

ETIOLOGICAL SPECTRUM OF COMMUNITY-ACQUIRED RESPIRATORY INFECTIONS IN HOSPITALIZED PEDIATRIC PATIENTS BY MULTIPLEX MOLECULAR APPROACH: A TERTIARY CARE CENTER BASED STUDY FROM NORTH INDIA

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ABSTRACT

Background: Respiratory infections are a leading cause of illness and death among children, globally. India, especially states like Uttar Pradesh (UP), bears a disproportionate burden due to factors such as high population density, environmental pollution, seasonal variation, and limited healthcare access. Despite national data availability, there is a lack of region-specific molecular epidemiological insights, particularly for older children (>5 years) and regions like Western UP. This study focuses on identifying and characterizing the viral and bacterial agents responsible for hospitalized children with respiratory distress in Western UP.

Methods: A hospital-based surveillance study was conducted in the neonatal and pediatric intensive care units (NICU/PICU) of a tertiary care center in Uttar Pradesh. A total of 210 clinical samples (Nasopharyngeal swab) were obtained from study population with clinical features consistent with acute respiratory infection (ARI). We employed multiplex real-time polymerase chain reaction (RT-PCR) for molecular detection of viral and bacterial agents, and conventional culture-based techniques for bacterial isolation and identification.

Results: A total of 210 nasopharyngeal swabs from pediatric cases (0–14 years) were evaluated for respiratory pathogens. Viral etiology was detected in 56(26.6%) of the cases. Among these, respiratory syncytial virus (RSV) was the predominant pathogen (42.85%), followed by Rhinovirus (21.42%), Adenovirus (17.85%), and Influenza B virus (7.14%). Mixed viral infections were identified in a subset of cases, specifically RSV/Rhinovirus (7.14%) and RSV/Adenovirus (3.57%). Bacteriological analysis demonstrated the presence of bacterial pathogens in 32 (15.23%) of the samples. The most prevalent being *Streptococcus pneumoniae* 14/32 (43.75%), followed by *Klebsiella pneumoniae* 08/32 (25%), *Moraxella Sps.* 06/32 (18.75%), *Escherichia coli* 02/32 (6.25%) and *Acinetobacter baumannii* 02/32 (6.25%).

Conclusion: The findings highlight the necessity for integrated diagnostic approaches, particularly synchronous testing methodologies, along with targeted preventive strategies to enhance the effective management of pediatric respiratory infections. Syndromic molecular testing is an effective approach to identify causative pathogen so that targeted treatment may be given timely.

INTRODUCTION

Acute respiratory infections (ARIs) are among the leading causes of morbidity and mortality in children worldwide, particularly affecting those under five years of age. Globally, these infections account for approximately 20% of all under-five mortality, with a higher concentration of cases observed in Southeast Asia and sub-Saharan Africa (1). In India, the persistence of these infections is exacerbated by factors such as suboptimal immunization coverage, the emergence of antibiotic-resistant pathogens, and significant regional disparities in healthcare infrastructure (2,3).

The etiology of these infections is influenced by social determinants such as maternal education, caste, and limited access to clinical healthcare resources across diverse geographic regions (4,5). Despite advancements in medical interventions, the high incidence rates (reaching approximately 5.74 episodes per child-year in certain rural settings) highlight the urgent need for targeted surveillance and standardized diagnostic protocols (6). Studies suggest that certain viruses like RSV and influenza, frequently necessitate intensive care interventions among hospitalized infants, reinforcing the critical requirement for improved local surveillance (8-10).

However, it may be caused by a diverse group of viral pathogens, including Respiratory Syncytial Virus (RSV), Influenza viruses, Parainfluenza viruses (PIVs), Adenoviruses, Coronaviruses, and Human metapneumovirus (Hmpv). [11] Although viruses are identified more frequently in acute respiratory infections, bacterial pathogens have historically been regarded as the primary causes of severe disease and mortality, particularly in children from developing countries. [12,13] *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*,

and *Staphylococcus aureus* are recognized as the predominant bacterial pathogens responsible for childhood acute respiratory infections (ARIs). [14]

Currently, comprehensive national data on acute respiratory infections (ARI) and pneumonia-related morbidity and mortality among Indian children aged over 5 years are limited. Most available statistics focus on children under 5 years of age. The integration of advanced computational and diagnostic technologies into clinical practice has created opportunities to improve disease detection, diagnostic accuracy, and clinical decision support [16]. Despite national data, there is a relative scarcity of comprehensive molecular epidemiological studies focusing on pediatric respiratory infections in different regions of India specially Uttar Pradesh. Localized epidemiological data support effective clinical management and guide public health interventions such as vaccination strategies and antimicrobial stewardship. [11-15]

In view of the aforementioned considerations, the present study was designed to investigate the molecular epidemiology of respiratory pathogens among hospitalized pediatric patients presenting with symptoms consistent with acute respiratory infection (ARI) and severe acute respiratory infection (SARI) at a tertiary care facility in Western Uttar Pradesh.

METHODS

Study Design and Setting

This was a cross-sectional, hospital-based study conducted from August 2022 to March 2026, at the Virology Diagnostic and Research Laboratory (VDRL), Department of Microbiology, and the Department of Pediatrics, Sarojini Naidu Medical College, Agra, Uttar Pradesh.

Study Population & Methodology

The study enrolled pediatric patients aged under the age of 14 years who were admitted to the Neonatal Intensive Care Unit (NICU), or Pediatric Intensive Care Unit (PICU) with clinical diagnosis of ARI/SARI. Case identification was guided by the World Health Organization (WHO) definitions of ARI and SARI. [20]

From each enrolled patient nasopharyngeal/oropharyngeal swab were collected in two sets .one set is placed in viral transport media VTM and second set is placed in sterile 15 ml falcon tube, and send to bacteriology lab for bacterial culture. Labeled samples were transported under cold chain conditions (2–8°C) to virology and bacteriology laboratories within 2 hours of collection. Samples not immediately processed were aliquoted and stored at -20°C to preserve viral nucleic acids.

Clinical and demographic information was systematically collected from all participants using a standardized case record form. Demographic variables included age, sex, and geographic location to facilitate epidemiological assessment.

The study protocol received ethical approval from the Institutional Ethics Committee (**ID: SNMC/IEC/2024/316**). Bacterial culture and sensitivity was performed as per standard criteria.

Nucleic acids were extracted using the automated nucleic acid extraction system (GENEFINE by Genaxy HT-96). Real-time reverse transcription PCR was carried out using the Respiratory Tract RT-qPCR MX-24T Panel (Bioeksan AR GE Teknolojileri A.Ş.) on the Biorad CFX-96 REAL Time System. The assay detected pathogens including influenza A/B, coronaviruses, parainfluenza viruses 1-4, metapneumovirus, rhinovirus, adenovirus, bocavirus, parechovirus, enterovirus, Legionella, Mycoplasma, Chlamydia, Haemophilus, Bordetella, Streptococcus pneumoniae, and RSV A/B. Data were entered into Microsoft Excel and analyzed using SPSS software.

RESULTS

In the present study, a total of 210 clinical specimens were obtained from pediatric patients of the NICU/PICU diagnosed with acute respiratory distress. The age-wise distribution of pediatric patients (n = 210) demonstrates a predominance of younger children within the study cohort. Infants aged 0–1 year constituted the largest group, comprising 82 patients (39.0%), indicating a higher burden of disease in this age category. Children aged 1–5 years formed the second largest group with 75 cases (35.7%), further emphasizing that the majority of infections occurred in children under five years of age. In contrast, older children were less frequently represented, with 27 patients (12.8%) in the 6–10 years' group and 26 patients (12.3%) in the 11–14 years' group. The distribution reveals a clear decline in case frequency with increasing age, highlighting that younger children, particularly those below five years, are disproportionately affected in the study population. Females constituted a marginally higher proportion, with 106 cases (50.4%), compared to 104 males (49.5%).

Table 1. Age Group Distribution of Pediatric Patients (n = 210)

Age Group	Number of Patients	Percentage (%)
0–1 year	82	39.0
1–5 years	75	35.7
6–10 years	27	12.8
11–14 years	26	12.3

Out of the 210 samples analyzed, the viral etiological agent was identified in a total of 56 (26.6%) cases. The distribution of the viral agents was found the highest positive for RSV (11.42%), followed by Rhinovirus (5.71%), Adenovirus (4.76%), and Influenza B virus (1.90%). Coinfection was detected to be RSV/Rhinovirus as (1.90%) and RSV/Adenovirus (0.95%). Culture-based diagnosis revealed 32 (15.23%) bacterial isolates. The

most prevalent being *Streptococcus pneumonia* (6.66%), followed by *Klebsiella pneumonia* (3.80%), *Morexella Sps.* (2.85%), *Escherichia coli* (0.95%) and *Acinetobacter baumannii* (0.95%). These results highlight the critical importance of intelligently integrating molecular and culture-based methodologies for accurate etiological diagnosis of pediatric respiratory infections.

Table 2a & 2b. Distribution of Detected Respiratory Viruses & Bacteria

Viral Etiology	Number Of Isolates	Percentage (%)
Respiratory Syncytial Virus (RSV)	24	11.42
Rhinovirus	12	5.71
Adenovirus	10	4.76
Influenza B	4	1.90
RSV & Rhinovirus	4	1.90
RSV & Adenovirus	2	0.95

Bacterial Etiology	Total Number of Isolates	RT-PCR Based Detection	Conventional Bacterio- Detection	Total Percentage (%)
<i>Streptococcus pneumonia</i>	14	10	04	6.66
<i>Klebsiella pneumoniae</i>	08	00	08	3.80
<i>Escherichia coli</i>	02	00	02	0.95
<i>Acinetobacter baumannii</i>	02	00	02	0.95
<i>Morexella Sps.</i>	06	06	00	2.85

Table 4. Etiological Distribution of Isolates (Viral and Bacterial) in Different Age Groups

Age Group	Viral Isolates	Percentage (%)	Bacterial Isolates	Percentage (%)
<12 months	20	9.52	12	5.71
1–5 years	18	8.57	10	4.76
6–10 years	10	4.76	5	2.38
11–14 years	8	3.80	5	2.38
Total	56	26.65	32	15.23

The etiological distribution of viral and bacterial isolates showed that the highest proportion of both viral and bacterial infections occurred in children aged less than 12 months. Among the viral isolates, 20 (9.52%) were detected in infants aged below 12 months, followed by 18 (8.57%) in the 1–5 years' age group, 10 (4.76%) in the 6–10 years' age group, and 8 (3.80%) in children aged 11–14 years. Similarly, among the 32 bacterial isolates, the highest number was observed in children aged less than 12 months, accounting for 12 (5.71%) isolates, followed by 10 (4.76%) in the 1–5 years' age group, while both the 6–10 years and 11–14 years' age groups contributed 5 (2.38%) isolates each.

These findings indicate that infants and younger children are more susceptible to both viral and bacterial infections compared with older children.

Table 5. Distribution of Co-Infections Among the Study Population (n = 210)

S. No.	Type of Co-infection	Number	Percentage (%)
1	Viral + Viral	06	2.85
2	Viral + Bacterial	12	5.71
Total	Co-infection Cases	18	8.56

Co-infection was observed in 18 (8.56%) of the 210 cases studied. Among these, viral-bacterial co-infections were more common, accounting for 12 (5.71%) cases, whereas viral-viral co-infections were detected in 6 (2.85%) cases. The findings indicate that mixed infections, particularly those involving both viral and bacterial pathogens, constitute a significant proportion of respiratory infections in the study population.

DISCUSSION

Acute respiratory infections (ARIs) represent a major public health concern in pediatric populations, with the majority presenting as upper respiratory tract infections (URTIs). Most ARI cases involve the upper respiratory tract and are predominantly of viral origin while bacterial pathogens play a critical role in severe lower respiratory tract infections (LRTIs). [16] Respiratory infections have become increasingly common in infants and are associated with significant morbidity worldwide. Hospitalization become necessary for 1 to 2 percent of newborns with LRTI (18). In these situations, antigen detection tests or PCR should be used to obtain a viral diagnosis at presentation in order to guide patient cohorting decisions and prevent nosocomial infections. (19)

Viral infections are ubiquitous and may manifest with or without symptoms such as fever and respiratory distress. The clinical differentiation between bacterial and viral infections is often difficult, which has led to the frequent and sometimes inappropriate use of antibiotics. Rapid viral detection has the potential to guide antiviral therapy, reduce unnecessary and invasive diagnostic testing, facilitate appropriate patient cohorting, and prevent

nosocomial transmission (18). Despite the burden of disease, there is a limited number of studies and a notable scarcity of data regarding the causative agents of these infections in Indian children. Furthermore, data from different regions in India remain fragmented and often lack reliability due to the absence of diagnostic tests with high sensitivity and specificity, limiting the simultaneous detection of a broad spectrum of pathogens (20). For bacterial pathogens, however, culture remains the primary diagnostic modality, as PCR often fails to distinguish between mere colonization and active infection, many studies have reported similar bacterial DNA loads in both cases and controls (21).

India has one of the highest rates of pneumonia and childhood death from ARI worldwide due to its large population, varied geography, and seasonal fluctuations. [21] The prevalence of Acute Respiratory Infections (ARI) among children is relatively higher in the northern states of India, Jammu and Kashmir (6.4%), Uttarakhand (4.9%), Uttar Pradesh (4.7%), and Punjab (4.6%) reporting some of the highest rates. [23]. According to the National Family Health Survey (NFHS-5), which was carried out between 2019 and 2021, ARI was responsible for almost 14.3% of fatalities in young children and 15.9% of deaths in children between the ages of one and five. Uttar Pradesh (UP) contributes significantly to the yearly national burden of ARI cases among children under five years of age (NFHS-5) among all Indian states [24].

In the present study we analyzed various etiological agents causing respiratory infections in critically ill infants and children (NICU/PICU). Among the 210 specimens analyzed, respiratory viruses were detected in **56(26.6%)** cases by multiplex RT-PCR and 32(15.23%) bacterial pathogens by combined conventional and molecular approach. The viral detection rate of 26.6% observed in the present study is consistent with findings from other Indian studies. Jambagi et al. and Barchha et al. reported a substantial viral contribution to acute respiratory infections requiring PICU admission, with prevalence estimates comparable to those observed in our study. [25,26] Similarly, Bharaj et al. documented a viral detection rate of 35.2% among children hospitalized with lower respiratory tract infections in Delhi. [27] Comparable findings have also been reported internationally, with Moynihan et al. demonstrating a viral positivity rate of 21% among critically ill children admitted to pediatric intensive care units. [28] Collectively, these studies highlight the significant role of respiratory viruses in severe pediatric respiratory infections and corroborate the viral prevalence observed in the present study.

Consistent with both global and regional epidemiological patterns, the highest incidence was observed among infants aged 0–1 year, likely reflecting their increased susceptibility to respiratory infections due to immunological immaturity and anatomical predisposition [19]. This substantial disease burden emphasizes the need for targeted preventive interventions in this particularly vulnerable population. Similar findings have been reported in other studies, which also demonstrated the greatest burden of respiratory infections in children aged ≤ 1 year, further supporting the value of multiplex molecular diagnostics in accurately defining the spectrum of respiratory pathogens. [29–31] These observations highlight the clinical importance of rapid and precise pathogen identification in young infants. Nevertheless, despite the improved pathogen detection rates achieved with multiplex PCR, empirical antibiotic use in the PICU remains high, indicating a persistent gap between enhanced diagnostic capabilities and their translation into clinical decision-making [32].

In our study, the most prevalent virus was RSV (11.42%). The trends were followed by Rhinovirus (5.71%), Adenovirus (4.76%), and Influenza B virus (1.90%). This corroborates global data highlighting RSV as the leading viral pathogen in pediatric lower respiratory tract infections [33]. The high prevalence of RSV in infants under one year is consistent with the known role in causing bronchiolitis and pneumonia, especially in neonates and young infants, who are immunologically vulnerable [34,35]. In India, RSV prevalence rates ranging from 20–40% have been reported in hospitalized children with ARI [36]. Rhinoviruses are frequently reported as common causes of upper respiratory infections and exacerbations of asthma in children [32]. On a contrary, National surveillance data from six sentinel sites (2016–2018) identified influenza viruses as the most frequently detected pathogens in pediatric ARI (15.4%) and SARI (12.7%) cases, followed by human rhinovirus (ARI 6.2%, SARI 6.1%) and respiratory syncytial virus (RSV) (ARI 5%, SARI 8.2%) [22]. Although Influenza A typically garners more focus due to its pandemic potential, Influenza B has also been recognized for causing significant morbidity in children [36]. This supports the necessity of including both strains in annual influenza vaccination programs to maximize protection, especially in regions with year-round circulation and seasonal peaks [38].

Additionally, coinfections have been increasingly documented in infants [32], with earlier studies reporting co-infection rates ranging from 2% to 82% (33–37). Similar to this data, in our study, co-infection was detected to be 2.85% (RSV/Rhinovirus as 1.90% and RSV/Adenovirus 0.95%). The clinical impact of such coinfections remains debated. While some studies indicate that coinfections contribute to increased disease severity (39), others report no significant difference in clinical outcomes between single and multiple pathogen infections. [40] Of the 210 cases that were included in the study, 18 (8.56%) had co-infections. Viral-bacterial co-infections accounted for twelve (5.71%) of these, whereas viral-viral co-infections were found in 6 (2.85%). These results imply that a significant percentage of respiratory infections in the study population are mixed infections, particularly those involving both viral and bacterial pathogens. Culture-based diagnostics revealed 15.23% bacterial isolates. The most prevalent being *Streptococcus pneumoniae* (6.6%), followed by *Klebsiella pneumoniae* (3.80%), *Escherichia coli* (0.95%) and *Acinetobacter baumannii* (0.95%). Furthermore, viral infections can predispose individuals to secondary bacterial infections by enhancing bacterial adhesion to respiratory epithelial cells, thus promoting coinfection. Thus, early and accurate diagnosis of both single and mixed infections remains essential to ensure appropriate therapeutic interventions and judicious drug use, particularly in

vulnerable infant populations. The use of rapid molecular PCR diagnostics has been shown in numerous studies to reduce unnecessary antibiotic usage (29). Moreover, the inappropriate administration of antivirals such as Oseltamivir may also be avoided through timely and accurate diagnostic testing. Therefore, molecular techniques for pathogen detection hold great promise in improving diagnostic precision and reducing the unwarranted use of antibiotics and antivirals (30,31). Consequently, there is an urgent need to demonstrate the clinical benefits of these novel, sensitive, and rapid molecular assays through well-designed studies (31). Such advancements would enable clinicians to optimize antibiotic stewardship and ensure timely and appropriate supportive care for immunocompromised paediatric populations.

Our results emphasize the **critical importance of integrating molecular diagnostics** for rapid viral detection with **conventional culture-based methods** to ensure comprehensive identification, especially when managing critically ill pediatric patients. Molecular detection of viruses allows timely therapeutic and isolation decisions while culture methods remain essential for uncovering bacterial pathogens and guiding antimicrobial management. A **dual diagnostic approach**, coupled with robust AMR surveillance and stewardship measures, is vital for informed clinical decision making and improved outcomes in this vulnerable population.

CONCLUSION

The findings of this study indicate that the clinical integration of such advanced diagnostics should be accompanied by rigorous stewardship programs to optimize antimicrobial usage in the high-acuity NICU and PICU environments. Ultimately, the implementation of these rapid diagnostic platforms facilitates a shift toward precision medicine, enabling clinicians to mitigate the risks of empirical therapy while improving surveillance of circulating microbial patterns. Furthermore, establishing standardized protocols for longitudinal surveillance can provide the epidemiological data necessary to guide seasonal immunization efforts and inform local antimicrobial stewardship policies. Given that co-infections are prevalent in up to 64% of symptomatic cases, diagnostic protocols should shift toward routine, broad-spectrum multiplex panels to capture both viral and bacterial loads simultaneously. Such comprehensive testing is particularly vital for identifying frequent viral-bacterial co-infections, which are often linked to more prolonged disease courses and increased clinical severity. Consequently, routine implementation of these rapid diagnostic panels at the point-of-care, rather than relying on centralized laboratory turn-around times, can significantly optimize the timing of antiviral administration and antibiotic de-escalation. Moreover, prioritizing the integration of syndromic panels into standard admission procedures will enable clinicians to transition from broad-spectrum empirical treatment to pathogen-directed therapy, thereby reducing unnecessary antibiotic exposure. Integrating these high-sensitivity molecular arrays into daily clinical workflows empowers multidisciplinary teams to refine therapeutic interventions and diagnostic accuracy in intensive care settings. Furthermore, adopting these high-sensitivity platforms allows for earlier implementation of infection control measures, which is critical in preventing the transmission of respiratory pathogens in the high-risk environment of the NICU.

ACKNOWLEDGEMENT

All authors are thankful to NIMS University, Jaipur, Rajasthan and Sarojini Naidu Medical College Agra, Uttar Pradesh for providing necessary support and laboratory facility for conducting these research work.

Statements and Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Disclosures and declarations

All authors declare that this work has not got any financial assistance from funding agency.

Ethical Responsibilities of Authors

All authors declare that this research article is original and has not been published elsewhere in any form or language (partially or in full).

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Declaration of Generative AI and AI-assisted technologies in the writing process:

During the preparation of this work, the authors had not used any AI-AI assisted technologies. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data transparency

All authors agree that all data, materials as well as software application has supported their published claims and complied with field standards.

Author contributions

Dr. Sonalika Singh, Dr. Ankur Goyal, and Dr. Neeraj Kumar have contributed to the study conception and design for this research work. Material's preparation, data collection and analysis were performed by scholar, Mr. Vinay Verma, Ms. Neha K. Mani and Dr. Ashish Singh. The first draft of the manuscript was written by Mr. Vinay Verma. Dr. Sonalika Singh, Dr. Ankur Goyal, and Dr. Neeraj Kumar have thoroughly reviewed and approved the final manuscript.

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