Section: Microbiology

Original Research Article

# MOLECULAR DIAGNOSIS OF CLINICALLY SUSPECTED PNEUMONIA CASES AT TERTIARY HEALTH CARE CENTRE

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## ABSTRACT

**Background:** Pneumonia remains a major global health burden, particularly among children and the elderly. Viral pathogens are increasingly recognized as primary or co-infecting agents in pneumonia cases, yet their clinical diagnosis is challenging due to overlapping symptoms with bacterial infections. Molecular diagnostic tools, such as multiplex real-time PCR, offer rapid and accurate pathogen identification, essential for appropriate clinical management and infection control. **Materials and Methods:** This prospective study was conducted over 12 months at a tertiary care hospital. A total of 100 patients of all age groups, clinically diagnosed with pneumonia and negative for bacterial cultures, were included. Nasopharyngeal and oropharyngeal swabs were collected and processed using multiplex real-time PCR (Fast Track Diagnostics, Siemens Healthineers) to detect a broad panel of respiratory viruses. Data were analyzed using descriptive statistics and chi-square test, with a significance level of $p < 0.05$. **Result:** Out of 100 samples, 78 tested positive for at least one viral pathogen by RT-PCR ($p < 0.01$). The most frequently detected organisms included Influenza A virus (11.22%), Human rhinovirus (9.18%), and Klebsiella pneumoniae (25.00%). Age distribution showed the highest prevalence in the 21–40-year age group (28.20%), with a mean age of 35.96 ± 22.36 years. Males constituted 56.41% of the cases, with a male-to-female ratio of 1.27:1. **Conclusion:** Multiplex RT-PCR significantly improves the etiological diagnosis of viral pneumonia, facilitating targeted therapy and reducing unnecessary antibiotic use. The detection of both viral and bacterial pathogens emphasizes the importance of integrated molecular diagnostics in clinical settings.

## INTRODUCTION

Pneumonia remains a formidable global health challenge, striking approximately 450 million individuals annually and accounting for around 3–4 million deaths, particularly impacting children under five and elderly populations.[1,2] Viral pathogens are increasingly recognized contributors to community-acquired and hospital-acquired pneumonia, implicated in up to 20–30% of cases among adults and frequently identified alongside bacterial co-infections. Age-related susceptibility follows a U-shaped curve, with incidence markedly high in infants and individuals over 65 years.

Historically, diagnosis of viral pneumonia relied heavily on culture, immunofluorescence, or serology, which were often time-consuming and limited in sensitivity.[2] The introduction of PCR-based diagnostics revolutionized the field by enabling rapid, highly sensitive, and multiplexed detection of respiratory viruses in a single assay. Numerous studies have demonstrated that multiplex real-time RT-PCR assays can identify a wide array of pathogens—including influenza A/B, respiratory syncytial virus, parainfluenza, metapneumovirus, coronaviruses, rhinovirus, and bocavirus—within hours, significantly improving diagnostic yield in both pediatric and adult populations.

In tertiary care settings, where severe or complex cases present, respiratory virus detection is critical


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for clinical management. One multicenter analysis revealed that nearly one-third of hospitalized adults with community-acquired pneumonia had viral pathogens, with nearly equal prevalence to bacterial agents.[4] Furthermore, molecular diagnostics have highlighted the seasonal variations of viral incidence (e.g., metapneumovirus peaks following RSV seasons), underscoring the need for ongoing surveillance for timely public health responses.[5,6]

Given that molecular diagnostics are underutilized in many resource-limited settings, there remains a substantial knowledge gap regarding the true burden and epidemiology of viral pneumonia at tertiary care centers in developing countries. Understanding the distribution of specific viral agents can inform empirical treatment decisions, antibiotic stewardship, and infection control measures. With these considerations, our study employed a standardized multiplex RT-PCR strategy to evaluate suspected cases of viral pneumonia at a tertiary care institution. The objectives were to determine the prevalence of respiratory viruses, assess their age–gender distribution, and explore diagnostic utility in a clinical context. These data aim to enhance regional epidemiological knowledge and support improved patient care strategies.

## MATERIALS AND METHODS

This prospective observational study was conducted over a 12-month period at a tertiary care hospital to evaluate the viral etiology in clinically diagnosed cases of pneumonia using molecular diagnostic techniques.

**Study Design and Setting:** The study followed a prospective design and was carried out at a microbiology department of a tertiary health care institution. Ethical clearance was obtained prior to initiation of the study, and all procedures were conducted in accordance with institutional and ethical guidelines.

**Study Population and Selection Criteria:** A total of 100 patients presenting with clinical features suggestive of pneumonia were enrolled. Inclusion criteria comprised individuals of all ages and both sexes who were clinically diagnosed with pneumonia by the attending physician. Patients with confirmed bacterial pneumonia, as indicated by a positive bacterial culture, were excluded from the study to ensure focus on suspected viral cases.

**Sample Collection and Transportation:** Clinical specimens, primarily nasopharyngeal and/or oropharyngeal swabs, were collected from each participant using sterile, synthetic fiber-tipped swabs with plastic shafts. All samples were collected under aseptic precautions and transported immediately to the microbiology laboratory under cold chain conditions, following the manufacturer's instructions provided with the diagnostic kit.

**Molecular Testing by Real-Time PCR:** All specimens were processed using a multiplex real-time polymerase chain reaction (RT-PCR) assay. The diagnostic kit used for this purpose was from Fast Track Diagnostics (a Siemens Healthineers Company), which is designed for the simultaneous detection of multiple viral respiratory pathogens. The protocol provided by the manufacturer was strictly adhered to, including steps for RNA extraction, reverse transcription, and amplification.

The RT-PCR assay targeted a broad range of respiratory viruses, including but not limited to Influenza A and B viruses, respiratory syncytial virus (RSV), adenovirus, human metapneumovirus, parainfluenza viruses, coronavirus strains, enterovirus, and rhinovirus. Internal controls were included in each run to validate assay performance and rule out the presence of PCR inhibitors.

**Data Collection and Statistical Analysis:** Demographic details including age and gender, along with RT-PCR results, were recorded for each patient. The collected data were entered into Microsoft Excel and subsequently analyzed using SPSS statistical software. Descriptive statistics were used to present frequencies and proportions. Chi-square test was applied to assess associations between categorical variables, and a p-value of <0.05 was considered statistically significant.

## RESULTS

A total of 100 patients clinically suspected of having pneumonia were enrolled in the study. RT-PCR analysis revealed that 78% of the suspected cases were positive for viral & bacterial pathogens, while 22% tested negative (Table 1). This positivity rate was statistically significant ($P < 0.01$), underscoring the relevance of RT-PCR as a diagnostic tool in suspected viral pneumonia.

**Table 1: RT-PCR positivity in suspected pneumonia cases**

| RT-PCR Result | n | % | P Value |
|---|---|---|---|
| Positive | 78 | 78 | <0.01 |
| Negative | 22 | 22 | |
| **Total** | **100** | **100** | |

**Table 2: Incidence of Co-infection in suspected pneumonia cases**

| Total Positive Cases | Mono-organism | | Poly-organism | |
|---|---|---|---|---|
| | n | % | n | % |
| 78 | 16 | 20.51 | 62 | 79.49 |


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**Table 3: No. of Patients having Co-infection in suspected pneumonia cases**

| Type of Infection | No. of Organism found | No. of Patient | Total |
|---|---|---|---|
| Mono-Infection | 01 | 16 | 16 |
| Poly-Infection | 02 | 27 | 54 |
| | 03 | 17 | 51 |
| | 04 | 10 | 40 |
| | 05 | 07 | 35 |
| **Total** | | **78** | **196** |

A wide spectrum of viral and bacterial organisms was detected among RT-PCR positive cases (Table 4). Influenza A virus was the most frequently isolated pathogen, identified in (11.22%) of cases, followed by Klebsiella pneumoniae (25.00%) and Human rhinovirus (9.18%). Notably, Streptococcus pneumoniae (7.14%), Haemophilus influenzae (8.67%), Staphylococcus aureus (6.63%), and Influenza A(H1N1) virus (6.63%) were also frequently detected. Other viral agents included Human bocavirus, Human metapneumoviruses A/B, and multiple serotypes of Human parainfluenza viruses, are at lower frequencies. The microbial diversity observed highlights the polymicrobial nature of respiratory infections in the study population.

**Table 4: Organisms isolated in suspected pneumonia cases**

| RT-PCR Result | n | % |
|---|---|---|
| Bordetella spp. | 1 | 0.51 |
| Chlamydophila pneumoniae | 3 | 1.53 |
| Enterovirus | 3 | 1.53 |
| Haemophilus influenzae | 17 | 8.67 |
| Human bocavirus | 7 | 3.57 |
| Human coronavirus 229E | 3 | 1.53 |
| Human coronavirus OC43 | 1 | 0.51 |
| Human metapneumoviruses A/B | 4 | 2.04 |
| Human parainfluenza virus 1 | 3 | 1.53 |
| Human parainfluenza virus 2 | 1 | 0.51 |
| Human parainfluenza virus 3 | 1 | 0.51 |
| Human parainfluenza virus 4 | 2 | 1.02 |
| Human rhinovirus | 18 | 9.18 |
| Influenza A virus | 22 | 11.22 |
| Influenza A(H1N1) virus (swine-lineage) | 13 | 6.63 |
| Influenza B virus | 11 | 5.61 |
| Influenza C virus | 1 | 0.51 |
| Klebsiella pneumoniae | 49 | 25.00 |
| Moraxella catarrhalis | 3 | 1.53 |
| Mycoplasma pneumoniae | 6 | 3.06 |
| Staphylococcus aureus | 13 | 6.63 |
| Streptococcus pneumoniae | 14 | 7.14 |
| **Total** | **196** | **100** |

The age of participants ranged from infancy to the elderly, with a mean age of 35.96 ± 22.36 years (Table 5). The majority of patients fell within the 21–40 years age group (28.20%), followed closely by the 41–60 years and 1–20 years age brackets. A statistically significant difference was observed in the age-wise distribution of cases ($P < 0.01$), indicating a higher burden in certain age groups.

**Table 5: Age distribution of suspected pneumonia cases**

| Age Group (Years) | n | % | P Value |
|---|---|---|---|
| < 1 year | 8 | 10.26 | <0.01 |
| 1 – 20 | 18 | 23.07 | |
| 21 – 40 | 22 | 28.20 | |
| 41 – 60 | 19 | 24.36 | |
| 61 – 80 | 10 | 12.82 | |
| 81 – 100 | 1 | 1.28 | |
| **Total** | **78** | **100** | |
| **Age (Years); Mean ± SD** | 35.96 ± 22.36 | | |

With regard to gender distribution, males constituted a slightly higher proportion (56.41%) compared to females (43.59%), resulting in a male-to-female ratio of 1.27:1 (Table 6). However, this gender difference was not statistically significant ($P = 0.23$).


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**Table 6: Gender distribution of suspected pneumonia cases**

| Gender | n | % | P Value |
|---|---|---|---|
| F | 34 | 43.59 | 0.23 |
| M | 44 | 56.41 | |
| **Total** | **78** | **100** | |
| **Male:Female Ratio** | 1.27:1 | | |

Figure 1 presents a graphical representation of the percentage distribution of organisms isolated by RT-PCR among the suspected viral pneumonia cases. The chart visually emphasizes the predominance of Klebsiella pneumoniae (24.62%) as the most commonly detected pathogen.

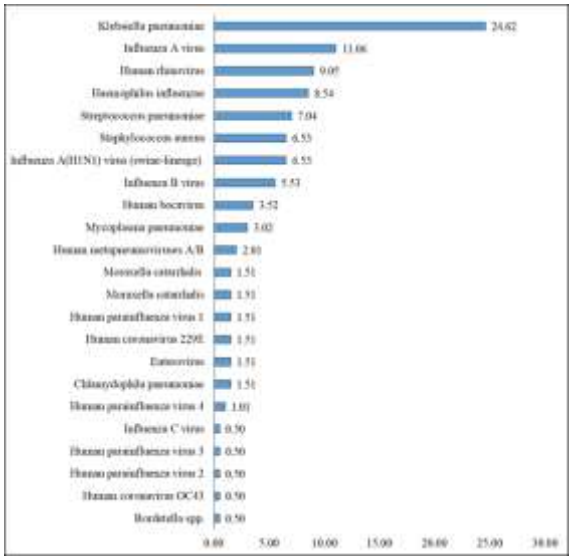


**Figure 1: Graphical presentation of % of organisms isolated by RT-PCR**

## DISCUSSION

In this cohort of suspected viral pneumonia cases, the high RT-PCR positivity rate (78%) underscores the diagnostic value of multiplex molecular assays in tertiary healthcare settings. Comparable studies have shown that incorporation of multiplex PCR panels can elevate detection rates by up to 30–50% over traditional methods,[7,8] demonstrating that molecular diagnostics effectively bridge the gap in etiological identification.

The predominance of Klebsiella pneumoniae (25.00%) is notable. Although typically considered a bacterial agent, it is frequently observed in severe or nosocomial respiratory infections.[9] Animal models have revealed that co-infection with influenza-like viruses and Klebsiella can exacerbate lung injury via exaggerated inflammatory responses and impaired immunity.[10] Clinically, secondary bacterial pathogens such as Klebsiella are well documented to complicate viral pneumonia, particularly in the elderly and immunocompromised.[11] Our data suggest a similar trend, even though all cases were initially suspected to have viral etiology.

Viral agents—especially Influenza A, H1N1, human rhinovirus, and human metapneumovirus—were identified in significant proportions. This pattern aligns with global surveillance data indicating that respiratory viruses, especially those mentioned, are key etiologies of community-acquired pneumonia, particularly among vulnerable age groups.[12] Moreover, human metapneumovirus was consistently detected across major cohorts, accounting for approximately 8–15% of hospitalized pneumonia cases.[13]

Age distribution data reveal a bimodal pattern—the U-shaped curve—common in respiratory infections, with heightened susceptibility in infants and the elderly. Our mean participant age (36 years) and age-specific distribution corroborate existing epidemiologic literature.[14]

Coinfection patterns further highlight the clinical complexity; viral infections can compromise host immunity, allowing bacterial superinfection, particularly by Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus influenza.[15] These trends were evident in our findings, reinforcing the importance of comprehensive pathogen profiling. Notably, studies have linked bacterial–viral coinfections to increased disease severity, ICU admissions, prolonged hospital stays, and higher mortality rates.

The predominance of Klebsiella observed may reflect factors such as local antimicrobial resistance patterns, hospital-based transmission, or underlying comorbidities such as diabetes or chronic lung disease, which were prevalent in our cohort. Given rising concern over carbapenem-resistant Klebsiella pneumoniae, especially in Asia, routine molecular detection can guide infection control and therapeutic decisions.

Clinical implications from our results are multifold: incorporation of multiplex RT-PCR testing can refine empirical antimicrobial use by reducing unnecessary antibiotic prescriptions in purely viral cases; identification of bacterial superinfections like Klebsiella can prompt targeted therapy; and routine surveillance can support hospital infection control efforts, especially in high-risk wards.

## CONCLUSION

This study highlights the utility of multiplex real-time PCR in accurately identifying viral pathogens in suspected pneumonia cases, with a high detection rate of 78%. The findings underscore the prevalence of both viral agents like Influenza A and Human Rhinovirus, and notable bacterial co-infections such as Klebsiella pneumoniae. Such molecular diagnostics enable timely, targeted clinical management, reducing reliance on empirical antibiotics. Age and gender distribution patterns


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support known epidemiological trends in viral respiratory infections. Integration of molecular testing in routine diagnostics can significantly enhance patient care and infection control in tertiary healthcare settings.

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