Original Article

Prevalence of Pathogens and Antimicrobial Resistance Genes in Lower Respiratory Tract Infections using Multiplex PCR: Insights from a Tertiary Care Hospital in Bangladesh

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Abstract:

Background: *Lower respiratory tract infections (LRTIs) are a major health concern worldwide. The identification of common pathogens and antimicrobial resistance (AMR) patterns is necessary for effective treatment and cost minimization in resource-limited developing countries such as Bangladesh.*

Objective: *This study aimed to detect the prevalence of pathogens and antimicrobial resistance genes in LRTIs using multiplex polymerase chain reaction (PCR) in a tertiary care hospital.*

Materials & methods: *This retrospective observational study analyzed 61 samples, including 46 tracheal aspirates and 15 bronchoalveolar lavage samples, collected between June 2024 and July 2025. The BIOFIRE FILMARRAY pneumonia panel was used to target 27 pathogens, including 9 viruses & 18 bacteria, and 7 antimicrobial resistance (AMR) genes. Data were analyzed using Microsoft Excel-2016.*

Results: *Of the 61 samples, 76.4% tested positive for one or more pathogens. Bacterial pathogens were detected in 64% of the samples, with Klebsiella pneumoniae (34.42%), Pseudomonas aeruginosa (21.31%), and Streptococcus pneumoniae (14.75%) being the most common. Viral pathogens, particularly Human Rhinovirus/Enterovirus (14.74%) and Influenza A (9.83%) were common. Co-infection with both bacteria and viruses was observed in 30% of the samples. AMR genes, particularly CTX-M, an extended spectrum β-lactamase (46.15%), OXA-48-like (38.46%), and NDM (35.89%), both of which are associated with carbapenem resistance, were frequently detected.*

Conclusion: *The high prevalence of multidrug-resistant organisms emphasizes the urgent need for targeted and appropriate antimicrobial therapy for the effective treatment of LRTIs. Continuous surveillance of pathogen prevalence and AMR patterns will help evaluate treatment protocols and local healthcare policies.*

Keywords: *Antimicrobial resistance, Bangladesh, BioFire FilmArray Pneumonia Panel, Lower Respiratory Tract Infections, multiplex PCR.*

Introduction:

Lower respiratory tract infections (LRTIs) are among the most prevalent infectious diseases worldwide. In 2019, there were 488.9 million incident cases and 2.4 million deaths due to LRTIs worldwide[1]. These infections not only impose a significant global health burden, resulting in considerable morbidity and mortality, but also lead to excess health expenses.[2-4] LRTIs are caused by heterogeneous microorganisms, including bacteria, viruses, fungi, and parasites. The majority of cases are caused by bacterial or viral pathogens. Common gram-negative bacteria associated with LRTI include *Pseudomonas spp., Acinetobacter spp., Klebsiella pneumoniae,* and Haemophilus influenzae. Conversely, *Staphylococcus aureus* and *Streptococcus* pneumoniae are the most prevalent gram-positive bacterial pathogens responsible for LRTI.[5-8]

The FilmArray system is an automated multiplex nucleic acid detection system. The BIOFIRE FILMARRAY Pneumonia Panel (PN Panel) is capable of simultaneously detecting multiple causative pathogens, including bacteria and viruses as well as antimicrobial resistance (AMR) genes within approximately one hour.[9-12]

Antimicrobials are commonly prescribed to treat LRTI; however, the patterns of causative organisms and their antimicrobial susceptibility vary across different geographical regions.[13,14] According to the World Health Organization

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(WHO), AMR responsible for over 700,000 deaths annually, and if left unaddressed, this figure is projected to escalate to 10 million by 2050.[15] Unfortunately, the burden of antimicrobial resistance is high in developing countries making treatment challenging and causing more morbidity and mortality.[16] Understanding the current trends in causative microorganisms and their antimicrobial resistance patterns is crucial for selecting appropriate antimicrobials, thereby reducing their misuse and overuse.

This study aimed to identify the common pathogens responsible for LRTIs and their AMR genes using a PN panel in a tertiary care hospital in Bangladesh, a developing country in Southeast Asia. The findings of this study would be helpful in the selection of appropriate empiric and definitive antimicrobials for more effective treatment of LRTIs and in the formulation of local guidelines.

Material and methods:

This retrospective observational study was conducted between June 2024 and July 2025. Ethical approval was taken from the hospital Institutional Review Board. Patients were not directly involved in this study, as only laboratory data were used, which were collected from the microbiology database.

Study subjects and sample collection

During the study period, patients admitted to the inpatient department with suspected LRTIs were enrolled. Tracheal aspirate and bronchoalveolar lavage (BAL) samples were collected and analyzed. The samples were received and processed by the microbiology department. A total of 61 samples were collected from patients of all age and sex groups.

BIOFIRE FILMARRAY Pneumonia Panel (PN Panel)

This multiplex single-pouch polymerase chain reaction (PCR) kit can identify 35 targets in approximately 1 hour. The assay tests for fifteen typical bacterial pathogens and three atypical bacterial pathogens, nine viruses, and seven antimicrobial resistance genes (Table -1) associated with LRTIs.[9, 10, 12]

Table I: *BIOFIRE FILMARRAY Pneumonia Panel targets*

Viruses	**Bacteria (Semi-quantitative)**	**Atypical Bacteria (Qualitative)**	**Antimicrobial Resistance Genes**
Adenovirus	Acinetobacter	Chlamydia	**Carbapenemases**
Coronavirus	calcoaceticus-baumannii	pneumoniae	IMP[a]
Human	complex	Legionella	KPC[b]
Metapneumovirus	Enterobacter	pneumophila	NDM[c]
Human Rhinovirus/ Enterovirus	cloacae complex	Mycoplasma pneumoniae	OXA-48-like
Influenza A	Escherichia coli		VIM[d]
Influenza B	Haemophilus influenza		**ESBL**[e]
Parainfluenza virus	Klebsiella aerogenes		CTX-M
Respiratory Syncytial virus	Klebsiella oxytoca		**Methicillin resistance**
Middle East Respiratory Syndrome	Klebsiella pneumoniae group		mecA/C and MREJ (MRSA)
Coronavirus (MERS-CoV)	Moraxella catarrhalis		
	Proteus spp.		
	Pseudomonas aeruginosa		
	Serratia marcescens		
	Staphylococcus aureus		
	Streptococcus agalactiae		
	Streptococcus pneumoniae		
	Streptococcus pyogenes		

[a] Imipenemase

[b] Klebsiella Pneumoniae Carbapenemase

[c] New Delhi Metallo-beta-lactamase

[d] Verona Integron –encoded Metallo-beta-lactamase

[e] Extended-Spectrum β-Lactamase

The BIOFIRE FILMARRAY pneumonia panel assay was performed according to the manufacturer's instructions. A 300 µL sample was added to the pouch and placed in the BIOFIRE torch system. The pouch is a multiplex PCR assay that contains all the reagents for sample preparation, reverse transcription PCR, and PCR.[12] During testing, the BIOFIRE system extracts and purifies all nucleic acids. Two-stage multiplex PCR was performed on the samples. A single large-volume multiplex reaction was performed in the first stage. The second stage involved singleplex reactions. The BIOFIRE system software then automatically analyzes the results using endpoint melting curve data and provide results.[12] The results for the typical bacterial pathogens are reported semi-quantitatively, according to the estimated abundance of the corresponding bacterial nucleic acids with bins allowing the approximate detection of 10^4, 10^5, 10^6, or 10^7 copies/ml.[10]

Data Analysis

The data collected in this study were analyzed using Microsoft Excel-2016. Basic statistical methods, such as percentage and frequency calculations, were used to analyze data. Excel functions, such as count, pie charts, bar charts, and tables, were used to organize and display the data. Some calculations were performed manually to double-check the results or to handle parts of the data that could not be analyzed using Excel automation. This approach helped identify key patterns and comparisons that were important for understanding the findings and addressing the research questions of the study.

Results:

A total of 61 samples were included, of which 46 were tracheal aspirates and 15 were BAL. Pathological microorganisms were detected in 46 samples and were absent in 15 samples. Only bacterial pathogens were detected in 21 samples, both bacteria and viruses were detected in 18 samples, and only viruses were detected in 7 samples (Fig 1). A minimum of one pathogen and a maximum of six pathogens were detected in each sample (Fig 2).

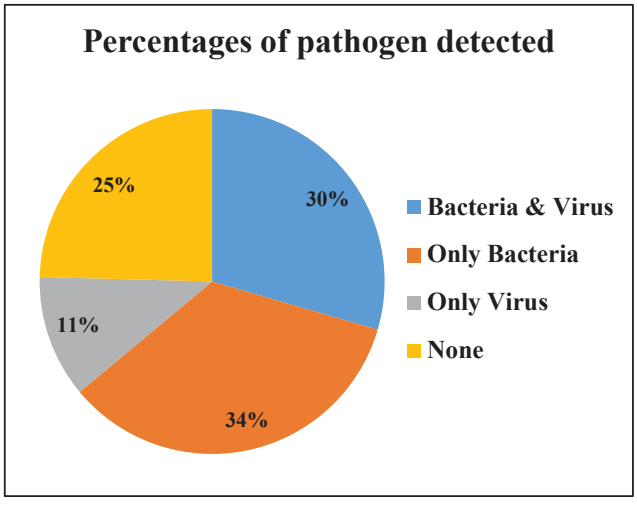

Figure 1: Percentages of Pathogens (Bacteria or Virus or Both) detection among the total of 61 samples.

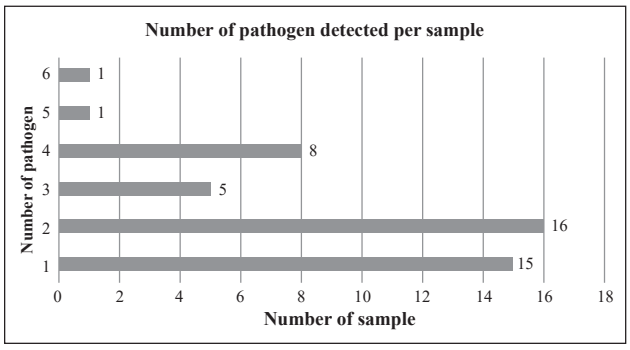

Figure 2: Number of pathogens detected per sample.

Detection of pathological microbial

The PN panel most frequently detected human rhinoviruses/enteroviruses, Influenza A virus, and coronaviruses. The frequencies of the detected viruses are listed in Table II.

Table II: Percentage of virus detection in regards to total of 61 samples

Virus	Percent Detected (%)	Count
Human Rhinovirus/Enterovirus	14.75%	9
Influenza A	9.83%	6
Corona virus	8.19%	5
Parainfluenza Virus	4.91%	3
Influenza B	3.27%	2
Respiratory Syncytial Virus	3.27%	2

The most frequently detected bacterial pathogens were *Klebsiella pneumoniae* in 21/61 samples, *Pseudomonas aeruginosa* in 13/61 samples, *Streptococcus* pneumoniae in 9/61 samples, and Acinetobacter *calcoaceticus-baumannii* complex and *Staphylococcus aureus* each in 7/61 samples. Table 3 shows the percentages of detected bacterial pathogens.

Table III: Percentages of bacteria detection in regards to total of 61 samples.

Bacteria	Percent Detected (%)	Count
Klebsiella pneumoniae group	34.42%	21
Pseudomonas aeruginosa	21.31%	13
Streptococcus pneumoniae	14.75%	9
Staphylococcus aureus	11.47%	7
Acinetobacter calcoaceticus baumannii - complex	11.47%	7
Streptococcus agalactiae	8.19%	5
Escherichia coli	8.19%	5
Enterobacter cloacae complex	6.55%	4
Haemophilus influenza	6.55%	4
Proteus spp.	1.63%	1
Klebsiella oxytoca	1.63%	1
Serratia marcescens	1.63%	1

Antimicrobial resistance Genes

A total of 57 AMR genes were identified in 39 samples that tested positive for bacterial pathogens. The most frequently detected gene was CTX-M (46.15% of samples), followed by OXA-48-like (38.46 %) and NDM (35.89 %). Other resistance genes, such as VIM, KPC, IMP *mec*A/C, and MREJ, were detected less frequently.

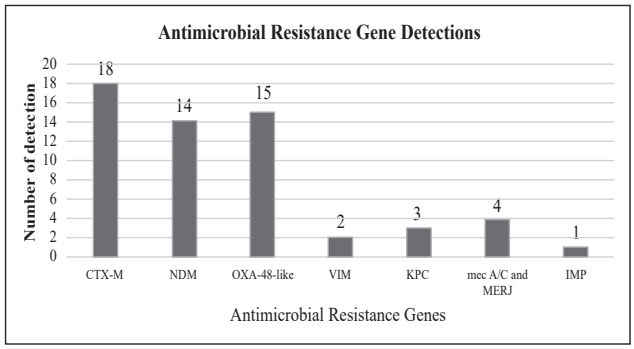

Figure 3: Frequency of Antimicrobial Resistance Gene detection in regards to total of 57 samples.

Discussion:

This retrospective observational study investigated lower respiratory tract samples to determine the prevalence of pathogens and antimicrobial resistance genes using multiplex PCR (BIOFIRE FILMARREAY pneumonia panel) in a tertiary care hospital in Bangladesh. Between June 2024 and July 2025, 61 samples, comprising 46 tracheal aspirates and 15 bronchoalveolar lavages, were analyzed. Of these, 46 samples (76.40%) tested positive for at least one pathogen, either a virus or a bacterium, or both, with bacterial pathogens detected in 64% of the samples. Consistent with the findings of Kosai et al. and Lee et al., our study effectively identified pathogens and AMR genes using the PN panel.[9,17] The PN panel detected a greater number of bacterial pathogens compared to the culture method (84 vs. 25, respectively), as reported by Kosai et al., who showed higher sensitivity compared to traditional culture methods.[9] These findings demonstrate the utility of multiplex PCR as a rapid diagnostic tool for guiding effective therapy in LRTIs, especially in resource-limited settings.

We observed co-infection with bacteria and viruses in 30% of samples, which, although lower than the 80% reported in a UK-based study, still indicates a substantial overlap in pathogen profiles.[18] Among the viral pathogens, Human Rhinovirus/Enterovirus (14.74%) and Influenza A (9.83%) were the most frequently detected, aligning with trends observed in other studies from different regions.[18,19] This overlap in viral detection supports the role of viruses in the exacerbation of LRTIs. Therefore, when treating LRTIs, antiviral therapy may be warranted in some cases in addition to antibacterial coverage.

Among the bacterial pathogens, Klebsiella pneumoniae (34.42%), Pseudomonas aeruginosa (21.31%), and Streptococcus pneumoniae (14.75%) were the most frequently detected. Similar findings were documented in a study conducted at Nagasaki University Hospital in Japan and another study at a specialized hospital in Ethiopia.[9,20] Similar to our study, a study conducted in Nepal by Bhatta et al. and an Italian study by Santella et al. on LRTI reported a high prevalence of Pseudomonas aeruginosa (16.1% and 11.6%–16%, respectively), while Acinetobacter spp. (29.4% and 17.9 to 20.1%, respectively) was the most dominant pathogen in their settings. In contrast, we found Klebsiella pneumoniae (34.42%) to be the most dominant pathogen.[21,22] However, we detected Acinetobacter spp. (11.47%) less frequently. We found Streptococcus pneumoniae in 14.75% of samples, representing the third most prevalent bacterial pathogen in LRTIs. Similar to our findings, Gadsby et al. also found Streptococcus pneumoniae to be one of the most frequently detected respiratory pathogens in a study conducted at a tertiary care hospital in the UK.[18] This regional variation may be due to differences in antimicrobial practices, infection control policies, and hospital environments.

A critical component of our study findings was the high detection rate of AMR genes. CTX-M, an extended-spectrum β-lactamase (ESBL) gene, was the most prevalent (46.15%), followed by OXA-48-like (38.46%) and NDM (35.89%), both of which are associated with carbapenem resistance. This pattern mirrors the resistance profiles documented in a prior Bangladeshi study by Safain et al., where CTX-M and NDM-1 were the predominant AMR genes.[23] Another study conducted in Italy also documented high resistance rates of beta lactams and carbapenems by Acinetobacter spp., Pseudomonas aeruginosa, and Klebsiella pneumoniae.[22] Notably, our study detected mec A/C and MREJ- markers of methicillin-resistant Staphylococcus aureus (MRSA) less frequently than studies from Japan and Korea, suggesting a greater challenge locally with ESBL and carbapenemase-producing organisms than with MRSA.[9,10]

This study has several limitations. One of the major limitations of this study was that we did not examine the pathogens detected by the PN panel using a reference method, such as culture, which limits direct comparison. Other limitations include the relatively small sample size and retrospective nature of the study. However, this study is one of the few studies to detect LRTIs causing pathogens and AMR gene patterns from a Bangladeshi perspective. A large-scale prospective study is required to validate and expand these findings.

Conclusions:

This study offers valuable insights into the prevalence of pathogens and AMR genes in LRTIs in Bangladesh. The rapid detection of pathogens and AMR genes can enhance patient outcomes by facilitating the use of targeted and appropriate antimicrobial therapy, thereby reducing unnecessary antibiotic use and mitigating the escalating threat of antimicrobial resistance. Compared with other relevant studies and in the context of the global antimicrobial resistance crisis, the findings of this study highlight the urgent need for comprehensive strategies, such as effective infection control, local resistance surveillance programs, and antimicrobial stewardship, to address this public health challenge in Bangladesh.

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Conflict of interest*:* None

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