agar, chocolate agar, and MacConkey agar, to encourage the growth of potential pathogens. Once growth was observed, organism identification and antibiotic susceptibility testing were performed using the automated VITEK 2 system.

Urine, Endotracheal (ET), or Tracheostomy Tube (TT) Secretions samples were cultured on conventional media using a calibrated loop (0.1 µL) to determine colony countS. Identification and antibiotic susceptibility testing (AST) were conducted using the automated VITEK 2 system.

Next-generation sequencing was performed using an amplicon-based long-read sequencing assay capable of simultaneously detecting bacteria, fungi, and clinically relevant antibiotic resistance genes (ARGs). Initial DNA extraction from clinical samples was conducted using a standard commercial kit for sterile body fluids, following the manufacturer's instructions. The NGS assay involved multiplex amplification of the bacterial 16S rRNA gene, the fungal internal transcribed spacer (ITS) region, and ARGs, followed by library preparation and sequencing on a nanopore-based long-read sequencing platform.Data analysis was conducted using proprietary automated sequence analysis and clinical report generation software. Only quality-filtered reads obtained after initial quality assessment were used for final pathogen and ARG identification.[16,17]

The sample size was calculated with due consideration of previous studies. Sensitivity and specificity were calculated with respect to culture for overall samples, and sub-sample analysis was also performed. Positive and negative predictive values were also calculated for the same samples.

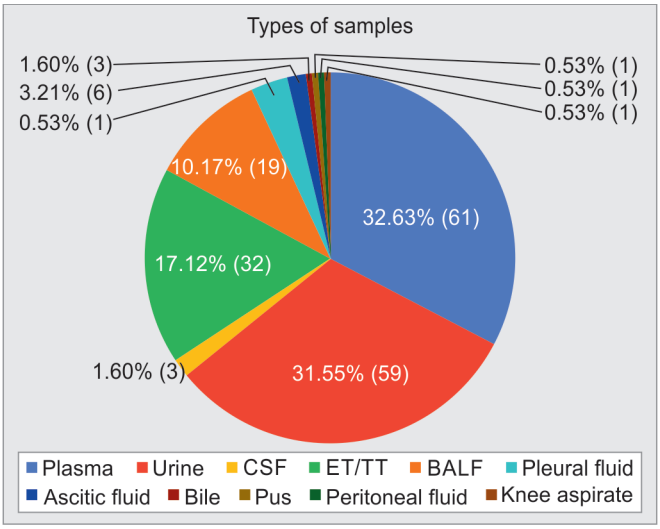

Fig. 1: Sample type distribution in the study population ($N = 187$). This pie chart represents the distribution of different sample types analyzed in the study, including plasma, urine, BALF, Endotracheal/tracheal secretions(ET/TT), CSF, and other body fluids (pleural fluid, ascitic fluid, bile, pus, peritoneal fluid and knee aspirate)

The concordance rate measures the agreement between two diagnostic methods in detecting the same outcome, expressed as a percentage. It is calculated by summing the matched positive results (both tests detect the same organism) and matched negative results (both tests yield negative findings), dividing by the total number of cases, and multiplying by 100.

Results

Based on the inclusion and exclusion criteria, 187 patients were included in the study. There were 116 males and 71 females, with a male-to-female ratio of 1.6. The mean age of the patients was 57.2 (range: 14–89 years).

Although endotracheal (ET) and tracheal tube (TT) secretions often contain numerous commensals, we included these samples and assessed them within the clinical context. Organisms identified by NGS were considered pathogenic if their presence aligned with the patient's clinical presentation.

All samples were subjected to both culture and NGS in a pairwise manner (Fig. 1).

Using culture as the reference standard, the sensitivity of NGS was 75%, with a specificity of 59.6%. The positive predictive value (PPV) of NGS was 62.23%, while the negative predictive value (NPV) was 72.84%.

Among the 187 samples analyzed, culture detected organisms in 88 samples, achieved a yield of 47.06%. In comparison, NGS detected organisms in 106 samples, yielding 56.68%. Of the total samples, only NGS was positive in 40 cases (21.4%), only culture was positive in 22 cases (11.8%), while both culture and NGS were negative in 59 samples (31.6%) and positive in 66 samples (35.3%).

Among the 66 double-positive samples, 48 were matched (concordant), detecting one or more matching organisms, while 18 were mismatched (discordant). Therefore, NGS and culture were concordant for 107/187 samples (59 double negative samples + 48 samples matched for organisms), resulting in an overall concordance rate of 57.2%. The following organisms were identified

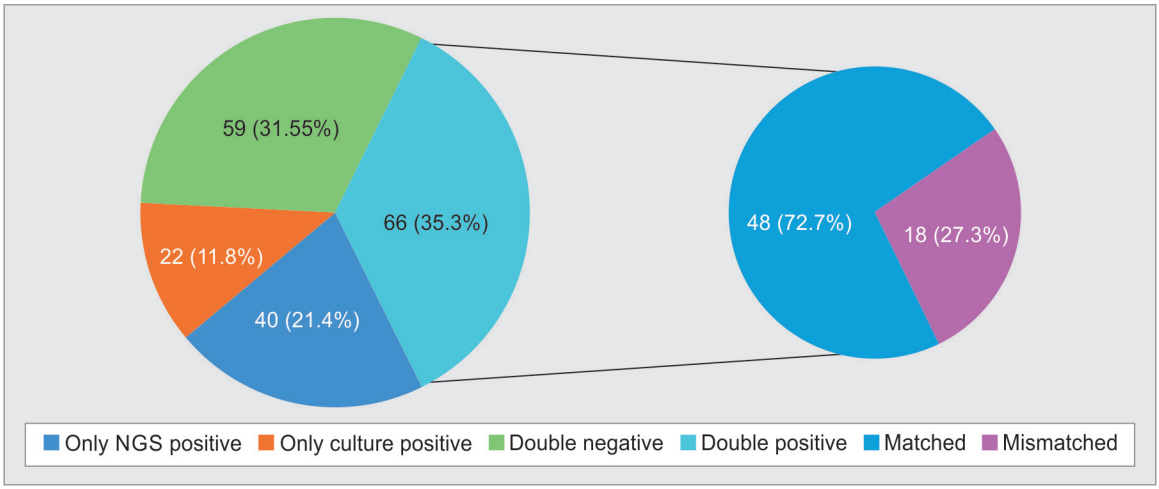

Fig. 2: Concordance between next-generation sequencing (NGS) and culture. This figure illustrates the concordance between NGS and culture for pathogen detection. The left pie chart categorizes results into double positive (both NGS and culture positive), double negative (both negative), NGS positive only, and culture positive only. The right pie chart splits double positive into the proportion of matched (identical pathogen detection by both methods) and mismatched results

by both NGS and culture from the same clinical samples, indicating overlap in detection: *Klebsiella pneumoniae* (10), *Acinetobacter baumannii* (8), *Candida albicans* (8), *Pseudomonas aeruginosa* (6), *Escherichia coli* (4), *Enterococcus faecalis/faecium* (4), *Providencia stuartii* (2), *Staphylococcus aureus* (2), *Stenotrophomonas maltophilia* (2), *Burkholderia cepacia* (1), and unidentified yeast species (1).

Next-generation sequencing has a significant advantage in detecting atypical organisms that are often missed by culture methods.[5,7] Our study supported this finding, with NGS identifying 17 atypical organisms that were not detected by culture. including *Abiotrophia defectiva*, *Veillonella* spp., *Prevotella* spp., *Achromobacter xylosoxidans*, *Delftia tsuruhatensis*, and *Stenotrophomonas maltophilia* (Fig. 2).

Based on Table 1, the highest sensitivity relative to culture was observed in cerebrospinal fluid (CSF) samples (100%, $n = 3$), followed by bronchoalveolar lavage (BALF) samples (87.5%, $n = 19$). Urine samples demonstrated a sensitivity of 83.87% ($n = 59$), and endotracheal/tracheal (ET/TT) samples showed 80.95% ($n = 32$). Lower sensitivities were recorded for ascitic fluid (66.67%, $n = 5$), plasma (52.38%, $n = 61$), and pleural fluid (50%, $n = 6$).

Specificity results varied significantly. Pleural fluid samples exhibited the highest specificity (100%), followed by plasma samples (87.5%). Cerebrospinal fluid and ascitic fluid samples had a specificity of 50% each, while urine, BALF, and ET/TT samples showed specificities of 35.71%, 36.36%, and 27.77%, respectively.

Discussion

In this study involving 187 ICU patients with suspected infections, NGS demonstrated superior diagnostic performance compared to culture. NGS achieved a sensitivity of 75% and a pathogen detection rate of 56.68%, whereas culture identified pathogens in 47.06% of cases. The specificity of NGS was 59.6%, with a PPV of 62.23% and a NPV of 72.84%. The overall concordance between NGS and culture was 57.2%. Notably, NGS uniquely identified 17 atypical organisms that were missed by culture. When analyzed by sample type, the highest sensitivity was observed in CSF samples (100%), followed by BALF (87.5%), urine (83.87%), and ET/TT secretions (80.95%). In terms of specificity, pleural fluid (100%) and plasma (87.5%) samples showed the best performance.

Numerous studies have demonstrated the successful application of NGS for pathogen detection in various specimens, including plasma, CSF, urine, respiratory secretions, BALF, and other body fluids.[3,7,13,18–24]

In India, prior studies on infectious etiology have primarily focused on evaluating the efficacy of NGS in detecting specific organisms or have concentrated on detecting organisms from only one sample type. Furthermore, the majority of these studies have been conducted in pediatric populations, with relatively few focusing on adult populations.[13,14,25]

Ramanathan et al.[12] conducted a large-scale study ($n = 490$) that compared NGS and culture results for infectious etiology across various sample types. However, this study did not include ET/TT samples as a sample type.

Our study is among the first cross-sectional studies in India to compare NGS and culture results across a diverse array of samples collected from patients admitted with infectious etiology. Notably, this study includes ET/TT samples for analysis and comparison – a feature uncommon in previous studies. We assessed the diagnostic accuracy of NGS relative to culture (the gold standard) across various sample types, including plasma, urine, BALF, ET/TT, CSF, and body fluid samples (such as ascitic fluid, pleural fluid, peritoneal fluid, bile, knee aspirate, and pus) from 187 patients with infectious etiology.

Taking culture as the reference standard, the sensitivity of NGS was 75%, the specificity was 59.60%, the PPV was 62.23%, and NPV was 72.84%. Our study revealed higher sensitivity and NPV when compared to previous studies,[6,7] whereas the specificity and PPV were lower than those reported by other research teams.[6,7] The difference in the results could be attributed to the following reasons: (1) smaller sample size, (2) an alternative approach to calculating the results of the statistical analysis, and (3) different standardization methods and criteria for positive NGS results.

Ramanathan et al.[12] analyzed a total of 490 samples, reporting a sensitivity of 94.1% and a specificity of 86.6%. These values were

Table 1: NGS vs. culture: sensitivity and specificity by sample type. This table compares NGS and culture-based pathogen detection across ICU sample types

		Culture		*Results*	
Sample type	*NGS result*	*Culture +*	*Culture –*	*Sensitivity*	*Specificity*
Overall	NGS+	66	40	75	59.6
	NGS–	22	59		
Plasma	NGS+	11	5	52.38	87.5
	NGS–	10	35		
Urine	NGS+	26	18	83.87	35.71
	NGS–	5	10		
ET/TT	NGS+	17	8	80.95	27.27
	NGS–	4	3		
BALF	NGS+	7	7	87.5	36.36
	NGS–	1	4		
Pleural fluid	NGS+	1	0	50	100
	NGS–	1	4		
Ascitic fluid	NGS+	2	1	66.67	50
	NGS–	1	1		
CSF	NGS+	1	1	100	50
	NGS–	0	1		

Next-generation sequencing (NGS) result: Detection outcome (NGS +: Positive, NGS –: Negative). Culture: Reference standard (Culture +: Pathogen detected, Culture –: No pathogen detected). BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; ET/TT, endotracheal/tracheostomy tube secretions; NGS, next-generation sequencing. The pus, bile, peritoneal fluid, and knee aspirate analyses were excluded from this table as each had only one sample, limiting statistical relevance.

higher compared to our study, likely due to the larger sample size in their analysis.

Our study compared NGS and culture results across different sample types. The highest sensitivity relative to culture was observed in CSF) samples (100%, $n = 3$), followed by BALF samples (87.5%, $n = 19$). Urine samples (83.87%, $n = 59$) and ET/TT samples (80.95%, $n = 32$) also showed high sensitivity. Lower sensitivities were noted in ascitic fluid (66.67%, $n = 5$), plasma (52.38%, $n = 61$), and pleural fluid (50%, $n = 6$).

Specificity results varied, with pleural fluid samples showing the highest specificity (100%), followed by plasma samples (87.5%). CSF and ascitic fluid samples each had specificities of 50%, while urine, BALF, and ET/TT samples showed specificities of 35.71%, 36.36%, and 27.77%, respectively.

Compared with previous studies, our study showed higher or comparable sensitivities in most sample types, except for ascitic fluid, where the sensitivity was lower, possibly due to the smaller sample size.[6,19,20,22–24,26]

Specificities for urine, ET/TT, ascitic fluid, and CSF were lower than the other studies, likely due to a higher rate of false positives from commensal or atypical organisms. Conversely, plasma, BALF, and pleural fluid samples showed higher specificity in our study compared to others.[6,19,20,22–24,26] Despite these variations, NGS proved to be a valuable tool, successfully detecting atypical organisms across sample types, including those that traditional culture methods often fail to identify.

The concordance rate (matched double positive + double negative) in our study was 57.2% (107/187) which was higher than or comparable to that observed in previous studies.[5,6]

Next-generation sequencing offers powerful capabilities for diagnostics but faces challenges, including complex data interpretation, high costs, and accessibility limitations, especially in resource-limited settings. While faster than some traditional methods, its high sensitivity can lead to false positives. Next-generation sequencing depends on comprehensive databases, skilled personnel, and quality control standards that are still evolving. Additionally, DNA from the patient's cells or past infections can interfere with detecting the actual infection. Despite these issues, improvements in technology and data analysis are making NGS increasingly useful for clinical diagnosis.[5,27,28]

In this study, we not only evaluated the overall diagnosis ability of NGS but also conducted a subgroup analysis of different pathogens and samples to provide further insights into the clinical application of NGS. That said, the study does have certain limitations, including the following: only the detection of bacteria and fungi was taken into consideration. The external validity of this study is limited, and a larger sample size would be needed to apply these findings to real-world situations. We did not account for the impact of antibiotic use before admission on culture results, which may lead to an underestimation of culture sensitivity and an overestimation of the sensitivity difference between culture and NGS. Lastly, this study did not evaluate the clinical relevance of the NGS results or assess whether early findings from NGS influenced treatment decisions. This aspect is being explored in an ongoing study.

Conclusion

Next-generation sequencing offers a comprehensive and rapid method for pathogen identification, detecting both bacteria and fungi from a single sample. This contrasts with culture, which often requires separate samples for different organisms. The quicker turnaround time of NGS is particularly beneficial in critical care settings, where timely initiation of appropriate antimicrobial

treatment is essential. Next-generation sequencing holds great potential for enhancing pathogen detection and supporting targeted antimicrobial therapy in selected subgroups of patients, potentially improving patient outcomes. Further research is needed to refine NGS protocols and assess its clinical impact in critically ill patients.

Clinical Significance

This study highlights NGS as a more sensitive (75%) but less specific (59.6%) tool than culture for pathogen detection in ICU infections. It helps in identifying atypical and unculturable organisms, potentially enabling prompt targeted treatment and reducing antimicrobial resistance. However, high costs, false positives, and standardization issues limit its standalone clinical use. Integrating NGS with traditional diagnostics could improve accuracy and patient outcomes in selective subgroups of patients.

Institutional Ethics Committee Clearance

- The Institutional Ethics Committee (Academic) approved the proposal on 09/12/2024.
- IEC Ref. no.: Dec/2024/2
- NECRBHR/DHR IEC Reg no.: EC/NEW/INST/2024/MH/0505

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