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ORIGINAL ARTICLE

Multiplex PCR for lower respiratory tract infection diagnosis in ICU and non-ICU settings: enhancing diagnostic stewardship in Indian tertiary care

Rajender Singh[1*], Barnali Kakati[2]

[1]Department of Microbiology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, India
[2]Department of Microbiology, All India Institute of Medical Sciences, Rishikesh, India

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ABSTRACT

Background and Objectives: Lower respiratory tract infections (LRTIs) are a leading cause of morbidity and mortality in both ICU and non-ICU patients. Timely identification of causative pathogens and antimicrobial resistance (AMR) genes is critical for optimizing therapy. This study evaluated the diagnostic performance of the BioFire FilmArray Pneumonia Panel (BFPP), a multiplex PCR assay, for pathogen and AMR detection in LRTI cases at a tertiary care hospital in India.

Materials and Methods: A retrospective study was conducted over 29 months (October 2022–February 2025) on 251 respiratory specimens from clinically suspected LRTIs. BFPP was performed for bacterial, viral, and AMR gene detection, with results compared to conventional aerobic bacterial culture. Diagnostic yield, co-infection rates, and concordance were assessed using kappa statistics.

Results: BFPP detected at least one pathogen in 81.7% of samples versus 44.2% by culture, with 55.8% showing polymicrobial infections. Sensitivity was 94.6%, with moderate agreement with culture (κ = 0.428). Among 251 cases, predominant bacteria included *Acinetobacter baumannii* (29.5%), *Klebsiella pneumoniae* (24.7%), and *Pseudomonas aeruginosa* (19.9%).

Major viral agents were human rhinovirus/enterovirus (15.1%) and influenza A virus (13.1%). Among 174 AMR-positive cases, $bla_{\text{CTX-M}}$ (59.2%), $bla_{\text{-NDM}}$ (56.9%), and $bla_{\text{OXA-48}}$ (46.6%) were the most frequently detected resistance genes.

Conclusion: The BioFire FilmArray Pneumonia Panel (BFPP) demonstrated high diagnostic sensitivity of 94.6% and detected pathogens in 81.7% of suspected LRTI cases, compared to 44.2% positivity by conventional culture. BFPP identified 69 additional pathogens missed by culture, enabling earlier targeted therapy and improved diagnostic stewardship in ICU and non-ICU settings.

Keywords: Multiplex polymerase chain reaction; Lower respiratory tract infection; Antimicrobial resistance; Diagnostic stewardship; BioFire film array

INTRODUCTION

Lower respiratory tract infections (LRTIs) continue to be one of the most significant global health concerns, especially in low- and middle-income countries such as those in South Asia. (1). The Global Burden of Disease (GBD) 2019 report highlighted that India experiences an exceptionally high burden of LRTIs, with more than 2.7 million cases and over 600,000 deaths each year, ranking among the lead-

*Corresponding author: Rajender Singh, MBBS, MD, Department of Microbiology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, India. Tel: +91-9997358899 Email: panwar.rajendra@gmail.com

ing infectious causes of death in both children and adults (1). A community-based cohort study in rural north India reported an LRTI incidence of 248 per 1,000 person-years and LRTI-associated hospitalization rates of 12.7 per 1,000 person-years among older adults, underscoring the impact in vulnerable adult populations (2). Similarly, population-based surveillance in Melghat, Maharashtra, demonstrated a high burden of severe RSV-associated LRTI in infants (<12 months), with community incidence of 22.4 per 1,000 child-years and hospitalization incidence of 14.1 per 1,000 child-years (3). These findings highlight the significant disease burden in both pediatric and adult populations in India, consistent with broader South Asian patterns. Accurate and early pathogen detection is essential for guiding antimicrobial therapy and improving outcomes. Although culture remains the traditional standard, it suffers from long turnaround times and reduced sensitivity, especially when patients have already received antibiotics.

The BioFire FilmArray Pneumonia Panel (BFPP) offers a rapid and comprehensive diagnostic alternative by detecting bacterial and viral pathogens along with antimicrobial resistance (AMR) genes directly from respiratory specimens. Previous studies have demonstrated that BFPP can significantly reduce the time to pathogen detection, enhance antimicrobial stewardship, and improve patient management in both pediatric and adult populations (4, 5). The panel's multiplex PCR-based approach enables simultaneous detection of bacterial and viral co-infections, which are common in LRTI patients and are often challenging to diagnose using standard microbiological methods (6, 7).

This study aims to evaluate the distribution of pathogens detected by BFPP among LRTI patients in ICU and non-ICU settings. Additionally, we assess the frequency of viral-bacterial and bacterial-bacterial co-infections and AMR gene detection patterns to determine their implications for patient management and antimicrobial stewardship.

MATERIALS AND METHODS

Study design and population. This retrospective study analyzed respiratory samples from 251 consecutive patients meeting the following criteria: inclusion—all age groups with clinically suspected LRTI admitted to ICU or non-ICU units during October 2022 to February 2025, with appropriate respiratory specimens collected and available for both multiplex PCR and conventional culture; exclusion—cases with purely upper respiratory or non-pulmonary infections, duplicate samples from the same episode, or incomplete clinical/sample data. Patient demographic details, pathogen distribution, and AMR gene detection were evaluated as well.

Sample collection and processing. Samples such as endotracheal and tracheal aspirates, bronchoalveolar lavage (BAL), mini-BAL, and sputum were collected aseptically from patients who were clinically suspected to have LRTIs. All samples were transported promptly to the microbiology laboratory for simultaneous processing by both multiplex PCR-based BioFire FilmArray Pneumonia Panel (BFPP) and conventional culture methods for aerobic bacteria only.

Molecular testing was performed with the BioFire FilmArray Pneumonia Panel (bioMérieux, USA), following the manufacturer's instructions. This automated multiplex PCR assay can identify 33 bacterial, viral, and resistance gene targets directly from respiratory samples without prior processing. Each test utilized a closed single-use pouch containing all required reagents. Approximately 200 μL of the specimen was loaded into the sample injection port, and the system performed nucleic acid extraction, nested multiplex PCR amplification, and real-time detection. Results were obtained within 60 minutes.

For conventional culture, an aliquot of each respiratory specimen was inoculated onto 5% sheep blood agar and MacConkey agar, followed by incubation at 35-37°C for 18-48 hours under aerobic conditions. Bacterial growth was assessed by colony morphology, and isolates were identified with antimicrobial susceptibility testing (AST) using the VITEK-2 Compact System (bioMérieux, France), interpreted according to CLSI 23rd standards. Susceptibility profiles were interpreted to determine phenotypic resistance patterns.

Patients with clinically and radiologically suspected LRTI were included, and their respiratory samples were tested using both BFPP and conventional aerobic culture. Results were correlated with clinical and radiological findings to ensure consistency in culture-positive and culture-negative cases. Agreement between methods was assessed using kappa (κ) statistics, with discordant results further analyzed for diagnostic yield.

Pathogen detection and AMR analysis. The prevalence of single bacterial and viral infections, bacterial-bacterial co-infections, and viral-bacterial co-infections was determined.

The detection rates of AMR genes, including bla_{CTX-M}, bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla-$_{VIM}$, and *mecA/C*, were assessed among bacterial mono-infections and co-infections.

Data analysis. Descriptive statistics were used to summarize patient characteristics, pathogen distribution, and AMR gene detection. Chi-square and Fisher's exact tests were used to compare categorical variables, while kappa statistics were applied to determine the agreement between BFPP and culture-based detection methods. A p-value of <0.05 was considered statistically significant.

This study was approved by the Institutional Ethics Committee of the Himalayan Institute of Medical Sciences (HIMS), Swami Rama Himalayan University (SRHU), under the ethics approval number SRHU/HIMS/RC/2025/131, dated 21/04/2025. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional review board and the Declaration of Helsinki.

RESULTS

Patient demographics and sample type distribution. The study population comprised 251 patients, with a slight male predominance (n=160, 63.7%). The mean age was 56.0 ± 14.6 years. Elderly patients (≥60 years) constituted the largest age group (51.8%), followed by adults (38.6%) according to WHO age classification criteria. Among the respiratory specimens evaluated, sputum specimens showed the highest detection rate (88.5%), followed by endotracheal/tracheal aspirates (84.3%) and BAL/mini-BAL samples (74.7%) (Table 1).

Pathogen distribution and co-infection patterns. BFPP detected pathogens in 205 (81.7%) of the 251 cases, while 46 (18.3%) patients showed no detectable pathogen. Among pathogen-positive cases, single viral infections were identified in 28 (11.2%) patients, and single bacterial infections in 37 (14.7%) patients. Viral–bacterial co-infections were the most frequent mixed pattern, occurring in 85 (33.8%) cases, followed by bacterial–bacterial co-infections in 55 (21.9%) cases (Table 2). The predominant bacterial pathogens detected were *A. baumannii* (29.5%), *K. pneumoniae* (24.7%), and *P. aeruginosa* (19.9%). Among viral pathogens, Human Rhinovirus/Enterovirus (15.1%) and Influenza A virus (13.1%) were predominant (Fig. 1). There was no statistically significant difference in pathogen distribution between ICU and ward patients.

Antimicrobial resistance gene detection. Among the 174 bacterial-positive cases, AMR genes were detected in 138 (79.3%). The most prevalent determinants were bla_{CTX-M} (59.2%), bla_{NDM} (56.9%), and bla_{OXA-48} (46.6%), followed by bla_{KPC} (21.8%), bla_{VIM} (14.9%), *mecA/C + MREJ* (8.6%), and bla_{IMP} (5.2%). The distribution of AMR genes did not differ significantly between viral–bacterial co-infections and bacterial mono/co-infections (all $p > 0.05$, Fisher's exact test) (Table 3).

Comparison of BFPP and culture-based methods. BFPP showed moderate agreement with bacteriological culture (κ=0.428). Among culture-positive cases, BFPP detected bacterial pathogens in 94.6%, while additional pathogens were identified in 69 culture-negative cases. Sensitivity was 94.6%, specificity 50.7%, PPV 60.3%, and NPV 92.2% (Table 4).

DISCUSSION

This study evaluated the diagnostic performance of the BioFire® FilmArray® Pneumonia Panel (BFPP) in patients with clinically suspected lower respiratory tract infections (LRTIs) based on compatible clinical and radiological features, with specimens subsequently processed by both multiplex PCR and conventional culture. It is important to clarify that the "sensitivity" values reported here are relative to culture positivity and reflect comparative diagnostic yield, not sensitivity against clinically confirmed infection. Among 251 patients, BFPP detected pathogens in 205 (81.7%), including 105 of 111 culture-positive cases (94.6%), whereas culture alone detected pathogens in 111 cases (44.2%). Comparable detection rates were observed in ward (81.5%) and ICU (81.8%) patients, underscoring the panel's utility across care levels. Similar high detection rates have been reported in prior studies, including Wu and Ling (94%) (7) and Kamel et al. (100%) (8), largely in ICU cohorts, while our findings extend this performance to ward settings.

Table 1. Socio-demographic and clinical characteristics of patients with clinically suspected LRTI (n=251)

Category	Subcategory	Ward (n=119)	ICU (n=132)	p-value
Gender	Male (n=160)	76 (63.9%)	84 (63.6%)	0.96
	Female (n=91)	43 (36.1%)	48 (36.4%)	
Mean age (yrs)	56.0 ± 14.6	53.6 ± 13.8	58.2 ± 15.1	0.07
Age group	Neonates (<1 mo, n=6)	4 (3.4%)	2 (1.5%)	0.21#
	Infants (1–12 mo, n=7)	1 (0.8%)	6 (4.5%)	
	Children (1–12 y, n=5)	2 (1.7%)	3 (2.3%)	
	Adolescents (13–19 y, n=6)	1 (0.8%)	5 (3.8%)	
	Adults (20–59 y, n=97)	44 (37.0%)	53 (40.2%)	
	Elderly (≥60 y, n=130)	67 (56.3%)	63 (47.7%)	
Sample type	ET/TT (n=108)	91 (84.3%)		
	BAL/mini-BAL (n=91)	68 (74.7%)		
	Sputum (n=52)	46 (88.5%)		

Fisher exact test applied

Table 2. Distribution of pathogens detected by BFPP among suspected LRTI patients in ward and ICU

Category	Ward (n=119)	ICU (n=132)	Total (n=251)	p-value (χ^2)
Single viral infection	13 (10.9%)	15 (11.4%)	28 (11.2%)	0.91
Single bacterial infection	18 (15.1%)	19 (14.4%)	37 (14.7%)	0.87
Viral–bacterial co-infection	43 (36.1%)	42 (31.8%)	85 (33.8%)	0.39
Bacterial–bacterial co-infection	23 (19.3%)	32 (24.2%)	55 (21.9%)	0.35
No pathogen detected	22 (18.5%)	24 (18.2%)	46 (18.3%)	0.92

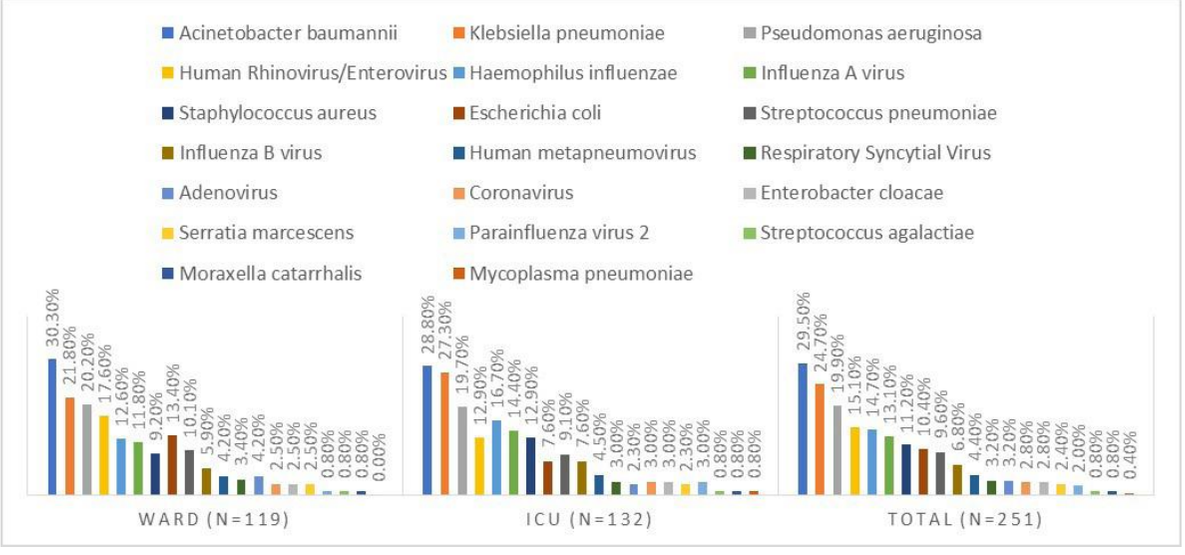

Fig. 1. Frequency table of microorganism detected in BFPP in ward and ICU admitted LRTI patients

Table 3. Antimicrobial resistance (AMR) genes detected in viral–bacterial versus bacterial mono/co-infections

AMR Gene	Viral–Bacterial (n=84)	Bacterial Mono/Co-inf. (n=90)	Total (n=174)	p-value
bla_{CTX-M}	46 (54.8%)	57 (63.3%)	103 (59.2%)	0.44
bla_{NDM}	43 (51.2%)	56 (62.2%)	99 (56.9%)	0.34
bla_{OXA-48}	32 (38.1%)	49 (54.4%)	81 (46.6%)	0.16
bla_{KPC}	13 (15.5%)	25 (27.8%)	38 (21.8%)	0.12
bla_{VIM}	11 (13.1%)	15 (16.7%)	26 (14.9%)	0.69
bla_{IMP}	4 (4.8%)	5 (5.6%)	9 (5.2%)	0.82
mecA/C + MREJ	7 (8.3%)	8 (8.9%)	15 (8.6%)	0.91
No AMR gene	17 (20.2%)	19 (21.1%)	36 (20.7%)	0.89

P-values calculated using Fisher's exact test

Table 4. Comparison of BFPP with conventional culture for bacterial detection

	Culture +	Culture –
BFPP +	105	69
BFPP –	6	71

The BFPP panel revealed that more than half of the patients had mixed infections involving two or more pathogens, while only about a quarter of the cases involved a single organism. Viral–bacterial co-infections were most frequent (33.8%), followed by bacterial–bacterial (21.9%). Such polymicrobial profiles have been widely reported, with Lim et al. reporting bacterial and viral coinfection in 13.6% of adults with CAP (9), Qin et al. noting 42% positivity in ICU BAL specimens (10), and Li et al. and Shen et al. reporting viral–bacterial co-infections in 22.4–58.9% of ICU cases (11, 12). Heitz et al. emphasized that early recognition of co-infections through multiplex assays supports timely antimicrobial tailoring and improved outcomes (13).

In critically ill patients, the most frequently detected bacteria were *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, all of which are common hospital-acquired pathogens. This aligns with Santella et al.'s large 5-year study of 7038 respiratory samples, where 39.1% were culture-positive, with Gram-negatives accounting for 72.5%. The prevalence rates for *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* were 18.6%, 14.2%, and 10.9%, respectively, confirming their predominant clinical role in ICU infections (14). Viral pathogens were led by Influenza A (13.1%), followed by Human Rhinovirus/Enterovirus (15.1%). While influenza holds major clinical relevance due to epidemic potential and antiviral options, the burden of rhinoviruses and enteroviruses is also notable, especially for triggering exacerbations of chronic lung diseases (1, 2).

BFPP's integrated AMR module provided rapid profiling in 174 bacterial-positive cases, detecting resistance genes in 138 (79.3%). The most frequent were bla_{CTX-M} (59.2%), bla_{NDM} (56.9%), and bla_{OXA-48} (46.6%), followed by bla_{KPC} (21.8%), bla_{VIM} (14.9%), bla_{IMP} (5.2%), and *mecA/C + MREJ* (8.6%). Among *Staphylococcus aureus* isolates (n=10), *mecA/C + MREJ* was found in 8 cases (80%).

These findings are consistent with Gong et al. (15) and Lee et al. (16), who demonstrated robust detection of ESBLs and carbapenemases, including bla_{IMP}, bla_{KPC}, and bla_{VIM}, in ICU pneumonia. Previous evaluations have shown variable AMR gene sensitivity (40.1-74.6%) and specificity up to 95.9% (17). Although AMR genes were slightly more common in bacterial mono/co-infections than in viral–bacterial infections, differences were not statistically significant. This underscores BFPP's potential role in real-time surveillance of resistance determinants, particularly ESBL- and carbapenemase-producing *K. pneumoniae* and *A. baumannii*.

Obtaining pathogen and resistance gene results within a few hours can greatly support antimicrobial stewardship. In our study, the BFPP detected 69 pathogens that culture failed to identify, enabling quicker adjustment of empiric therapy and reducing unnecessary antibiotic use. Although our study did not directly measure changes in prescribing, prior published studies demonstrated that BFPP-guided stewardship facilitated de-escalation in 25-45% of

cases, with reductions in broad-spectrum antibiotic exposure and length of stay (18-20). Importantly, these findings apply to the overall patient cohort rather than specific species. Such early stewardship interventions are critical in high-resistance settings such as India (2).

BFPP outperformed culture in diagnostic yield, detecting pathogens in nearly all culture-positive samples and additionally in 69 culture-negative specimens. Agreement between methods was moderate ($\kappa = 0.428$). Similar performance has been noted in multicenter evaluations, with BFPP sensitivity ranging from 93–96.4% (21, 22) and specificity from 45.9-100% (17, 21, 23). Additional detections in culture-negative cases may reflect fastidious organisms, antibiotic suppression, or colonization. Distinguishing colonization from infection remains a challenge for syndromic panels, as highlighted by Crémet et al. (23) and García-Martínez et al. (24). We addressed this by correlating results with clinical and radiological features, but future prospective studies should evaluate outcomes such as antibiotic duration and survival.

This study has inherent limitations. Its retrospective design restricted control over sample handling and precluded collection of key clinical outcomes, such as mortality, length of stay, or BFPP-guided stewardship interventions. Although detection rates were reported across BAL, sputum, and ET aspirates, a comparative performance analysis by sample type was not undertaken. Differentiation between colonization and infection, while potentially aided by BFPP's semi-quantitative thresholds ($\geq 10^7$ copies/μL) and clinical correlation, was not systematically assessed. The panel also excluded important pathogens such as *Stenotrophomonas maltophilia* and fungi (e.g., *Aspergillus* spp.), necessitating complementary testing in high-risk patients. Furthermore, incomplete demographic and clinical data limited assessment of confounding factors, and some subgroup analyses (e.g., AMR gene comparisons) were underpowered, reducing statistical precision.

CONCLUSION

Our findings highlight the substantial diagnostic and stewardship benefits of the BFPP in LRTI management. With its rapid turnaround, broad pathogen coverage, and integrated AMR detection, BFPP facilitates targeted therapy and supports infection control decisions. Given the high burden of antimicrobial resistance and polymicrobial pneumonia in ICU and ward settings, integrating syndromic molecular diagnostics into routine practice represents a transformative step toward precision respiratory care.

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