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# Unraveling Human Metapneumovirus: A Full-Spectrum Review Of Its History, Global Burden, Bioinformatics Advances, And Current Gaps

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## ABSTRACT

Human metapneumovirus (HMPV) is one of the primary sources of severe respiratory infections worldwide. The threat level of the virus is almost the same as that of influenza and RSV, and it is mostly difficult for the vulnerable trio of neonates, the elderly, and immunocompromised patients to deal with. Although it took approximately four decades from the time it had been circulating before it was identified in 2001, it still has no vaccine or antiviral drugs. The current review sheds light on HMPV's genetic lineages and worldwide transmission, emphasizing how bioinformatics has changed its research. Advanced sequencing enables detailed phylogenetic tracking of the virus, whereas computational tools, such as AlphaFold2, help obtain an accurate protein structure. Such breakthroughs can attract the development of drugs through molecular docking and epitope generation for vaccine design. The molecular dynamics simulations also show the viral fusion protein conformational transitions, among other key processes, On the road to success there are still some major gaps in knowledge, such as host receptor identification Integrating these computational approaches is essential to accelerate the development of therapies and reduce the global HMPV burden.

**Abbreviations:**
**HMPV:** Human Metapneumovirus
**RSV:** Respiratory Syncytial Virus
**MD:** Molecular Dynamics
**N:** Nucleoprotein
**M2-1:** Transcriptional Regulator (Matrix 2-1)
**M2-2:** Transcriptional Regulator (Matrix 2-2)
**SH:** Small Hydrophobic
**G:** Glycoprotein
**L:** Large Polymerase
**RADT:** Rapid Antigen Detection Test
**POC:** Point-of-Care
**NAAT:** Nucleic Acid Amplification Test

## Introduction

The identification of the Human Metapneumovirus (HMPV) was the result of isolating this virus following cases of respiratory tract infections in children, which had no known cause (van den Hoogen et al., 2001). The virus was first identified in the Netherlands in 2001. After its discovery, there have been several retrospective serological

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and molecular studies that have also detected the presence of the virus in human populations over 50 years ago, indicating that HMPV has a very long history as a human respiratory pathogen, but was heavily underestimated. Presently, HMPV is recognized as one of the major sources of respiratory infections worldwide. It is a main etiologic agent of respiratory tract disease morbidity, with a variety of clinical exacerbations. These range from mild symptoms of the upper respiratory tract to severe infections of the lower respiratory tract, such as pneumonia and bronchiolitis (Williams et al., 2004).

The human metapneumovirus (HMPV) is a viral respiratory infection that severely affects immunocompromised people, neonates, young children, and elderly people (Walsh and Falsey,2022). Infections in these groups most often lead to hospitalization and high healthcare costs. At present, no specific antiviral medications or vaccines are available, regardless of the clinical significance of the virus. Thus, supportive care forms the basis of treatment (Edwards and Talbot, 2023). Furthermore, the breakthrough in the exploitation of HMPV knowledge has occurred in the last twenty years, during which virology, epidemiology, and molecular processes have advanced significantly due to computational methods and genome sequencing (Schildgen and Lüsebrink, 2020). There is already a broad spectrum of bioinformatics approaches that are utilized to discover the character, usefulness, development, and immune response of the virus. Such methods as phylogenetic reconstruction, homology modeling, molecular docking, dynamic simulations, and epitope prediction are available. (Huck and Scharf, 2022; Alam and Lee, 2023).

## *Historical Perspective and Genomic Foundations*

In 2001, the detection of human metapneumovirus (HMPV), an entirely new human respiratory virus of the Pneumoviridae family, was a milestone in respiratory virology (van den Hoogen et al., 2001).Briefly, the first genome characterization demonstrated that metapneumovirus has a close relationship to avian metapneumovirus (AMPV), which has led to the assumption that the new virus may have come from animals and that the species change would be very recent, probably in the last century (de Graaf and Fouchier,2014).Different sequencing data from 2001 to 2005 from geographically diverse places allowed researchers to classify the HMPV phylogenetic tree into two major genotypes, A and B (Peret et al., 2002). Later, these lineages were divided further into four subgroups, A1, A2, B1, and B2. These subdivisions are the basis of all molecular epidemiological studies that are currently being done and represent the temporal and spatial circulation types which constitute one of the most intriguing aspects of these viruses (Skiadopoulos et al., 2004). The very first period of HMPV research also revealed several gaps in knowledge about virus biology. In the absence of detailed genomic architecture, the lack of high-resolution structures for the fusion (F) and attachment (G) glycoproteins, which were considered the major viral proteins, greatly hindered the mechanistic understanding of viral entry, immune evading, and antigenicity. The situation was similar concerning the uncertain functions of SH and M2-2, which were among the auxiliary proteins(Johnson and Kolli, 2020; Jones and Lamb, 2014). At that time, global burden estimates were still very rudimentary and early surveillance data were confined to local regions (Boivin et al., 2003). The unanswered questions that stretched the way for a fresh period of HMPV research have been the source of issues resolved using computational biology and bioinformatics, which have become indispensable since then (Schildgen and Lüsebrink, 2020). These open questions have turned into the road that led researchers to the new era of HMPV research, which depends heavily on computational biology and bioinformatics for the protein structure prediction, the protein dynamics simulation, the epitope mapping, and the logical countermeasure development facilitation (Alam and Lee, 2023; Huck and Scharf, 2022).

## *Genome Organization and Protein Function*

The nine proteins N (Nucleoprotein), P (Phosphoprotein), M (Matrix), F (Fusion), M2-1 and M2-2 (Transcriptional Regulators), SH (Small Hydrophobic), G (Glycoprotein), and L (Large Polymerase) are encoded by the roughly 13 kb non-segmented, negative-sense, single-stranded RNA virus (van den Hoogen et al., 2001). These proteins can be divided into two groups: one consisting of auxiliary proteins, where M2-1 and M2-2 are included, and the other of structural proteins, i.e. N, P, M, F, G, SH, and L( Fig. 1.The accessory proteins not only can transcriptional activity but also replication balance (Biacchesi et al., 2006; Buchholz et al., 2016), i.e. structural proteins are necessary for viral entry, replication, and assembly. Among the proteins, F, G, and SH were the three most significant proteins in the treatment group. The F protein, which is involved in viral fusion, is the primary target of neutralizing antibodies and antiviral drugs (Cox and Williams, 2013; Williams et al., 2019). The purpose of the SH protein has not yet been identified; however, it is considered to be an ion channel that is a part of the immune evasion process (Thammawat et al., 2015).G protein facilitates the virus attachment and helps in the immune response of the host (Schowalter et al., 2009; Chang et al., 2012).The lack of structural information for some human metapneumovirus (hMPV) proteins, especially G and SH, has made it very difficult to develop drugs and vaccines that can effectively combat the virus (Jaber et al., 2021).The use of computational methods, such as dynamics simulations, homology modelling, and molecular docking for structural prediction,

ligand interaction research, and epitope identification, can noticeably speed up the next generation of therapeutic approaches for hMPV is very suitable. (Alam and Lee, 2023; Pavlović and Büttner, 2022; Huck and Scharf,2022).

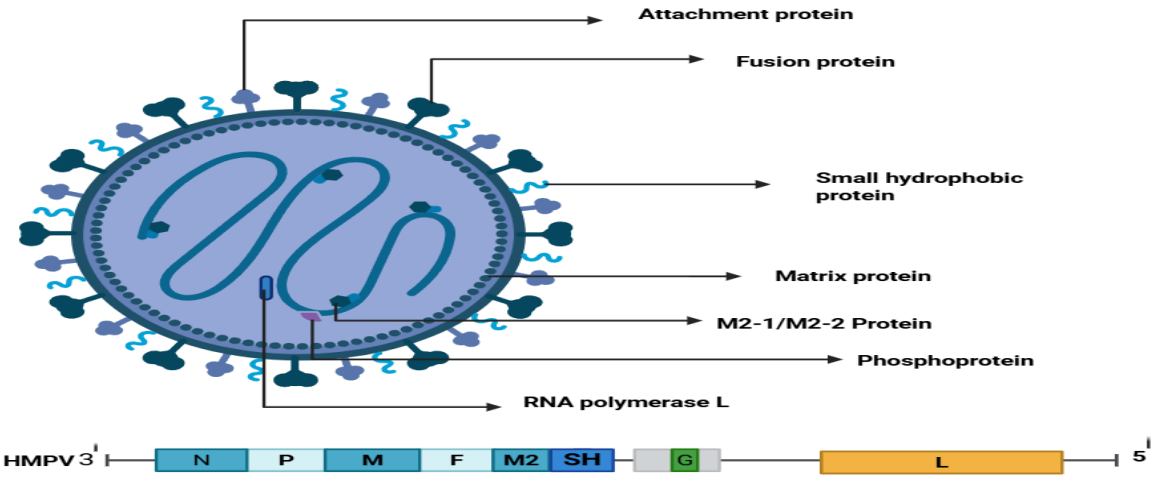


**Fig. 1:** Schematic diagram of HMPV virion structure and genome organization. Structural and non-structural proteins are shown along the viral RNA. Key: F, fusion protein; G, attachment protein; L, large protein (polymerase); M, matrix protein; M2, putative transcription factor (M2-1) and RNA synthesis regulatory factor (M2-2); N, nucleoprotein; P, phosphoprotein; SH, small hydrophobic protein.

## *Pathogenesis and Host Interaction of Human Metapneumovirus (HMPV)*

The Human Metapneumovirus (hMPV) replication cycle which refers to the process by which the hMPV, a negative-sense RNA virus, propagates in the host cell. The viral surface glycoproteins initially coordinate to the host cell receptors and after that, the virus is delivered via membrane fusion or endocytosis. When the virus enters, it unzips its RNA genome to be put in the cytoplasm. Moreover, replication is initiated by the viral RNA-dependent RNA polymerase which thus produces both positive-sense RNA (protein synthesis templates) and negative-sense RNA (genomic copies). At the same time, transcription is running and producing polyadenylated and capped viral mRNAs, which after that are translated into viral proteins by the host machinery. These proteins that viruses produce and are essential to genome replication, assembly, and budding are among others the structural proteins (F, G, SH, and M) and the non-structural proteins (N, P, L, and M2). The proteins are going through maturation after they have been synthesized. Phases in virion assembly. Synthesis of RNPs happens when assembly of the ribonucleoprotein complexes, a merger of structural proteins and viral genomic RNA, budding virions is formed. Overall, the cycle closes and thus, new cells can be infected because new virus particles are released from the host cell by budding (Fig. 2).

The crosstalk between the host inflammatory response and viral replication is the main driver which determines the clinical course of HMPV infection (Cox and Williams, 2013). Clinical manifestations may differ depending on the severity of the disease from a mild viral infection of the upper respiratory tract with cough and nasal discharge, to more severe lower respiratory infections such as pneumonia and bronchiolitis where the airway obstruction is caused by the combined action of inflammatory debris, edema, and cell death. In addition to this, the virus-triggered inflammation is that which aggravates airway hyperresponsiveness and bronchoconstriction in the susceptible population, hence, HMPV ranks as one of the leading causes of acute exacerbations of chronic disorders like asthma and COPD (Papadopoulos and Gourgiotis, 2023).

## *Epidemiology*

*Human metapneumovirus* (HMPV) was first identified in 2001 and since then it has been labeled as one of the most common respiratory pathogens, (Fig.3a ),which is found all over the world (van den Hoogen et al., 2001). Exposure to it is practically universal by the age of five as stated by seroprevalence studies, which means that it is well-distributed in all the continents where there are people (Hamelin and Boivin, 2005). HMPV retains considerable periodicity in the areas with a temperate climate, where epidemics happen almost every year, generally starting from late winter up to early spring (Li and Wang, 2022). Such outbreaks are at times overlapping with the peaks of influenza and respiratory syncytial virus (RSV) or being shortly after them. The seasonality in the areas around the equator is not so evident, but hot and humid weather are the factors that are most frequently associated with a higher incidence and continuous transmission of the virus (Nair and Simões, 2020).

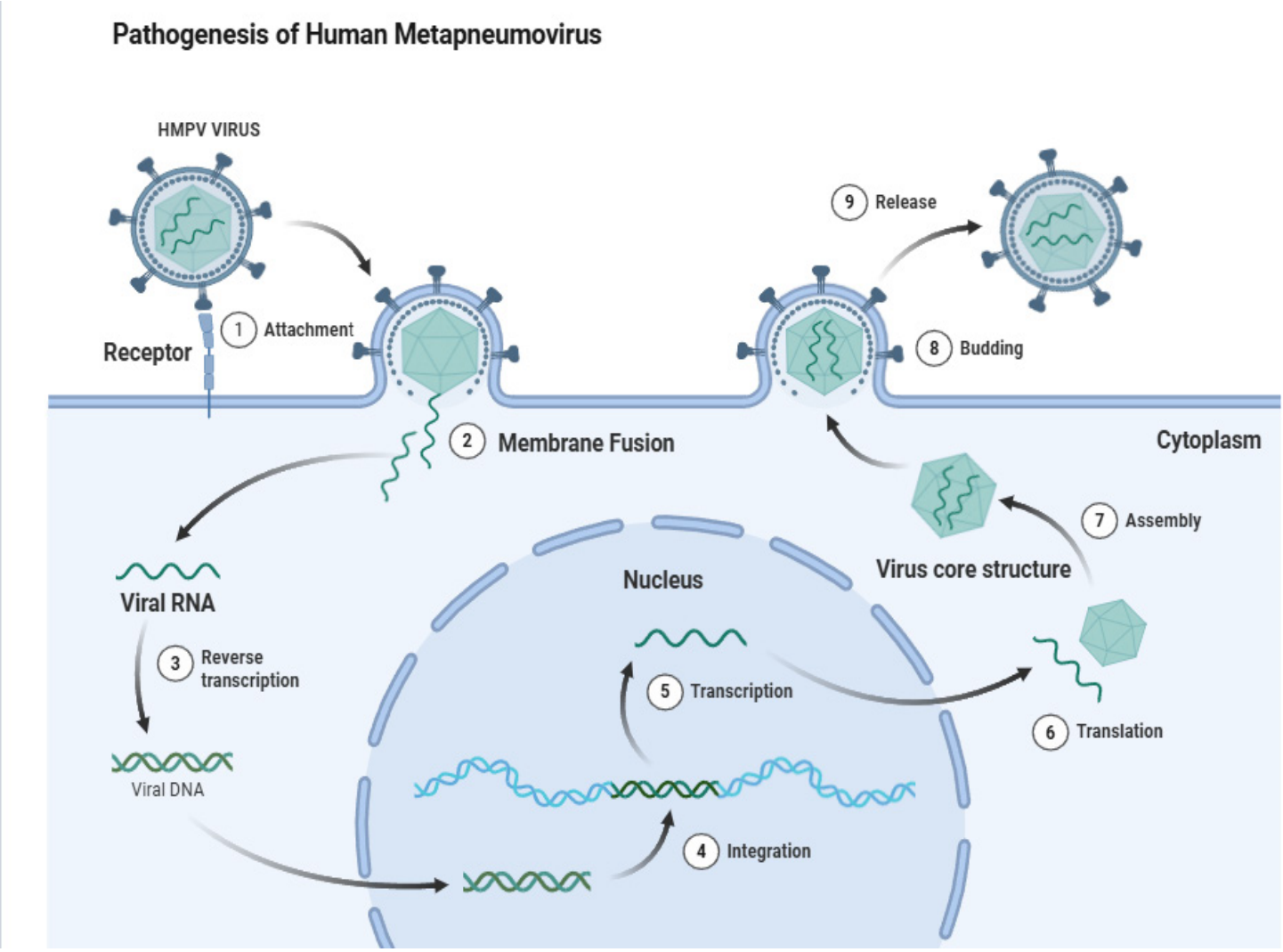


Fig. 2: Pathogenesis of Human Metapneumovirus (HMPV). The virus attaches to host cell receptors (1) and fuses with the cell membrane (2), releasing viral RNA into the cytoplasm. Viral RNA undergoes reverse transcription to form viral DNA (3), which integrates into the host genome (4). Transcription (5) and translation (6) produce viral proteins, which assemble into new virions (7). The virions bud from the host cell (8) and are released to infect other cells (9).

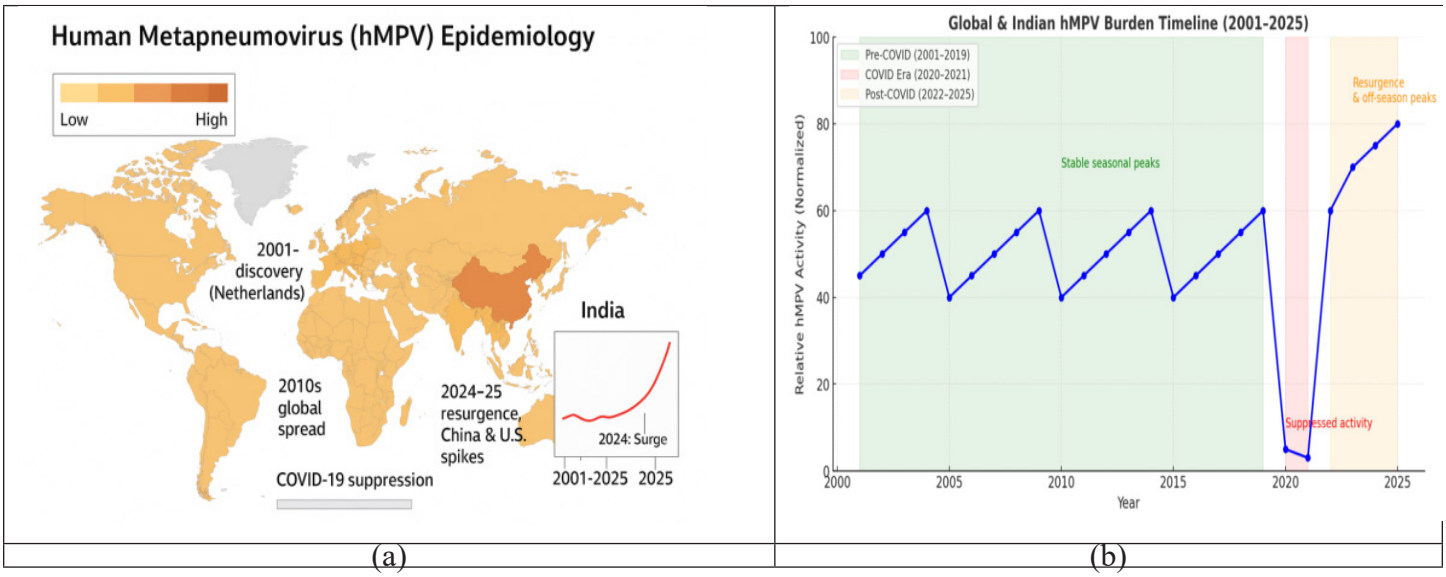


**Fig.3:a)**Global epidemiology of *Human Metapneumovirus (*hMPV), from its 2001 discovery to worldwide spread, COVID-19 suppression, and recent 2024–25 resurgence with notable surges in China, the U.S., and India. **b)**Timeline of global and Indian hMPV burden (2001–2025), highlighting pre-COVID seasonal peaks, sharp suppression during the COVID era, and a strong post-COVID resurgence with atypical off-season spikes.

## Burden in Vulnerable Populations

*Human metapneumovirus* (hMPV) is the main causative agent of acute lower respiratory infections, which accounts for 5-10% of pediatric outpatient visits and 10-15% of pediatrics hospitalizations. (Nair and Simões, 2020). Besides, immunocompromised people and older adults with cardiopulmonary co-morbidities are a high-risk group for severe illness (Walsh and Falsey, 2022). Although deaths are rare in wealthy countries and mostly among high-risk newborns and the frail elderly, the problem is still remarkably heavy in low and middle-income countries conditioned by poor diagnostics and healthcare access that leads to higher case fatality rates (Nair and Simões, 2020).

***Impact of the COVID-19 Pandemic and Post-Pandemic Resurgence***

Non-pharmaceutical measures during the COVID-19 pandemic significantly lowered the circulation of hMPV, leading to an "immunity gap" in young infants (Bouscambert-Duchamp and Lina, 2020). Pediatric hospitalizations have gone up after the easing of NPIs, between 2022 and 2024, due to off-season outbreaks (Choudhary and Chadha, 2022; Ren and Wang, 2023). Genomic surveillance has been very effective in keeping an eye on these atypical transmission patterns and shifts in lineage distribution (Thongpan et al., 2021). The changes reflect the importance of continued epidemiological and genomic surveillance and the vulnerability of populations with little exposure to the virus. (Fig. 3b) and (Table 1;). It is extremely necessary to know the changed seasonality and activities of the outbreak of hMPV-related respiratory infection to guide public health interventions, plan vaccination scheduling for the future, and facilitate the healthcare system in coping with the next wave(Li and Wang, 2022).

**Table 1:** Shows the burden of hMPV globally and in India across three phases: pre-COVID (2001–2019), during the COVID-19 pandemic (2020–2021), and post-COVID (2022–2025). Data illustrate the sharp suppression of hMPV circulation during the pandemic, followed by resurgence with atypical, off-season peaks in the post-pandemic period.

| Parameter | Pre-COVID (2001–2019) | COVID Era (2020–2021) | Post-COVID (2022–2025) |
|---|---|---|---|
| Global Pediatric Hospitalizations (ARI) | 10–15% of all pediatric ARI [WHO, CDC] | <1–2% (sharp decline due to NPIs & reduced circulation) | 12–18% (resurgence with off-season peaks and immunity gaps) |
| India Pediatric Hospitalizations (ARI) | 8–12% of pediatric ARI [ICMR surveillance] | <1–2% (almost absent during strict lockdown) | 10–14% (resurgence, especially in 2022–2023 off-season outbreaks) |
| Outpatient Visits (Global) | 5–10% | <2% | 8–12% |
| Outpatient Visits (India) | 4–8% | <2% | 6–10% |
| Overall ARI Positivity (PCR/serology) | 3–10% (global); 5–12% (India) | 0.5–1% | 7–15% (surge after restrictions lifted) |
| Co-Infections | 15–30% | Very low (few ARIs circulating) | >30% (frequent RSV, influenza, SARS-CoV-2 overlaps) |
| Mortality | Rare, confined to high-risk | Extremely rare (hMPV circulation suppressed) | Slight rise in frail elderly and high-risk infants (due to delayed exposure) |

## *Molecular Epidemiology and Viral Lineages*

The two main lineages of hMPV are A and B, each of which has the sub lineages A1, A2, B1, and B2. Multiple sub lineages co-circulate, with A2 and B2 (Peret et al., 2002; Skiadopoulos et al., 2004). predominating in recent years, according to global surveillance. (Panda and Mohakud, 2021; Darniot and Pitoiset, 2021). Immune evasion is made possible by antigenic diversity, particularly in the F and G glycoproteins, which also leads to recurrent infections throughout life(Boivin et al., 2004).

## Co-infections and Clinical Implications

Co-infections are frequently observed in Human Metapneumovirus (hMPV) infections, occurring in approximately 15–30% of cases. (Hashem and Hall, 2022). The most reported co-pathogens include respiratory syncytial virus (RSV), influenza viruses, rhinoviruses, and SARS-CoV-2. (Fig. 4b),Such co-infections may exacerbate clinical severity, prolong illness, and pose challenges for accurate diagnosis and effective management.Recognizing the prevalence and impact of these co-infections is therefore critical for improving patient outcomes and informing clinical and public health strategies (Hashem and Hall, 2022).

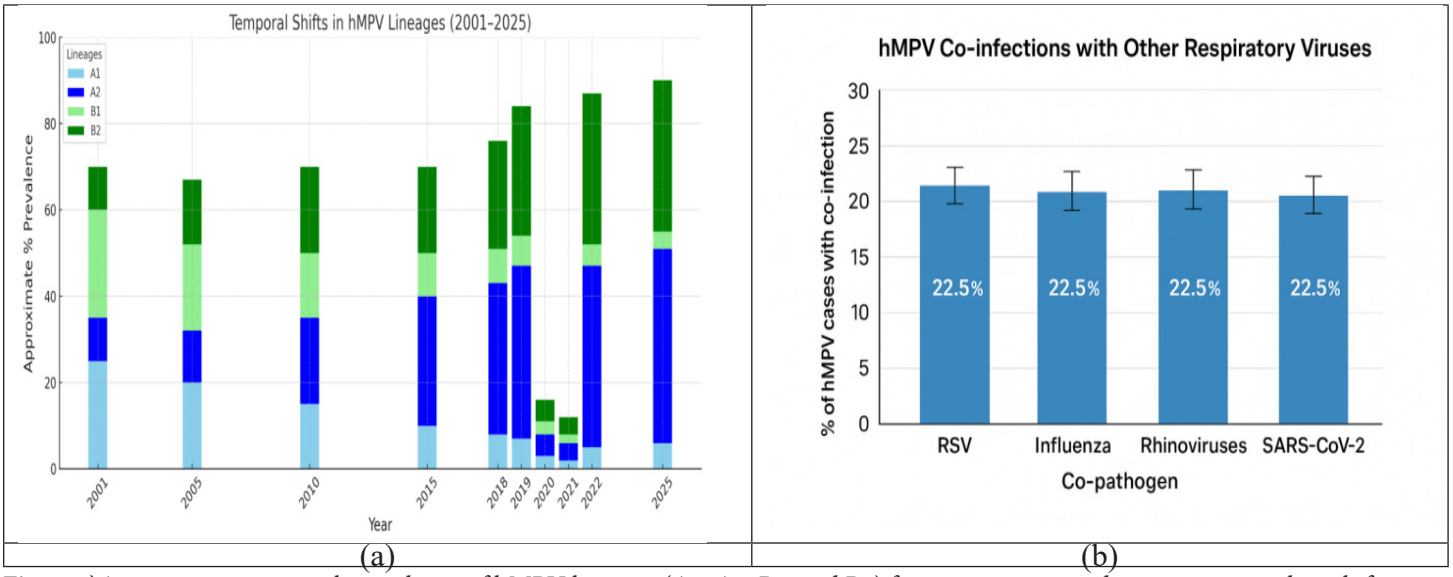


**Fig. 4: a)**Approximate temporal prevalence of hMPV lineages (A1, A2, B1, and B2) from 2001 to 2025, demonstrating a clear shift toward the dominance of Lineages A2 and B2. **b)** Percentage of *human Metapneumovirus* (hMPV) cases presenting with co-infection by common respiratory pathogens.

## Epidemiology and Burden in India (2001–2025)

It has been known since the beginning of the 2000s that the Human Metapneumovirus Virus (HMPV) is among the main reasons for respiratory diseases in India and that it has been triggered by the virus. Based on the findings of systematic surveillance studies, 5–12% of acute respiratory infection (ARI) incidents are identified as HMPV-positive, and the most affected group is children below five years of age (Panda and Mohakud, 2021).According to the global trend, besides the young children, those with weak immune systems and elderly persons are the most vulnerable to getting severe diseases that are also caused by HMPV (Choudhary and Chadha, 2022).

### *Lineage Distribution in India*

The existence of the two main lineages (A and B) has been established beyond doubt by molecular epidemiological studies, which indicate that the sub lineages A2 and B2 have been predominant for the last few years. (Fig. 4a) Variations in lineage superiority over time have been observed through several years of local monitoring, and these changes are consistent with the global patterns of viral evolution and spread (Darniot and Pitoiset, 2021).

## Materials and methods

## Homology Modeling and Structural Predictions

Before the experimental structures were resolved, the basis for predicting the three-dimensional structure of hMPV proteins was homology modelling. In those days, there were not so many templates available for the models, but nevertheless functional fragments for the three proteins, SH, G, and M2-2, created by the mentioned in their abstracts and results part of the publications SWISS-MODEL, Modeller, and Phyre2, were given for the SH, G, and M2-2 proteins. The finding of one or two quite a few structures, the crystal ones, of the hMPV F protein combining pre-and post-fusion conformations during the 2013–2019 period was an evident turning point (Jaber et al., 2021)These structures facilitated drug and vaccine design by showing major conformational changes and antigenic areas. (Table2;), Besides that, from 2021 the usage of deep learning-based methods such as RoseTTAFold and AlphaFold2 has really revolutionized this area to the point where the predictions of the entire hMPV

proteome, including such targets as the G protein, which was not yet identified, are now extremely accurate (Alam and Lee, 2023). This opens new possibilities for drug discovery and mechanistic studies.

Table 2: Structural studies of hMPV proteins over time. Early homology modeling (2010s) and X-ray crystallography (2013–2019) provided preliminary and atomic-level insights, while AI-based predictions (2021 onward) now offer high-confidence models for nearly the entire proteome, enabling advanced functional and therapeutic research.

| Year Period | Protein's Studied | Method/ Approach | Key Insights & Significance |
|---|---|---|---|
| 2010s | SH, G, M2-2 | Homology Modeling | produced ideas for functional research and provided preliminary predicted structures for proteins without experimental evidence. |
| 2013– 2019 | F (Fusion) | X-ray Crystallograpy | identified key processes for drug targeting and membrane fusion by resolving the atomic-level structures of pre- and post-fusion conformations. |
| 2021 onward | Entire hMPVproteome | AI-Based Prediction (Alpha-Fold2, Rosetta Fold) | Almost all unresolved proteins now have highly accurate, high-confidence models, allowing for a thorough understanding of the viral proteome for functional and therapeutic research. |

## *Phylogenetics and Molecular Evolution*

Bioinformatics tools have greatly aided in the understanding of the concept of genetic diversity, investigation of the evolutionary background, and tracing of hMPV worldwide (Schildgen and Lüsebrink, 2020). First, phylogenetic analyses that aimed to determine evolutionary relationships and validate the origin of hMPV in the avian metapneumovirus (AMPV) group were based on software such as MEGA, PAUP, and BEAST (de Graaf and Fouchier, 2014). The estimated time of divergence from AMPV to hMPV and subsequently, the separation of A and B lineages of the present day has been determined using molecular clocks to be between 200 and 400 years. The birth of the NeXT strain and similar real-time genomic surveillance platforms has made the observation of spatiotemporal transmission patterns and lineage dynamics (post-2020) so detailed and close to continuous as never before (Thongpan et al., 2021). Furthermore, the use of computational methods for selection pressure identification (e.g., FEL, FUBAR, MEME) has led to the discovery of several key positions in the F and G proteins that are under immune pressure, which consequently causes the progressive change of epitopes and thus immune evasion, which is among the factors of hMPV reinfections and persistence (Boivin et al., 2004).

## Epitope Prediction and Immunoinformatics

Immunoinformatics has been a major tool in mapping the hMPV immunogenic landscape and designing novel vaccine candidates (Huck and Scharf, 2022). B-cell epitope prediction methods (e.g., IEDB, ElliPro, and BepiPred) have led to the identification of both linear and conformational epitopes, especially on the F and G proteins (Bach and Boivin, 2022). Predictions made by in silico technologies, such as NetMHCpan and NetCTL, of peptides that can bind different HLA alleles have paved the way for the design of broad-coverage vaccines (Ullah and Khan, 2023). The in silico fabrication of multi-epitope vaccine constructs, which are usually equipped with immunogenicity enhancers such as adjuvants and linkers, was the outcome of this study (Sharma and Kamath, 2022). Additionally, the identification of conserved neutralizing epitopes on the F protein through structural mapping has provided a blueprint for the development of universal hMPV vaccines that can circumvent antigenic heterogeneity (Tao and Wang, 2022).

# Molecular Docking and drug Discovery

## *In Silico Docking Approaches*

In silico studies using numerous computer-aided docking methods have focused on human metapneumovirus (hMPV) RNA-dependent RNA polymerase and fusion (F) protein (Pavlović and Büttner, 2022). These computer-aided operations have substantially facilitated the discovery of novel probable inhibitors, opening of easily accessible binding sites, and providing detailed quality at the atomic level of the protein-ligand bonding. These steps have paved the way for the design of antivirals with the help of the structure.

### *Drug Repurposing Strategies*

To speed up the discovery process, researchers have implemented the drug repurposing method, wherein they have tried known drugs against hMPV targets. These fusion inhibitors were developed based on antigens of other viruses, such as RSV and HIV, and nucleoside analogs, such as ribavirin, a familiar drug that can be used to reprogram viral RNA synthesis. In addition, researchers have sought the potency of fragments of monoclonal antibodies to neutralize the virus by binding to the already specific epitopes of the F protein (Lee and Chang, 2023). This approach paved the way for short leads of possible hMPV into clinical trials, as it is a quick and affordable method for screening therapeutic agents with already established safety profiles.

## Natural Compound Screening

Nature is a major source of chemical variety that can be used to develop antiviral drugs. Various studies of the ligand, alkaloid, and flavonoid interactions with hMPV conducted dicking the derived substances from Indian Ayurveda medicinal herbs showed strong binding to the target shown in (Table 3),(Kumar and Dhama, 2021; Banerjee and Mukhopadhyay, 2021). Some of these compounds also showed docking scores that were comparable to or better than those of already known antivirals, thus, these being relicts in the pharmaceutical drugs.

Table 3: Plant-derived compounds showing potential anti-HMPV activity through strong binding, docking affinity, or inhibition of viral replication.

| Compound | Plant Source | Antiviral Mechanism / HMPV Potential |
|---|---|---|
| Andrographolide | Andrographis paniculata (Kalmegh) | Strong hydrogen bonding and π-stacking with HMPV target proteins |
| Amarogentin | Swertia chirayita | High antiviral activity, effective in molecular docking with HMPV |
| Arjunolic acid | Terminalia arjuna | Potent docking affinity and dynamic stability against HMPV |
| Cirsimaritin | Artemisia scoparia | Inhibits viral replication/protein synthesis in similar respiratory viruses |
| Tangeretin | Citrus reticulata (peel) | Potent flavonoid, interferes with viral replication and host interaction |

### *Genomic Sequencing and Metagenomics for Surveillance and Discovery*

Sequencing technologies have transformed the detection of hMPV from a simple process to a detailed genomic characterization. Besides the strain identification and epidemiological surveillance that Sanger sequencing allows, it follows PCR of variable regions such as the F gene. NGS, as well as whole genome and metagenomic methods, enable the detection of the presence of variants, of recombination events and of co-infecting pathogens in a non-biased manner (Feuillet and Lina, 2022). NGS, though still expensive and complex, plays a vital role in outbreak tracing, the monitoring of antiviral resistance, and vaccine development (Thongpan et al., 2021).

# RESULTS AND DISCUSSION

## Global Epidemiology and Disease Burden

Most of the hMPV robust surveillance data is supported by high-income countries in Europe and North America. The low- and middle-income countries (LMICs) have limited and, quite often, non-systematic surveillance (Nair and Simões, 2020). This results in a huge blind spot regarding the true global burden of disease in such areas as infectious disease hotspots, particularly the issue of pediatric deaths due to bronchiolitis and pneumonia. Further, the gap limits our understanding of the virus evolution, the occurrence of recombination events, and the global distribution of genotypes (A1, A2, B1, B2) (Panda and Mohakud, 2021). Without a coordinated global genomic surveillance network, like those for influenza and SARS-CoV-2, the release of antigenically different variants or new lineages with greater transmissibility or virulence might be happening silently for a long time before they have caused widespread epidemics (Thongpan et al., 2021).

### *Viral Evolution and Genetic Diversity*

Molecular epidemiology studies have delineated HMPV into two primary genetic lineages (A and B) with four major subgroups (A1, A2, B1, B2). Surveillance data indicates consistent co-circulation of multiple sub-lineages, with A2 and B2 emerging as the predominant strains in recent years. This genetic diversity, particularly in the F and G glycoproteins, facilitates antigenic variation and contributes to the virus's ability to cause re-infections throughout life. The high frequency of co-infections (15-30% of cases) with pathogens like RSV, influenza, and SARS-CoV-2 complicates clinical presentation and management, potentially exacerbating disease severity and prolonging recovery.

## Structural Characterization of Viral Proteins G and SH

Despite the fusion (F)and nucleocapsid proteins being well characterized, the SH (small hydrophobic) and G (attachment glycoprotein) proteins remain the major unknowns of hMPV biology (Johnson and Kolli, 2020; Liu and Osterhaus, 2020). In comparison with other paramyxoviruses, the G protein role in the viral binding as well as its main host receptor or receptors are still not defined (Liu and Osterhaus, 2020). The SH protein, despite the lack of its exact structure and functions, is believed to be the virus channel (a viroporin) that modifies the host immune response such as TNF-alpha signaling and apoptosis (Johnson and Kolli, 2020). Only a limited understanding of viral pathogenesis and immune evasion tactics is available and greatly structure-based drug design is obstructed by the non-existence of high-resolution structural data (like from X-ray crystallography or cryo-EM) for these proteins (Jaber et al., 2021).

## Therapeutic Development Challenges Beyond the F Protein

One of the major advancements that led to numerous predictions about the interactions of drugs and natural compounds with human metapneumovirus (hMPV) proteins is the use of in silico modeling, especially AI-based tools like AlphaFold2 (e.g., binding to the F protein to inhibit fusion), (Alam and Lee, 2023; Pavlović and Büttner, 2022).But what stands as a major hurdle is the experimental validation of those computational hits. The step from a predicted binding affinity to a confirmed therapeutic agent is through in vitro testing in cell-based assays (e.g., plaque reduction, cytopathic effect inhibition) as well as in vivo validation in animal models (e.g., cotton rats, hamsters, non-human primates). This is a very slow and resource-consuming process resulting in many compounds that look computationally promising but do not have enough empirical support (Pavlović and Büttner, 2022). Besides that, most of the research concentrates just on the F protein; to have a strong antiviral armory, it is necessary to carry out a complete screening against other potential targets such as the polymerase complex (Lee and Chang, 2023).

## Bioinformatics-Driven Design of Molecular Diagnostics for HMPV

Bioinformatics is the main pillar that enables the accuracy of modern molecular diagnostics. Presently, the computational workflow for primers and probes designing greatly relies on the analysis of huge genomic databases (for instance, GenBank, GISAID) (Schildgen and Lüsebrink, 2020).The in silico methods are used for the multiple sequence alignment to identify the highly conserved sequences that are common to all hMPV strains found in different countries which, in turn, ensures a high detection range and gives the tests the ability to be resistant against genetic drift. The above procedures are additionally used to perform a thorough false-positive elimination because there might be a cross-reaction with the human genome and other microbial flora. A digital method like this enables the quick re-targeting, as well as the easy upgrading, of diagnostic assays to keep on their utility in the ongoing battle against a virus that is constantly mutating.

## Point-of-Care Diagnostics for HMPV: Assessing Rapid Antigen Tests

The lateral flow immunochromatographic assays or the rapid antigen detection tests (RADTs) are hMPV nucleoprotein antigen identification tools by a direct route from a nasal swab or nasopharyngeal samples. Their chief advantage is time; they provide results at the point-of-care (POC), which is 15 to 30 minutes, thus allowing for immediate clinical decision-making (Piyaratna and Tollefson, 2021). These tests are particularly useful for quick screening in pediatric setting, are the Pediatric Emergency Department, the Emergency Department, and the Outpatient Clinics. Rapid identification of the viral etiology can guide prompt patient placement and administration of antibiotics. Nevertheless, their sensitivity in comparison with that of NAATs is very low, thus, their use is drastically limited. They are thus very reliable in children if used during the first three to four days after symptom onset, i.e. when shedding is at the highest, and their efficiency is dependent on the viral load. As a result, the sensitivity of the test is enormously lowered in adults as well as in the later stages of the disease when infectivity is low. Hence, a molecular assay is always required as a confirmation in a high clinical index of suspicion cases because a negative RADT result cannot be used for the exclusion of hMPV infection.

## Molecular Assays as the Gold Standard for HMPV Detection and Characterization

The earliest, accurate, and human metapneumovirus (hMPV) diagnosis which is the source of infection epidemiological surveillance as well as reliable clinical therapy is the basis of safety in hospital practice, infection prevention and control, besides surveillance. The area has changed a lot and is now dominated by molecular and computational biological approaches, whereas it was once based on virus culture and serology, which are both insensitive in nature. In fact, this revolution has been very important in the case of hMPV, as the virus can be clinically distinguished only from a few other respiratory viruses namely influenza and Respiratory Syncytial Virus (RSV). The changes in diagnostic methods,

the methods utilized, and the advancement in technology which is influencing hMPV detection in the future are the main topics of this section. Among all the methods, RT-PCR is considered as the fittest one and is referred to as the gold standard because of its top sensitivity and specificity. Generally, the conserved regions of polymerase (L), fusion (F), and nucleoprotein (N) genes are targeted. (Schildgen and Lüsebrink, 2020). The use of multiplex PCR panels for the simultaneous identification of hMPV and other respiratory infections leads to accurate diagnosis and clinical management. Nowadays, real-time quantitative RT-PCR (qPCR) viral load data is employed for monitoring antiviral treatment efficacy, determining the period of virus shedding, and estimating disease severity (Geller and Vabret, 2021).

### *The Translational Gap in HMPV Prophylaxis and Treatment*

Though human metapneumovirus (hMPV) was identified in 2001, no vaccines or specific antiviral treatments have been approved for it (Edwards and Talbot, 2023). Some of the obstacles are the need for an extremely safe vaccine approach, learning from the history of the RSV vaccine, and the possibility of vaccine-facilitated disease, particularly in very young infants (Kandasamy and Pichichero, 2022). The situation becomes more complicated with the target population, e.g., immunization of the elderly, immunization during pregnancy, or seronegative newborns (Walsh and Falsey, 2022). Besides, deciding on the best immunogen for a strong protective immunity remains a challenge, even with pre-fusion versus post-fusion F protein or other antigens (Tao and Wang, 2022).

## Conclusion

The rapid advancement in human metapneumovirus (hMPV) research, fueled by bioinformatics, has created a paradox: an abundance of molecular knowledge coexists with a complete absence of clinical countermeasures (Schildgen and Lüsebrink, 2020; Edwards and Talbot, 2023). This stark translational gap highlights that understanding a pathogen is not synonymous with controlling it. The failure to develop a vaccine or treatment underscores a critical disconnect between basic virological discovery and applied clinical innovation, leaving public health vulnerable. Bridging this divide demands a strategic pivot towards integrated, next-generation solutions. The most promising path forward lies in the concerted convergence of high-resolution structural biology, systems immunology, and artificial intelligence (Alam and Lee, 2023; Lee and Chang, 2023). This multidisciplinary framework will enable the rational design of novel antigens, a comprehensive understanding of host-pathogen interactions, and the predictive power to accelerate development. By fostering such collaboration, the scientific community can finally translate decades of research into effective vaccines and therapeutics, transforming patient care for hMPV. The journey of human metapneumovirus (hMPV) research exemplifies the accelerating power of modern molecular biology and bioinformatics. In a remarkably short timeframe, scientists have sequenced its genomes, elucidated its protein structures through homology modeling, and mapped its phylogenetic history, creating a detailed portrait of the virus's biology and evolution. However, this wealth of in-silico knowledge has starkly failed to transition from the database to the bedside. This translational gap is not merely an oversight but a reflection of the immense complexity of vaccine and drug development, where factors like achieving robust mucosal immunity in the respiratory tract, ensuring safety in vulnerable populations like infants and the elderly, and the virus's own mechanisms of immune evasion present formidable hurdles. Consequently, the absence of any licensed vaccine or specific antiviral treatment stands as a persistent and troubling reminder that scientific discovery, while necessary, Is insufficient on its own to impact public health.

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