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Prevalence of respiratory viruses among children hospitalized with acute respiratory infections (ARIs) in central India.

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ABSTRACT

Background: Acute respiratory infections (ARIs) remain a major cause of morbidity and mortality among children under five years, particularly in low- and middle-income countries. Viruses are the predominant etiological agents, and their circulation is influenced by seasonal and sociodemographic factors.

Objective: This study aimed to investigate the prevalence, seasonal variation, and co-infection patterns of respiratory viruses in hospitalized children under five years of age in the Sagar district of central India from June 2023 to January 2025.

Methods: A hospital-based prospective study was conducted on 417 children presenting with ARIs. Nasopharyngeal/throat swabs were collected and analyzed using real-time reverse transcription PCR to detect 18 respiratory viruses. Demographic, clinical, and environmental data were collected and statistically analyzed.

Results: Viral pathogens were identified in 332 (79.6%) of the samples, with single-virus infections in 225 (53.9%) and co-infections in 49 (14.7%). The most prevalent viruses were human respiratory syncytial virus (hRSV, 35.76%) and human rhinovirus (HRV, 27.81%), which exhibited consistent year-round circulation with winter and autumn peaks. hRSV and HRV accounted for 65% of all infections. Seasonal trends revealed the highest viral diversity during the monsoon. A significant increase in adenovirus and emergence of HPIV4 were noted in autumn 2024, while other viruses like H1N1 and HPIV1 showed complete disappearance. Sociodemographic analysis indicated that younger age, household crowding, undernutrition, lack of breastfeeding, and positive family history of respiratory illness were significantly associated with higher ARI incidence ($p < 0.05$).

Conclusions: hRSV and HRV are the leading viral pathogens in children under five with ARIs in central India, with clear seasonal trends. These findings highlight the need for continuous viral surveillance, targeted vaccination timing (ideally pre-monsoon), and region-specific intervention strategies to reduce ARI burden. Improved diagnostics and epidemiological monitoring are critical for timely management and outbreak preparedness..

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INTRODUCTION:

Acute respiratory infections (ARIs) are considered one of the leading causes of illness and death in children around the world[1,2,3]. The pathogens that cause ARIs vary depending on geographic area and seasonal changes, with viruses being the predominant contributors globally. Human Respiratory syncytial virus (hRSV) is the most commonly found pathogen associated with severe respiratory diseases such as bronchiolitis, worsened asthma, and pneumonia in early childhood, making it a major cause of hospitalization in children under two years of age[4]. Influenza viruses are particularly capable of causing severe respiratory illnesses in very young children, the elderly, and individuals with preexisting chronic conditions[5]. Enteroviruses, including human rhinoviruses (HRV) and human enteroviruses (hEV), have been previously identified in upper respiratory infections in children and are often associated with milder ARIs[6]. Human metapneumovirus (hMPV) is responsible for roughly 5-10% of all acute respiratory infections (ARIs) in both children and adults[7]. Moreover, adenoviruses (hAdv) contribute to 5-15% of respiratory infections in children[8]. Other viruses, such as parainfluenza virus (hPIV) and human coronaviruses hCoV-229E and OC43, can also lead to respiratory illnesses[7]. With advancements in molecular diagnostics, newly identified viruses, including human bocavirus (hBoV), human coronaviruses (hCoV-NL63 and hCoV-HKU1), human parechoviruses (hPeV), and polyomaviruses WU (WUPyV) and KI (KIPyV), have been detected in children suffering from respiratory infections, although the evidence for causation varies[9]. Hospital-based studies conducted over the last decade have shown that viruses are identified in up to 95% of ARI cases in children, with a single virus present in 40-60% of cases and multiple viruses in 1-40% of affected patients[7,8,10]. Coinfection seems to be associated with seasonal variations when multiple viruses are circulating.[11]

Some studies have suggested that the incidence of co-infections does not align with the overall prevalence of individual viruses[12]. Factors such as younger age, male sex, and a history of immunosuppression are linked to an increased likelihood of viral coinfections[11,13,14]. There may be notable interactions among climatic, environmental, and behavioral elements as well as a complex relationship between circulating viruses and population-level immunity regarding viral co-infections. Understanding these factors could help prevent the spread of these infections. Recent etiological research on pediatric respiratory infections has mainly reported the prevalence among hospitalized children and the seasonal trends of viruses, without exploring viral co-infection. Consequently, the significance of identifying multiple viral pathogens in acute respiratory infections (ARIs) remains unclear. In this study, we analyzed 18 respiratory viruses among pediatric inpatients in central India during the years 2023–2024 and their seasonal distribution.

Acute respiratory infections (ARI) are a disease of considerable public health importance. It arises from a variety of organisms that affect human airways[15]. While it can affect individuals across all age groups, the consequences are especially perilous for children under five years of age[16]. Globally, ARIs, primarily pneumonia, result in a 20% mortality rate among children younger than five. When neonatal pneumonia is factored in, the mortality rate increases to 35–40% for under-five children, leading to an estimated 2.04 million deaths each year. The highest incidence of ARI is found in Southeast Asia, followed by countries in sub-Saharan Africa, which collectively contribute to more than 80% of worldwide cases[17]. A range of social and environmental factors play a role in the morbidity and mortality rates of ARI in children. These factors include poverty, inadequate nutrition, poor housing conditions, indoor air pollution (including parental smoking), insufficient ventilation, overcrowding, industrialization, sociocultural values, overuse and misuse of antibiotics, lack of basic health services, and a lack of awareness[18].

Approximately 40 million cases of acute respiratory infections (ARI) are reported annually in India. These infections represent a considerable share of healthcare usage, accounting for 30-60% of all outpatient visits and 20-40% of pediatric hospital admissions[19]. Research in developed nations has shown that younger siblings of school-aged children frequently experience higher infection rates as older children bring pathogens into their homes. Children of lower socioeconomic backgrounds are generally more vulnerable to these infections. Pre schoolers who attend day care centers are also at an increased risk. Notably, urban populations typically have a higher incidence of these infections than rural communities[19].

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Therefore, the present study was carried out to assess the prevalence of ARI among children aged 1-5 years living in the Sagar district and to explore any associations between ARI and sociocultural and sociodemographic factors.

An estimated 1.9 million childhood ARI deaths are reported in developing countries, of which 20% occur in India. In India, approximately 14.3% of infant deaths and 15.9% of deaths in under-fives were caused by ARIs. Factors associated with high mortality and morbidity in childhood ARI include poverty, overcrowding, poor nutrition, poor air quality, and misuse of antibiotics[20].

The 21st century has witnessed multiple emerging and re-emerging respiratory viruses, such as the ongoing SARS CoV-2 pandemic, influenza A/H1N1pdm09, avian influenza (H5N1), severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome coronavirus {MERS-CoV}[21,22]. Improved surveillance and the employment of sensitive detection systems have helped detect and control emerging viruses.

The clinical diagnosis of respiratory tract infections is difficult because of the overlap of symptoms caused by different respiratory viral and bacterial pathogens. ARI bacterial agents have largely been controlled by an effective combination of sensitive diagnostic systems, antibiotics, and vaccines. Viral ARIs are the most common reasons for hospitalisation of children in India yet their aetiology and burden in children <5 years remains poorly understood due to the absence of affordable and efficient diagnostic systems [21,22,23]. India has a substantial burden of acute respiratory infections in all age groups. The present study aimed to describe the prevalence and seasonal patterns of respiratory viruses in a hospital-based surveillance over a period of 20 months (June 2023 – Jan 2025) in children under five years of age.

MATERIAL AND METHODS:

Study site and study population:

This prospective observational study was performed over 16 months, from June 2023 to January 2025, at the Virology Laboratory, Department of Microbiology, Bundelkhand Medical College Sagar, which is recognized as the largest tertiary care hospital in the Sagar Division of Madhya Pradesh. A total of 444 subjects aged < 5 years presented with acute respiratory illness (ARI) and were admitted to the inpatient department (IPD). Conversely, patients with severe acute respiratory infection (SARI) were admitted from the emergency room, pediatric intensive care unit (PICU), and high-dependency unit (HDU) ward of the Department of Pediatrics at Bundelkhand Medical College Sagar. Among these individuals, 27 were rejected because they did not fulfill the inclusion criteria and because of inappropriate sample handling. Real-time PCR analysis was performed on the remaining 417 samples. The study protocol was approved by the Institutional Ethics Committee of the Bundelkhand Medical College, Sagar, M.P.. (EC ID: IECBMC/EC/2023/125). As the participants were children under the age of five, their parents were informed about the study in their native language and provided with a patient information sheet in the same language. Before enrolling the children in the study, written consent was obtained from their parents, ensuring adherence to ethical standards and safeguarding the rights of the participants. All identifiers were anonymized for analysis and the samples were collected by qualified medical professionals.

Inclusion and Exclusion criteria

Participants in the study were recruited from the age group of 1–60 months, with parents providing informed consent for their participation. This study specifically excluded any previous cases of respiratory infections to eliminate the possibility of detecting residual nucleic acids. Children over 60 months of age with a history of respiratory infections or other chronic conditions, including HIV, Tuberculosis, cardiac failure, primary cardiac failure, severe metabolic acidosis without signs of respiratory tract infection (RTI), empyema, hydropneumothorax, or tuberculosis, as well as non-respiratory causes of respiratory distress, underlying chronic conditions, hospitalization exceeding 7 days, and lack of written consent from parents were excluded from the study.

Sample collection:

All swabs were collected in accordance with WHO guidelines, utilizing a plastic-shaft, rayon-budded swab placed in a transport tube containing a foam pad reservoir saturated with a viral transport medium, specifically the HiViral Transport Kit (HiMedia, Cat. No: MS2760A-50NO). Proficient clinical personnel collected nasal and/or throat respiratory specimens from enrolled patients with ARTI. Subsequently, these samples were transported while maintaining the cold chain to the VRDL lab at the Department of Microbiology to ensure their integrity and were stored at -80°C until further analysis. The VTM samples were subjected to batch testing for 18 viruses and 14 bacteria using validated real-time PCR assays.

Nucleic acid extraction was conducted utilizing the Macherey-Nagel™ NucleoSpin™ Virus kit (Takara Bio USA, Inc Cat no.) in accordance with the manufacturer's guidelines. Viral nucleic acids

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(DNA/RNA) were extracted and purified from 200µl of VTM sample viral RNA collected from VTM samples using a DNA Nucleospin Virus kit (MACHEREY-NAGEL GmbH & Co, Germany). In summary, 5 µL of liquid proteinase K was added to 200 µL of the sample and mixed gently, followed by the addition of 200 µL of VL Buffer to 1.5 ml microcentrifuge tubes, which were vortexed for 15 s. Subsequently, 5.6 µl of Carrier RNA was added to the mixture and vortexed for another 15 s. The suspension was incubated at room temperature (15-25°C) for 3 min, after which brief centrifugation was performed to eliminate droplets from the inner side of the lid. Absolute ethanol (200 µL) was then added to the sample and mixed by vortexing for 15 s, followed by another brief centrifugation step to remove droplets from the inner side of the lid. Careful application of 610µl of the solution to the nucleospin virus column was performed using a 2 ml collection tube, ensuring that the rim remained dry. The cap was secured and centrifuged at 4000 g for 3 min. The Nucleospin virus column was subsequently placed into a new 2 ml collection tube, and the tube containing the filtrate was discarded. The Nucleospin virus column was then opened with care, and 400µl of VW1 Buffer was added, followed by centrifugation at 11000 g for 30 s. This was succeeded by replacement with a fresh 2 ml collection tube, discarding the tube containing the filtrate. 400µl of VW2 Buffer was added to the column and centrifuged at 11000 g for 30 s. Following this, a fresh 2 ml collection tube was used to replace the previous one, discarding the tube containing the filtrate. The Nucleospin virus column was opened carefully, and 400µl of VW2 Buffer was added, followed by centrifugation at 20,000 g for 5 min. Finally, the Nucleospin virus column was placed in a sterile, DNAase, RNAase-free 1.5 ml microcentrifuge tube. The previous collecting tube that held the filtrate was disposed of and substituted with a new 1.5 ml microcentrifuge tube (MCT). 30µl of RNase-free water was introduced into the nucleospin virus column and allowed to equilibrate at room temperature for 3 min, after which centrifugation was performed at 20,000 × g for 3 min. The eluate collected in the MCT contained DNA/RNA and was preserved at -80°C until further use.

Multiplex Real Time PCR for Respiratory Pathogens:

The nucleic acid that was purified and extracted underwent amplification utilizing the TRUPCR Respiratory Pathogen Panel kit (3B BlackBio Biotech India Ltd., version 1.0) on the CFX 96 Bio-Rad Real-time PCR (Bio-Rad Laboratories, CA, USA) for the identification of 18 respiratory viruses. These included Human Parechovirus, Human coronavirus (both alpha and beta strains), Human Parainfluenza viruses 1, 2, 3, and 4 (hPIV-1, 2, 3, 4), Influenza A virus (Inf A), Enterovirus, Influenza A (H3N2), Human Metapneumovirus (A/B) (hmPV), Pandemic H1N1 Influenza virus (InfV), Influenza B virus (Inf B), Influenza C virus (Inf C), Human Adenovirus (hAdv), Human Respiratory Syncytial Virus (A/B) (hRSV), Human Rhinovirus (Hrv), and Human Bocavirus (hBoV). The assay included *RNaseP* gene as an endogenous internal control. The reaction mixture consisted of 9.65 µl of primer-probe mix, 10 µL of master mix buffer, 0.35 µl of SuperScript III enzyme, and 5 µL of nucleic acid templates, resulting in a total volume of 25 µL for each PCR reaction. Thermal cycling was conducted under the following conditions: reverse transcription at 50°C for 20 minutes, initial denaturation at 94°C for 10 minutes, followed by 40 cycles comprising two stages (10 seconds at 94°C and 60 seconds at 60 °C, with data acquisition) on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific MA, USA). A PCR result was classified as positive or negative based on whether the Cq value was below or above 36 cycles, respectively, with the positive control yielding the expected Cq value of 20 ± 4, the negative control being negative, and the internal control reflecting the anticipated Cq value of 32 cycles.

SARS-CoV-2 was identified utilizing the Viral Detect –II Multiplex Real-Time PCR kit (GENES2ME, HR, India Cat no. G2M020220). In particular, 10 µl of 2x one step Master Mix was mixed with 1 µl of primer probe mix, followed by the addition of 9 µl of RNA to the 11 µl reaction mix in accordance with the kit's instructions. Real-time PCR was conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific MA, USA). The thermal cycling protocol was as follows: reverse transcription at 55°C for 10 minutes, initial denaturation at 95°C for 3 minutes, and then 40 cycles comprising two phases (10 seconds at 95°C and 60 seconds at 60°C, with data acquisition). A Ct value of 35 is to be regarded as the threshold for distinguishing between positive and negative samples.

Statistical Analysis

The data collected from the patients was analyzed using Epi-info 7.2. Various statistical tests, including the Chi-square test and Fisher's exact test, were employed for categorical data. Descriptive statistics, such as percentages, means, and standard deviations, were calculated for continuous data. Inferential statistical tests, including the Chi-squared test and Fisher's exact test, were utilized to investigate factors related to

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ARI in children under the age of five. A p-value of less than 0.05 was considered statistically significant. The results were presented through appropriate tables and graphs.

RESULTS:

Study Population and Demographic Characteristics:

Around 417 samples fulfilling the inclusion criteria and with appropriate quality were analysed through reverse transcriptase polymerase chain reaction (RT-PCR) analysis.The demographic distribution of the study population revealed a male predominance, with 287 participants (68.82%) being male and 130 participants (31.17%) being female, yielding a male-to-female ratio of approximately 2:1. The age distribution ranged from 1 month to 60 months, with the highest prevalence observed in infants aged 1 to 12 months, representing 51.55% of the total cohort. The comprehensive socio-demographic and clinical profiles of the ARI patients (n = 417) are systematically presented in Table 1.

Socioeconomic Risk Factors and ARI Prevalence:

The analysis of socioeconomic determinants revealed several significant associations with ARI prevalence. Age emerged as a critical factor, with younger children, particularly those in the 1-12 month age group, demonstrating a markedly elevated ARI prevalence of 51.55%. Maternal characteristics showed notable correlations with infection rates: mothers younger than 25 years of age were associated with higher ARI rates (72%), while maternal unemployment status corresponded to an ARI prevalence of 70% (Table 1).

Interestingly, the distribution of ARI cases across different socioeconomic strata demonstrated relative uniformity, with comparable rates observed across all three socioeconomic classes, suggesting that economic status may not be the primary determinant of infection risk in this population. However, household composition emerged as a significant risk factor, with families comprising more than 10 members showing a substantialy elevated ARI prevalence of 90.9%.

Perinatal and Birth-Related Risk Factors: Birth weight analysis revealed an Ushaped relationship with ARI occurrence. Children born with extremely low birth weight (<1500g) demonstrated the highest ARI prevalence at 83.3%, while those with birth weights exceeding 4 kg showed an occurrence rate of 72.7%. Birth spacing intervals also demonstrated significant associations, with children born within 1-2 years of a previous sibling exhibiting an ARI prevalence of 80.6%. Birth order analysis indicated that second born children faced an elevated risk, with an ARI occurrence rate of 72.5%. Additionally, families with more than two children under five years of age displayed a higher ARI prevalence of 77.4%, suggesting that the presence of multiple young children in a household may contribute to increased transmission risk (Table 1).

Familial and Community Transmission Factors:

The presence of similar illness within the family or community environment, coupled with viral positivity, demonstrated statistically significant associations with ARI occurrence ($p < 0.05$). This finding underscores the importance of household and community-level transmission dynamics in the epidemiology of acute respiratory infections among pediatric populations.Statistical significance was determined at $p < 0.05$ level. Detailed statistical analyses and complete demographic breakdowns are presented in Table 1.

Table 1 Demographic and Socioeconomic Variables

		ARI condition		
Variables	-	present	Absent	p-value
Study cohort, n=417 (100 %)	-	405	12	-
Age (Years)	1-12	204 (34)	23 (11)	0.345
	13-24	115 (84)	21 (16)	
	25-36	48 (82)	10 (18)	
	> 36	7 (87)	1 (15)	
Gender	Male	208 (72)	80 (28)	0.482
	Female	98 (76)	31 (24)	
Mothers age	≤ 25 years	157 (72)	62 (28)	0.246
	26-30 years	97 (67)	47 (33)	
	> 30 years	32 (58)	24 (42)	
Mothers education	Illiterate	16 (48.4)	13 (39.3)	0.334
	Literate	265 (68.29)	123 (31.70)	
Mothers occupation	Unemployed	208 (70)	88 (30)	0.168
	Employed	63 (52)	58 (47)	
Socioeconomic status	Lower class	68 (65.38)	36 (34.61)	0.762
	Middle class	147 (63.36)	67 (28.87)	
	Upper class	54 (66.6)	27 (33.3)	
Type of Family	Joint family	287 (74.2)	100 (25.83)	0.579
	Nuclear Family	21 (72.6)	9 (30)	
Birth space between children	< 1	12 (60)	8 (40)	0.408
	1-2 yrs	27 (71)	11 (29)	
	> 2 years	81 (72)	32 (28)	
	None	163 (67)	83 (33)	
Siblings	Present	159 (75.71)	51 (24.28)	0.079
	Absent	129 (61.42)	78 (37.14)	
Number of under five children	1	14 (73)	5 (26)	0.050
	2	244 (90)	28 (10)	
	> 2	98 (78)	28 (22)	
History of	Present	66 (67)	32 (33)	0.548

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smoking in household	Absent	203 (64)	114 (317)	
Breastfeeding	Yes	210 (67)	102 (32)	<0.05
	No	44 (42)	61 (58)	
Nutritional status	Under Nourished	12 (41)	17 (58)	<0.05
	Normal	323 (83)	65 (17)	
Birth weight	≥ 2.5	215 (68)	102 (32)	0.865
	≤ 2.5	64 (70)	28 (30)	
	< 2	7 (88)	1 (12)	
Family history of similar illness	Present	140 (63)	79 (36)	<0.05
	Absent	23 (12)	175 (88)	

Viral Etiologies in Acute Respiratory Infections: The reverse transcription polymerase chain reaction (RT-PCR) method successfully identified 332 positive samples, representing 79.6% of the total sample population. Human respiratory syncytial virus (HRSV) emerged as the most frequently detected pathogen, identified in 108 out of 417 cases (25.9%). This molecular diagnostic approach demonstrated the capability to detect both single infections and complex coinfections, providing comprehensive pathogen identification capabilities essential for clinical management and epidemiological surveillance.The viral distribution analysis revealed distinct prevalence patterns among respiratory pathogens. The most commonly identified viral agents included hRSV (35.76% of positive cases), human rhinovirus (HRV, 27.81%), human adenovirus (hAdv, 6.29%), and influenza B virus (Inf B, 3.6%). These four viral pathogens collectively accounted for approximately one third of all positive detection results, highlighting their significance as primary etiological agents in acute respiratory infections. Infection pattern analysis revealed important epidemiological characteristics of respiratory viral infections. Single viral infections were identified in 225 samples (53.95%), while multiple viral infections occurred in 49 samples (11.7%).

Rhino virus (39.3 %), HRSV (22.5%) and Adeno (12.6%) virus were dominant during winter season and rhino virus is dominant with 39.3% mean with highest in all seasons and reaches to peak 48% in November. Highest diversity of viruses was observed during monsoon with seven different viruses which may be certainly due to hot and humid weather. This Highest viral diversity requires strict surveillance. Inf A (H3N2) activated during monsoon has significant monsoon pattern.

During autumn dual dominance of HRV (41.4%) and hRSV (35.6%) was observed. Moreover, we observed a 58% increase in hRSV as compared to monsoon. Rhino virus emerged as a dominant pathogen during autumn with a 23.7% increase and reaching to 48% prevalence. Strikingly, there is a dramatic increase of 166.6% was observed in case of adenovirus in 2024 as compared to previous year which requires continuous monitoring for potential outbreaks. However, hRSV maintained as a major pathogen with slight 5.2% increase in 2024. Surprisingly, there is an emergence of HPIV4 in autumn 2024 with 5.55% prevalence. Remarkably there is a complete disappearance of InF A (H1N1), HPIV3, Enterovirus and HPIV1 in 2024. Strong increase in Rhinovirus in 2024 suggests enhanced circulation, possibly due to viral evolution. Whereas, Complete absence of H1N1 while persistence of H3N2 indicates a significant seasonal influenza pattern change. Additionally, disappearance of HPIV1 and HPIV3 coupled with HPIV4 emergence suggests an altered viral ecology.

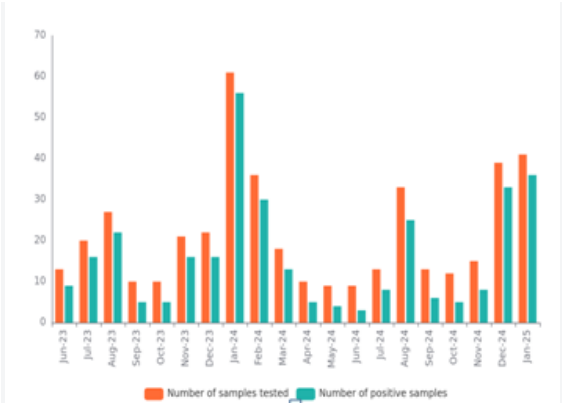

Figure: 1 Virus distribution by months

Figure 1 displays the month wise recruitment of ARI cases alongside the positive samples for viruses. The peak recruitment and positive samples were recorded in January and February of 2023, as well as in December and January of 2024, with a notable seasonal peak in August 2024.

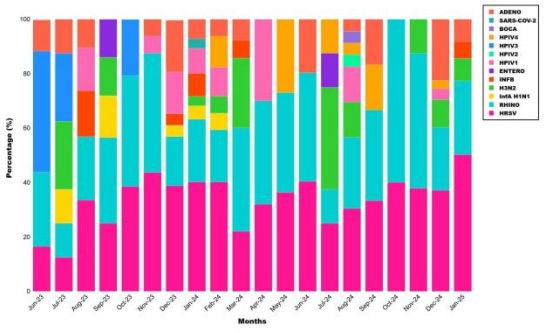

Figure: 2 Virus distribution by months

Analysis across seasons revealed a two tier viral circulation pattern over the study period. Human Respiratory syncitial virus emerged as the dominant pathogen (33.52% average circulation) with autumn-winter peaks with 50% and 52% peak

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prevalence in January 2023 and 2024 respectively. Rhinovirus ranked second (31.26% average) with winter and spring peaks.

Together, hRSV and rhinovirus accounted for 65% of respiratory illness burden, maintaining 100% activity rates with consistent year-round circulation. In contrast, episodic pathogens including H3N2 (8.24% average), adenovirus (7.1% average), and HPIV1 demonstrated 40-65% activity rates with outbreak-driven patterns (Figure 2)

Adenovirus showed predictable winter vulnerability with peak outbreak activity (24.5% in December 2024), while H3N2 displayed unpredictable seasonal patterns with an unusual July 2024 peak. The remaining 11 viral pathogens showed ≤ 25% activity rates, representing minimal clinical impact. Maximum viral diversity (7 pathogens) occurred during monsoon season (Figure 2)

These findings support stratified surveillance strategies: continuous monitoring for hRSV and Rhinovirus, outbreak detection protocols for episodic pathogens, and routine surveillance for low-activity viruses.

DISCUSSION:

Acute lower respiratory infection (ALRI) stands as the foremost cause of illness among children under five years old worldwide, with around 156 million new cases each year [24]. Severe ALRI cases impose a significant burden on the global healthcare system, serving as the primary reason for pediatric referrals and hospitalizations [25]. Acute respiratory infections (ARIs) predominantly affect the pediatric population, occurring three to eight times annually in infants and young children, with frequency diminishing as they age[26,27].While substantial regional differences are anticipated in large countries with diverse climates, these variations may also stem from differences in study design.

In India, acute respiratory tract infections (ARI) account for 69% of all communicable diseases, with severe acute respiratory infections (SARI) that significantly contribute to mortality among children less than five years of age[20]. Research on pediatric ARI in India is limited, especially regarding prevalence, surveillance, disease burden, and diagnostics for viral respiratory illnesses. This study aimed to investigate the clinical and viral profiles of ARI in children under five at a tertiary care center in Sagar, M.P. A statistically significant difference was observed in the number of male and female participants ($p < 0.05$), which is consistent with previous studies[28].

The understanding of how common viral lower respiratory tract infections (LRTIs) are among infants in central India is limited, primarily because of a lack of published studies. This report employs cutting-edge technology to identify viral agents to bridge this knowledge gap. Research revealed that up to 65% of samples from infants with respiratory symptoms were positive for either single or multiple viral infections, with hRSV, HRV, Inf B (H3N2), HPIV1, and hAdv being the most frequently detected viruses. hRSV (A/B) was found in 35.5% of the samples as a single infection, which is consistent with previously reported rates of 10–58% [29,30,31,32,33]. This indicates that maternal antibodies may not sufficiently protect infants from hRSV infection, leading to a significant illness.

In India, the routine laboratory diagnosis of viral acute lower respiratory infections (ALRI) is both inadequate and under-researched, even in advanced healthcare facilities. The viral causes of ALRI often remain undetected, leading to the frequent empirical use of antibiotics. Although viral isolation and RT-PCR are renowned for their high sensitivity and are considered the gold standards, these tests are rarely performed in clinical diagnostic laboratories in low- and middle-income countries (LMICs) because of their high costs[34]. The early detection of these conditions is crucial for timely management and is essential for addressing ALRIs. In India, antibiotics are commonly prescribed for pediatric ALRI even when a viral cause is suspected. A Cochrane review examining the effectiveness of antibiotics in children under two years with bronchiolitis found insufficient evidence to support their use[35]. Antibiotics should only be administered when there is clear evidence of secondary bacterial infection[36]. Early diagnosis of viral respiratory infections could help reduce the overuse of antibiotics. Understanding epidemiology enhances the awareness of pathogens, aids in accurate diagnosis, and ensures timely management. Our findings revealed that approximately 16% of the samples were coinfected with multiple viruses. However, no significant differences in clinical symptoms or laboratory results have been observed between single viral infections and confections, and the clinical significance of confections remains uncertain.

For many years, researchers have explored and debated the factors contributing to the seasonality of respiratory viral infections. Temperature and humidity can affect the stability and transmission rate of these viruses. Influenza virus circulation varies significantly in the Asia-Pacific region, with

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an unusually high prevalence of influenza B[37]. Overall, influenza B accounted for 31.4 percent of cases in this area[38]. hRSV is responsible for a substantial number of hospitalizations worldwide, affecting both developed and developing nations. Symptoms of hRSV can range from asymptomatic to flu-like symptoms, or even acute respiratory distress[39]. In most studies, the simultaneous detection of two or more respiratory viruses in pediatric patients ranged from 10 to 30% [40, 41]. Previous research has suggested that co-infection with respiratory syncytial virus and human metapneumovirus offers protection against prolonged hospital stays and hypoxia compared to infection with a single virus[41]. Additionally, a study found that children with both rhinovirus and RSV infections had shorter hospital stays than those infected with only hRSV[42]. Co-infections with the lowest incidence rates have been observed in individuals older than 16 years. Each virus follows a seasonal cycle, peaking in specific seasons. A study in the Canary Islands indicated that hRSV infections displayed an epidemiological pattern similar to that of temperate climates[43]. In a previous study, most viral pathogens showed seasonal incidence patterns. Comprehensive studies are required to determine the prevalence and seasonal variability of respiratory viral agents in developing countries [44]. The district's location near the ropic of Cancer results in a subtropical and humid climate (Köppen climate classification Cwa), characterized by hot summers, a moderately wet monsoon season, and cool winters [45]. The Sagar region receives rainfall from the southwest monsoons, and hMPV and Inf B virus were reported during the winter season. During winter (November–February), hRSV and HRV were common, followed by HAdv, while during the pre-monsoon period (March-May), InfA (H3N2) was the common viral etiology among ARI/SARI cases. During the summer monsoon (June-September), hRSV, HRV, H1N1, HPIV3, hAdv, and Inf B, followed by HPIV4, are common etiologies. However, the rhinovirus had two peaks, one significant in October–November and the other in March. Under conditions of high humidity and precipitation with moderate temperatures and rainfall, a surge in respiratory viruses has been reported.

The Sagar district experiences average relative humidity that fluctuates between 25% and 86%. The highest levels of precipitation are typically recorded from June to September[46]. hRSV and InfA viruses thrive within a specific humidity range, whereas hPIV type 3 prefers drier conditions. Cold temperatures are most favorable for hAdv, Inf A and B, hRSV, and hMPV[47]. Our research indicated that the distributions of hRSV, HRV, and hAdv were consistent throughout the year. hMPV and influenza B viruses have been reported during winter. In winter (November–February), hRSV and HRV were prevalent, followed by hAdv, while the pre-monsoon period (March-May) saw Inf A (H3N2) as the predominant viral etiology among ARI/SARI cases. During the monsoon summer (June-September), common viral etiologies include hRSV, HRV, H1N1, hPIV3, hAdv, InfB, and hPIV4. Nevertheless, the rhinovirus exhibited two peaks: a significant peak in October–November and a moderate peak in March. An increase in respiratory viruses has been documented during periods of high humidity and precipitation accompanied by moderate temperatures and rainfall.

In this investigation, respiratory virus infections were documented in 302 of 417 samples, corresponding to a rate of 72.42%. Compared to other studies, a higher infection rate was found among pediatric patients in Saudi Arabia (12 viruses, 109 out of 135, 80.7%) [48], Honduras (16 viruses, 260 out of 345, 75.4%)[49], China (16 viruses, 248 out of 490, 50.6%)[50], and Greece (17 viruses, 428 out of 611, 70.0%) [51]. Our findings are consistent with those of previous research that reported viral detection rates ranging from 47 to 95% [52,53,54,55]. The considerable differences in viral pathogen detection rates may be attributed to the heterogeneity of the study population, genetic variability, types and numbers of viral pathogens tested, and methodologies used for testing[52,56,57]. Most respiratory infections were observed in the 1–5-year-old age group, followed by those older than 1 year (Table 1), whereas a previous study[25] found a higher prevalence of respiratory pathogens in children under 1 year of age. The elevated infection rates in infants and young children may be associated with their underdeveloped or weaker immune systems, less favorable living conditions, increased exposure to pathogens, and inadequate hygiene[52,58,59,57]. The identification of the viral pathogens responsible for diseases is vital for patient management and treatment. The predominant respiratory viruses detected were hRSV (35.7%), HRV (27.81%), hAdv (6.29%), and Inf B (3.6%). A previous study [48] reported the detection rates for hRSV (23.9%), HRV (14.7%), and hAdv (11%) in Saudi Arabia. Another study in China identified influenza viruses (18.50%), hRSV (7.86%), and hAdv (3.47%) as the most common respiratory pathogens [60].

Among various viral infections, HRV (42.85%), hRSV (18.36%), and InfA H3N2 (16.32%) were the most commonly identified. In cases of single-virus infections, hRSV (35.76%), HRV (27.81%), and HAdv (6.29%) were the most frequently

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detected (Table 3). The prevalence of hAdv and hEV was significantly higher than that of other viruses, suggesting that these viruses may facilitate the infection of patients with other viral pathogens. Our study found that the rate of mixed viral infections was 14.7% across all samples. The most frequently coinfected groups were HRV (42.85%), hRSV (18.36%), and InfA, H3N2 (16.32%). Another study reported the detection of multiple viral infections involving rhinovirus, hAdv, and HCoV-OC43[61]. HRV and hAdv have also been identified as primary viral pathogens associated with mixed viral infections in children[62]. These findings further suggest that certain viral groups may promote infection or colonization by other viruses in the same patient. Viral coinfection or the identification of two or more viral pathogens in a single patient may result from asymptomatic persistence or virus shedding[53]. The significant variations in the detection rates of multiple viral infections across different studies may be attributed to differences in study populations, geographical locations, study durations, environmental factors, the number of viral pathogens examined, and variations in diagnostic methodologies[61,63,64]. The similarities in clinical presentation among patients infected with various respiratory viruses complicate the accurate diagnosis of the specific etiological agent based solely on clinical signs.

In this study, hRSV and hPIV1 showed peaks of activity in December, HRV peaked in October, and hAdv peaked in June. Circulation of hRSV, HRV, and AdV was observed throughout the year, with winter seasonal peaks noted for hRSV and hPIV1. An increase in the detection frequency of viral pathogens was observed during the cold and rainy seasons. The highest diversity of viruses was found during the monsoon season, with hRSV and HRV being the most frequently identified pathogens in all seasons. Other studies have reported that most influenza viruses are detected in November and December, with hRSV most commonly detected between December and February[58,63,65,48]. According to a study by Saleh et al. in Riyadh, hRSV A, hRSV B, HRV, and hEV peaked in December, whereas hMPV and hBoV peaked in March and April. HRV, hAdV, and hBoV circulations were noted throughout the year, with winter seasonal peaks recorded for hRSV A and hRSV B[66]. In a study conducted in Rajasthan[67], hMPV (25.7%) and Inf A (19.9%) were the most prevalent SARI cases, whereas in the present study, hMPV and Inf A accounted for only 1.4% and 4.9% of cases, respectively. In India, findings regarding the prevalence of viral etiologies in cases of respiratory illnesses vary. A previously published comprehensive literature review found hRSV (26%) to be the major etiology of respiratory illnesses among children older than 5 years, followed by influenza (11%) and parainfluenza (10%)[68]. However, in South India, hRSV (45.69%) and rhinovirus (17.88%) were identified as the most common viral etiologies for respiratory illnesses among children under 5[69].

Before the onset of the COVID-19 pandemic, the seasonal trends of respiratory virus circulation were consistent with previously published studies, which noted annual peaks of influenza and hRSV during the late autumn and winter months[70,71]. Our research also highlighted that hRSV was more prevalent during winter months. Human respiratory syncitial virus is particularly common among children under five years of age, and we recorded a positivity rate of 28.6% in this demographic, mainly during late autumn and winter. This aligns with an earlier study[72,76] that also observed hRSV-B in the winter and spring months, with a positivity rate of 9.52%. In our study, there was no detection of Parecho, Hcorona, or Inf C in children with ARI and SARI cases. The results of this study also support the positivity prevalence documented in another study[73], which reported rates of 2.4% to 3.5% and 2% to 3.8% in SARI and ARI patients, respectively, when considering our age group population. The rate of respiratory virus coinfections varies significantly across studies worldwide[74,75,76]. In this study, we identified a total of 49 (14.7%) co-infections, which is lower than the findings from a Rajasthan study [67] but higher than those from a pan-India study [73], which reported a 0.4% co-infection rate among SARI cases in children aged < 5 years. The highest coinfection rates in our study were linked to hRSV (42.85%), HRV (18.36%), and Inf A H3N2 (16.32%). Young children, especially infants, have immune systems that are mature. In addition, their smaller lungs and airways make them more susceptible to viruses that affect the respiratory system. We found that patients younger than 2 years of age were more frequently infected than those older than 2 years. According to another study [74], the detection rate of respiratory viruses was highest in children aged one year or younger. Furthermore, another study indicated that the highest rates of viral infections were found in children under five years of age[75].

This study provides valuable insights into the prevalence of acute respiratory infections (ARI) among children under five. The research was strengthened by a robust sample size and the recruitment of participants over a 20-month span, which included two consecutive winter seasons. Nevertheless, this study has certain limitations, such as its focus exclusively on children from the inpatient department, the exclusion of data on virus

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reinfection rates, the collection of samples from a single center, and the absence of an investigation into concurrent or secondary bacterial infections. Additionally, since the research was conducted solely in the Sagar District, the patterns of virus transmission and circulation might not be representative of other regions in India, suggesting that a multicenter study could offer more comprehensive insights.

CONCLUSIONS:

Respiratory viruses are the primary cause of hospitalization for acute lower respiratory tract infections in children under five years old worldwide. To effectively manage the prevention and control of these viruses in India and globally, it is crucial to clarify their regional prevalence, clinical profiles, and seasonal variation. The Sagar district, situated in the north-central region of Madhya Pradesh, is bordered by six intrastate districts and one interstate district, which increases the likelihood of respiratory infection transmission. Additionally, a predominantly humid subtropical climate may significantly influence the transmission dynamics of respiratory viruses in this area. In this study, we assessed the prevalence and varying transmission dynamics, including seasonal patterns, of different respiratory viruses and their clinical presentations. We also documented distinct seasonal epidemic patterns of various respiratory viruses among children less than five years of age. This has clear implications for vaccine administration, which should ideally occur in April – May, before the onset of the monsoon season in this region. These seasonal patterns may help to reduce the disease burden of various respiratory viruses in the ARI/SARI pediatric population through the development of vaccines and/or therapies. These findings underscore the need for ongoing surveillance of respiratory viral infections. Such research is crucial for improving and optimizing diagnostic strategies and developing approaches for preventing emerging respiratory viral infections. Seasonal data would be beneficial in predicting and preventing respiratory virus outbreaks.

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