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Short Communication

Emerging lineages A2.2.1 and A2.2.2 of human metapneumovirus (hMPV) in pediatric respiratory infections: Insights from India

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ABSTRACT

Objectives: Human metapneumovirus (hMPV) is recognized as a significant cause of acute respiratory infections among infants under 5 years of age.

Methods: Nasal swabs collected from January 2021 to June 2024 were screened to detect hMPV using reverse transcription-quantitative polymerase chain reaction. Furthermore, representative positive samples were sequenced and subjected to phylogenetic analysis.

Results: Of 4519 samples tested, 113 were positive for hMPV. Notably, an outbreak occurred between November 2022 and March 2023, where 56 of 583 (9.6%) patients tested positive. Analysis of the outbreak samples revealed that majority (6.3%) of cases occurred in December and January. hMPV infection was more prevalent in less than 1 year, with 29 (67%) patients with a history of wheezing and 3 (6.9%) with seizures. On the genetic analysis of F protein, 37 samples identified two genotypes as A and B, with subclusters of 29 (85.29%) samples to A2.1, 1 (2.94%) to A2.2.1, and 4 (11.76%) to A2.2.2 within genotype A and one sample clustered with B1 and 2 samples to B2 within genotype B.

Conclusions: The study underscores the significant prevalence and genetic diversity of hMPV in children in Puducherry, India. Notably, the identification of novel A2.2.1 and A2.2.2 lineages highlights the evolving nature of hMPV.

Introduction

Acute respiratory infections (ARIs) pose significant global health challenges, particularly, in children under five years old in developing countries, with 13 million cases reported annually [1,2]. Human metapneumovirus (hMPV) is a prominent cause of upper and lower respiratory tract infections across all age groups, in which 5-7% of hMPV infections occur in infants [3]. hMPV is classified into genetic groups A and B, further divided into lineages A1, A2.1, A2.2.1, A2.2.2, B1, and B2 based on the F gene sequence, demonstrating significant antigenic diversity [4]. Recent studies have identified novel subtypes such as A2.2.1 and A2.2.2, highlighting its genetic variability [4]. The global COVID-19 pandemic has influenced the spread of seasonal respiratory viruses, including hMPV, prompting the need for comprehensive epidemiologic studies [5]. Despite its known prevalence worldwide, there are limited data on hMPV in Puducherry, South India. This study aims to fill this gap by investigating the prevalence, genetic diversity, and clinical characteristics of hMPV during outbreak periods in Puducherry to better inform public health interventions and preparedness for respiratory outbreaks.

Methodology

Children aged ≤5 years with symptoms of influenza-like illness and sub-ARIs were enrolled at JIPMER, Puducherry, India from January 2021 to June 2024 and children aged >5 years were excluded (IEC No JIP/IEC/2021/341). Nasopharyngeal swabs in viral transport medium were processed, where RNA extraction utilized the HiPurA Viral RNA kit (Himedia, India) on the KingFisher Flex system (Thermo Scientific, US). Samples were screened for respiratory pathogens including hMPV, influenza A (H1N1, H3N2), influenza B, and respiratory syncytial virus. hMPV was detected by targeting the nucleoprotein gene; during the outbreak, samples with a cycle threshold (Ct) <35 were further characterized by sequencing the fusion protein using established primers [6,7].

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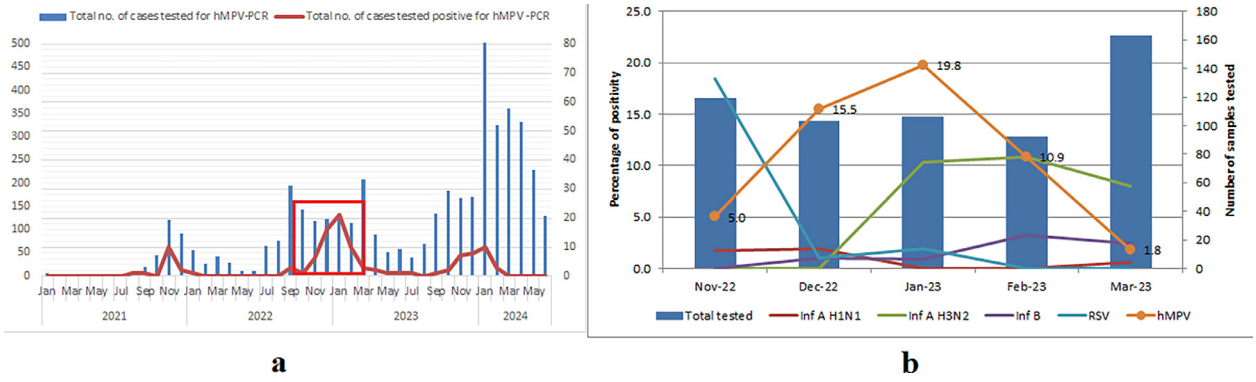

Figure 1. (a) Distribution of hMPV from 2021 to 2024. (b) Distribution of respiratory viruses among children aged 0-5 years from November 2022 to March 2023. hMPV, human metapneumovirus; RSV, respiratory syncytial virus.

Site-specific selection pressures were evaluated by using the (SLAC) method and (MEME), with a *P*-value cutoff of 0.1. Phylogenetic analysis of partial F gene in MEGA v.11 by neighbor-joining method. Amino acid variations were assessed against NC_039199.1. Predictions of O- & N-glycosylated residues sites were utilized by NetOGlyc 4.0 and NetNGlyc 1.0 servers (https://services.healthtech.dtu.dk/services/), respectively.

Results

From January 2021 to June 2024, (n = 4519) swabs from children aged ≤5 years were tested for respiratory panel, with 113 (2.5%) positive for hMPV (Figure 1a). An outbreak occurred from November 2022 to March 2023, with 56 of 129 cases (43.1%) positive for hMPV, making it the most prevalent pathogen, followed by H3N2 (n = 34, 26.2%) (Figure 1b). The hMPV positivity rate was 10.1% in males and 9.1% in females. Children aged ≤1 year constituted the majority (68.9%) of the study population, with 10.7% (n = 43) testing positive for hMPV. Total hMPV positivity in children aged ≤1 and >1-5 years were 10.7% (n = 43) and 7.3%, respectively (n = 13) (Supplementary Table 1). The common clinical findings of hMPV infection among inpatients were flu-like illness (n = 40, 93%), followed by history of wheezing (n = 29, 67%), seizures (n = 3, 6.9%), bronchitis (n = 30, 70%), and respiratory distress (n = 40, 93%). On further examination, 36 patients (83%) showed bilateral crepitation. Among the inpatients positive for hMPV, 24 (55%) were diagnosed with pneumonia, 12 (27%) with bronchiolitis, four (9%) with bronchitis, and three (7%) with upper respiratory tract infection. Detailed outpatient demographic data were not available. In the phylogenetic tree, of 37 sequences, 34 (91.89%) were genotype A, with 29 (85.29%) sequences clustering with A2.1, 1 (2.94%) sequence clustering with A2.1.1, and four (11.76%) sequences clustering with A2.2.2 The remaining three sequences (8.11%) were grouped into genotype B, with two sequences with B1 and one sequence with B2 (Figure 2). The generated sequences were deposited in GenBank under accession numbers PP444876-PP444912 for the F gene.

On the genetic analysis of the F gene, protein sequences show mutations such as A61T, R82K, V122I, T135N, N139G, K143T/Q, D167E, and E175S in B1/B2 and T223N and N233Y in B2. A2.1 showed G42V, D62E, E96K, K179R, A185D, and M250R mutations. A2.2.2 and A2.1 had O-linked glycosylation at position 191; N-linked glycosylation was at 16 and 131 in B1/B2. F gene selection pressures revealed seven negatively selected sites in (SLAC) and one positively selected site at position 191 (MEME).

Discussion

The present study provides valuable insights into the prevalence, genetic diversity, circulating lineages, and clinical characteristics of hMPV infections among children under 5 years of age in south India. A total of 4519 samples were screened for hMPV, with a 2.5% prevalence rate, which was comparable with study from Spain from 2014 to 2021 [8]. For different viral respiratory pathogens, the positive rate of 21.75% (129 of 583) was high compared with other south Indian studies (7.1-13.0%) [2,9,10] and low (50.7%) compared with eastern Indian studies [2,4,9]. Worldwide, studies from Nepal and China reported a higher rate of viral infection rates, from 21.9-44.7% in children with ARI [2,4,9].

The hMPV prevalence of 9.7% was high compared with those from different states in India [2,9,10]. Worldwide, several studies were conducted in different parts and reported frequencies of hMPV ranging from 2.2% to 43% [2,4,9].

A slightly higher rate of hMPV infections was noticed in children aged less than 1 year than the age groups 1-5 years (10.6% and 7.7%, respectively). More studies have documented a higher prevalence of hMPV in children aged less than 1 year due to an underdeveloped immune system and the environment [9,11]. In these studies, in the male to female ratio (1.8:1), male preponderance was observed, which was concurrent with the study by Hindupur et al. [9]. Peak infections occurred in December and January, aligning with studies suggesting a winter-spring prevalence [12]. In contrast to these results, Hindupur et al. [9] found that the peak incidence of hMPV occurred in February in northwestern India. Co-infections were infrequent in this study, contrasting with reports of up to 70% co-occurrence [2]. Notably, a history of wheezing was prevalent, contrasting with findings on post-tussive emesis absence. Several studies have used the F gene; Groen et al. [4] found that comparing whole genomes with partial or full-length F gene sequences showed no differences in lineage topology, suggesting that F gene sequences alone provide enough information to accurately determine the genotype. The phylogenetic analysis revealed the co-circulation of A (A2.1, A2.2.1, A2.2.2) and B (B1, B2) genotypes of hMPV co-circulating in Puducherry, India, consistent with global findings [9]. In A2.2 lineage, notably, A2.2.1 and A2.2.2, showed gradual evolution over time, marking its first detection in India in this study. These strains exhibited similarities with isolates from the United States in 2010 and Japan in 2018 [4]. The exact evolutionary relationships between these subtypes remain uncertain, although distinctions such as those between A2.2.2 and A2c have been noted [4]. Amino acid substitutions and variations in N or O glycosylation cause genetic changes and may impact immune evasion, strain stability, and clinical severity in affected children, underscoring the need for ongoing genetic surveillance.

Our study underscores the role of hMPV as a significant cause of ARIs in India, particularly, in young children. It highlights hMPV's association with pneumonia and bronchiolitis, which is especially severe in infants under 1 year old with a history of wheezing. We reveal the co-circulation of diverse hMPV lineages in Puducherry and document ongoing mutations in local strains. Notably, to the best of our knowledge, our findings mark the first identification of novel hMPV sub-clusters (A2.2.1 and A2.2.2) within the A genotype in India.

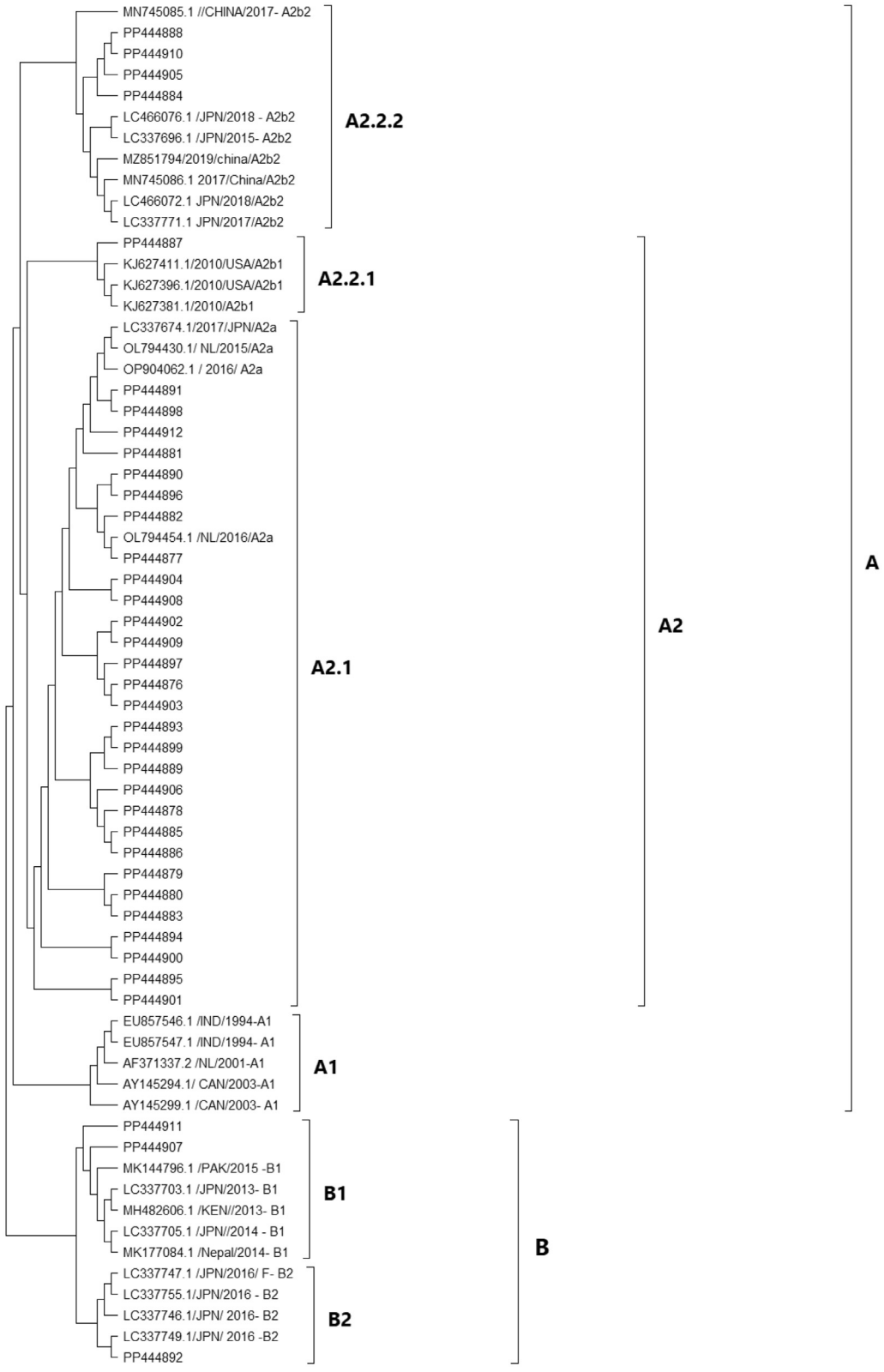

Figure 2. Phylogenetic relationships of 37 human metapneumovirus strains obtained in this study. Neighbor-joining method with 1000 bootstrapping replicates of partial F gene sequences.

Declarations of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Nivedha Devanathan: Methodology, Formal analysis, Writing – original draft. **Ferdinamarie Sharmila Philomenadin:** Methodology, Formal analysis, Writing – original draft. **Gokul Panachikuth:** Methodology. **Sangitha Jayagandan:** Data curation. **Narayan Ramamurthy:** Methodology, Writing – original draft. **Vimal Raj Ratchagadasse:** Methodology, Supervision. **Venkatesh Chandrasekaran:** Data curation. **Rahul Dhodapkar:** Conceptualization, Funding acquisition, Supervision, Writing – original draft.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100486.

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