

Human Metapneumovirus Diagnosis, Pathogenesis, and Therapeutics

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Human metapneumovirus (hMPV) is a globally prevalent respiratory pathogen that causes acute respiratory infections across all age groups, with the greatest disease burden in young children, older adults, and immunocompromised individuals. Clinical manifestations range from mild upper respiratory tract symptoms, including cough, wheezing, and sore throat, to severe lower respiratory tract disease such as bronchiolitis, pneumonia, acute respiratory distress syndrome (ARDS), and secondary bacterial infections that may require intensive care and mechanical ventilation. Despite its widespread circulation and substantial healthcare impact, important aspects of hMPV transmission, pathogenesis, and host immune responses remain incompletely understood, hindering the development of effective therapeutics and vaccines. This review summarizes the current knowledge of hMPV epidemiology, clinical presentation, diagnostics, pathogenesis, and prevention strategies, with emphasis on recent outbreaks that highlight its seasonal and geographic variability. We discuss the viral life cycle and the contributions of the major surface glycoproteins—fusion (F), attachment (G), and small hydrophobic (SH) proteins—to viral infectivity, immune evasion, and disease progression. Advances in molecular diagnostics, including reverse-transcription polymerase chain reaction (RT-PCR), next-generation sequencing, metagenomic approaches, and nanopore sequencing, are also examined. While supportive care remains the cornerstone of clinical management, existing antiviral therapies demonstrate limited and inconsistent efficacy. Emerging preventive and therapeutic strategies, including mRNA-based vaccines and neutralizing monoclonal antibodies, offer promising avenues for disease control. Improved understanding of hMPV biology and host-pathogen interactions will be critical for developing effective interventions to reduce the global burden of this increasingly recognized respiratory pathogen.

Keywords: hMPV; respiratory viral infection; glycoproteins; pathogenesis; transmission; detection; therapeutics

Introduction

Human metapneumovirus (hMPV), a negative-sense single-stranded RNA virus belonging to the family *Pneumoviridae*, genus *Metapneumovirus*, and species *Human metapneumovirus*, is a significant respiratory pathogen that primarily affects young children, older adults, and immunocompromised individuals. Since its discovery in 2001, hMPV has been recognized as a major cause of upper and lower respiratory tract infections worldwide, contributing substantially to morbidity and healthcare burden. Clinical manifestations of hMPV infection often overlap with those of other respiratory viruses, particularly respiratory syncytial virus (RSV), leading to frequent underdiagnosis and challenges in disease surveillance [1]. Despite the global impact of hMPV, treatment remains largely supportive, as no approved antiviral therapies are currently available. Similarly, no vaccine has been licensed for routine clinical use, although several vaccine candidates and the fusion (F) protein-targeted immunotherapeutic strategies are under active investigation for both hMPV and

RSV. Recent advances in molecular diagnostics, including reverse-transcription polymerase chain reaction (RT-PCR) and metagenomic sequencing, have significantly improved viral detection and epidemiological monitoring [1]. Furthermore, passive immunization approaches targeting the viral F protein have emerged as promising preventive and therapeutic strategies. This review provides a comprehensive overview of hMPV biology, epidemiology, pathogenesis, clinical manifestations, diagnostic approaches, disease transmission, and current management strategies, while highlighting recent progress in antiviral development, vaccine research, and future directions aimed at improving prevention and clinical outcomes.

Since its initial identification in the Netherlands in 2001 during an outbreak of respiratory tract illness among young children, hMPV has emerged as a globally prevalent respiratory pathogen and has been reported across multiple regions, including China and North America. The virus primarily causes upper respiratory tract infections but can also lead to more severe lower respiratory tract diseases, such as bronchiolitis, pneumonia, and asthma exacerbations. In

vulnerable populations, including older adults and individuals with underlying respiratory conditions, hMPV infection may contribute to the progression or worsening of chronic obstructive pulmonary disease (COPD) [2]. Owing to its widespread distribution and substantial clinical impact, hMPV is now recognized as an important cause of respiratory morbidity worldwide [3]. The same research group, together with findings from subsequent studies, demonstrated that hMPV had been circulating in human populations since at least the late 1950s. Clinical manifestations of hMPV infection were found to closely resemble those associated with other respiratory viruses, particularly parainfluenza virus infections, which likely contributed to its delayed recognition as a distinct human pathogen. hMPV infection has been reported across all age groups [4], but predominantly in immunocompromised individuals [5,6]; however, younger populations appear to be most affected.

As of 2021, hMPV was estimated to account for approximately 3–10% of hospital admissions among children younger than five years of age presenting with acute lower respiratory infections (ALRIs). Globally, hMPV was responsible for an estimated 11.1 million ALRI cases in 2018 (uncertainty range 8.0–15.7 million), resulting in approximately 502,000 hospitalizations (UR: 332,000–762,000) and 11,300 ALRI-associated deaths (UR: 4000–61,600). These findings underscore the substantial burden of hMPV infection on pediatric health worldwide and highlight its significance as a major contributor to respiratory morbidity and mortality in young children [7]. In early 2025, hMPV was identified as a significant cause of respiratory disease in China, representing 6.2% of laboratory-confirmed respiratory infections and 5.4% of respiratory-related hospital admissions. The highest rates of infection were reported in northern regions of the country, underscoring the continued epidemiological importance of hMPV as a respiratory pathogen. Surveillance data on acute respiratory infections reported by the China Center for Disease Control and Prevention (China CDC) through December 29, 2024, indicated a growing incidence of respiratory viral infections, including those attributable to RSV and hMPV. This upward trend highlighted the increasing contribution of these pathogens to the respiratory disease burden during the surveillance period [8]. Recent global epidemiological syntheses have highlighted hMPV as a persistent and significant cause of acute respiratory infections worldwide [9]. This increasing trend has not been confined to a single region but has also been observed in multiple Northern Hemisphere countries, as reported by the World Health Organization (WHO), emphasizing the ongoing global public health relevance of hMPV [10]. An increase in hMPV infections was observed across several regions of the United States, including Kansas, Iowa, Missouri, and Nebraska, beginning in November 2024. By December 28, 2024, hMPV was detected in 1.94% of weekly respiratory diagnostic tests, consistent with the seasonal epidemiology of respiratory

viral infections. Although hMPV activity was increasing, its prevalence remained lower than that of influenza and SARS-CoV-2, which exhibited positivity rates of 18.71% and 7.10%, respectively, highlighting the continued dominance of these pathogens during the respiratory virus season [11].

A previous review reported that hMPV was among the ten most detected viral pathogens in pediatric oncology patients and was associated with severe clinical complications, including post-transplant mortality in a subset of cases [12]. This finding highlights the substantial risk posed by hMPV infection in immunosuppressed populations and underscores the need for improved preventive and therapeutic strategies [12]. Globally, hMPV infection is typically associated with relatively low rates of morbidity and mortality. However, in hematopoietic stem cell transplant (HSCT) recipients, progression to lower respiratory tract infection can result in severe clinical outcomes. Reported mortality rates among post-transplant patients with hMPV-related lower respiratory tract disease range from 10% to 43%, highlighting the significant burden of hMPV infection in this highly immunocompromised population [13,14,15], which increases to 80% when the virus is detected in bronchoalveolar tissues [13]. The clinical manifestations of hMPV infection are generally similar to those of the common cold and closely resemble those caused by RSV, another member of the *Pneumoviridae* family. Genetic diversity among hMPV subtypes is primarily attributed to sequence variations in the attachment (G) and F surface glycoproteins, as well as in the small hydrophobic (SH) protein. Functionally, the G protein facilitates viral attachment to host cells, whereas the F protein plays a critical role in mediating viral fusion, entry, and subsequent infection. These surface glycoproteins are major determinants of viral infectivity, immune recognition, and vaccine development efforts.

Although hMPV represents a significant global respiratory pathogen, vaccine development remains an unmet clinical need. Numerous vaccine candidates are currently in various stages of preclinical and clinical testing; however, no licensed hMPV vaccine is presently available for widespread use. Consequently, prevention relies largely on supportive measures, underscoring the importance of accelerating vaccine research and development, particularly for populations at increased risk of severe disease [16,17]. Despite the significant clinical and public health impact of hMPV, there are currently no FDA-approved vaccines available for widespread use. Furthermore, antiviral treatment options remain scarce, and those that have been investigated have demonstrated limited efficacy, highlighting the need for continued research into novel vaccines and antiviral strategies [18].

Symptoms

Clinical manifestations of hMPV vary according to the region of the respiratory tract affected, ranging from mild upper respiratory symptoms to severe lower respiratory tract disease. Despite its substantial clinical burden and broad spectrum of presentations, hMPV remains frequently under-recognized in clinical practice. This limited recognition, combined with the nonspecific nature of its symptoms, continues to pose challenges for accurate diagnosis and appropriate patient management [1].

The clinical manifestations of hMPV vary depending on the affected respiratory organs. Upper respiratory tract disease is commonly characterized by pharyngitis, rhinitis, cough, coryza, rhinorrhea, and sore throat. In contrast, lower respiratory tract infections (LRTIs) may present with respiratory distress, tachypnea, wheezing, crackles, rhonchi, rales, retractions, decreased breath sounds, and, in severe cases, respiratory failure. Patients frequently experience nasal congestion and increased work of breathing. Extrapulmonary manifestations may include tachycardia, poor peripheral perfusion, conjunctivitis, otitis media, abnormal tympanic membrane findings, and cervical lymphadenopathy. Systemic symptoms such as fever, fatigue, irritability, anorexia, vomiting, diarrhea, reduced urine output, ocular irritation (watery or erythematous eyes), ear pain, and apnea have also been reported [19,20]. The key symptoms are summarized in Fig. 1.

The first reported outbreak of hMPV in the Netherlands in 2001 revealed a clinical spectrum ranging from mild upper respiratory tract illness to severe lower respiratory tract infections, such as bronchiolitis and pneumonia. In some cases, these infections progressed to respiratory failure, especially among children under five years of age [3]. In a 2004 comparative study, cough was the predominant clinical symptom of hMPV infection, with coryza and rhinitis also commonly reported [19]. Notably, hMPV was more strongly associated with bronchiolitis and asthma exacerbations than with pneumonia, distinguishing its clinical presentation from that of other respiratory pathogens such as RSV, influenza virus, parainfluenza virus, and adenovirus [19]. The incidence of fever was higher in hMPV cases than in infections caused by RSV and other respiratory pathogens, underscoring potential differences in clinical presentation among respiratory viruses [21].

Although hMPV accounts for nearly 2% of all respiratory illnesses throughout the year, its contribution to respiratory disease burden can rise to approximately 6% during the winter months [22]. Age-related increases in hMPV incidence have been observed, with infection rates reaching 4.8% in adults older than 60 years and climbing to 14.2% during seasonal outbreaks [23]. A 2014 study from Germany documented age-related differences in the clinical presentation of hMPV infection. Fever and chills were more commonly observed in older adults (>60 years),

whereas cough and bronchitis were the predominant manifestations among young children (<4 years) [23]. A Norwegian study published in 2017 reported a substantial burden of hMPV infection, with disease severity varying according to age [24]. Children aged 12–23 months exhibited more prolonged and severe symptoms, supporting the hypothesis that maternally derived antibodies provide partial protection during the first six months of life. Additionally, a recent retrospective audit from Australia found that 41.2% of hospitalized patients with hMPV infection had underlying comorbidities, including prematurity, cerebral palsy, and malignancies, highlighting the increased vulnerability of individuals with pre-existing medical conditions.

Pre- and post-COVID-19 studies yielded contrasting results regarding the clinical presentation and seasonality of hMPV. A study from Switzerland analyzed retrospective data from 2011 to 2023 and found no differences in the presentation of hMPV infection in hospitalized children; however, there was an increased off-seasonal occurrence in the post-COVID-19 pandemic era (2021–2022 and 2022–2023) [25]. This study also reported that pneumonia and upper respiratory tract infections occurred more frequently than bronchitis and bronchiolitis post-pandemic compared to the pre-pandemic COVID-19 period, without much change in clinical course. Furthermore, a group from Spain reported prolonged hospitalization and severe clinical courses in children with hMPV infection post-COVID-19 pandemic era [26]. The COVID-19 pandemic has altered hMPV patterns, with regional differences observed, highlighting the need for further investigation.

Transmission

hMPV spreads primarily via respiratory droplets through direct or close contact with infected individuals and can also be transmitted through contact with contaminated objects, surfaces (fomites), or environmental reservoirs [27]. Based on evidence from nosocomial infection studies, the incubation period of hMPV infection is estimated to be 3–6 days [28,29]. Although symptoms may resolve, recovered healthy individuals can continue to shed hMPV for several weeks after infection [24,30] and for months in immunocompromised patients [31]. Long-term or latent hMPV infections have not been documented in humans. Although the timing of peak transmission varies across the USA and Central Asia, hMPV infections are predominantly observed during the winter and spring seasons [32,33,34]. In northern Europe, the peak season was reported from January to March [35], in contrast, studies from Central Europe have reported alternating biennial peaks of hMPV infection occurring in winter and late spring [36]. However, in West Africa, the highest incidence of hMPV infection has been reported between July and September [37]. In contrast, no distinct seasonal pattern of hMPV infection has been observed in Italy [38]. A recent report

Clinical Signs and Symptoms associated with hMPV in the affected regions

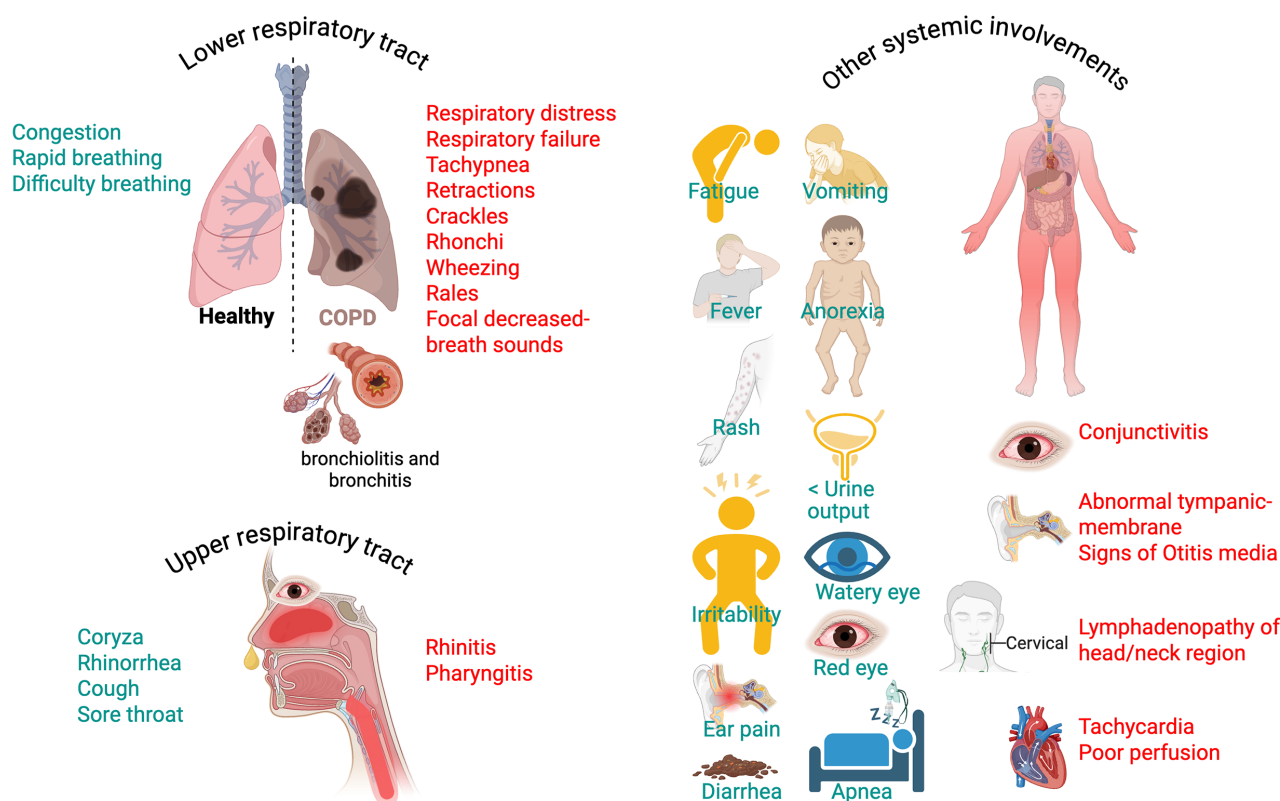


Fig. 1. Clinical Signs and Symptoms associated with hMPV. Summary of key clinical signs and symptoms as reported in the literature in the various affected regions of patients belonging to different age groups. Signs are color coded as green and symptoms are color coded as red. This figure was created by the authors using BioRender (BioRender Inc., Toronto, ON, Canada; <https://www.biorender.com/>). hMPV, human metapneumovirus.

from the Republic of Korea demonstrated that hMPV infections among older adults reached peak incidence during the spring and summer seasons [39].

The widespread adoption of non-pharmacological interventions, including frequent hand hygiene, facial mask use, and social distancing, was associated with a significant decrease in hMPV infections in the post-COVID-19 period relative to the pre-pandemic period (odds ratio, OR = 1.81) [40], and the difference in mean positivity was around 2.5 [41]. Reports of hMPV circulation were largely absent until 2021, coinciding with the implementation of COVID-19 mitigation measures. Subsequently, multiple studies have described alterations in the seasonal epidemiology of hMPV in the post-pandemic era [42,43,44]. Emerging data further highlight substantial variations in transmission dynamics between community-acquired and hospital-acquired respiratory infections, particularly relative to other seasonal respiratory viruses such as RSV and influenza [45].

hMPV Pathogenesis

Virus Structure and Innate Immune Sensing

hMPV is an enveloped, non-segmented, negative-sense single-stranded RNA virus belonging to the family Pneumoviridae. Its genome is approximately 13.2 kb in length and encodes eight genes that give rise to nine viral proteins, making it slightly smaller than the closely related RSV, which possesses a genome of approximately 15.2 kb [46,47]. The viral envelope contains three major glycosylated surface proteins: the F, attachment (G), and small hydrophobic (SH) proteins (Fig. 2A). The G protein facilitates viral attachment to host-cell glycosaminoglycans, whereas the F protein mediates membrane fusion and viral entry through interactions with integrin receptors, particularly integrin α -V β -1 (α v β 1) [48,49].

Infection is initiated in the respiratory epithelium of the upper airway, where viral attachment and fusion permit entry into susceptible host cells. Following primary infection, viral replication can extend to the lower respiratory tract depending on factors such as inoculum size, duration

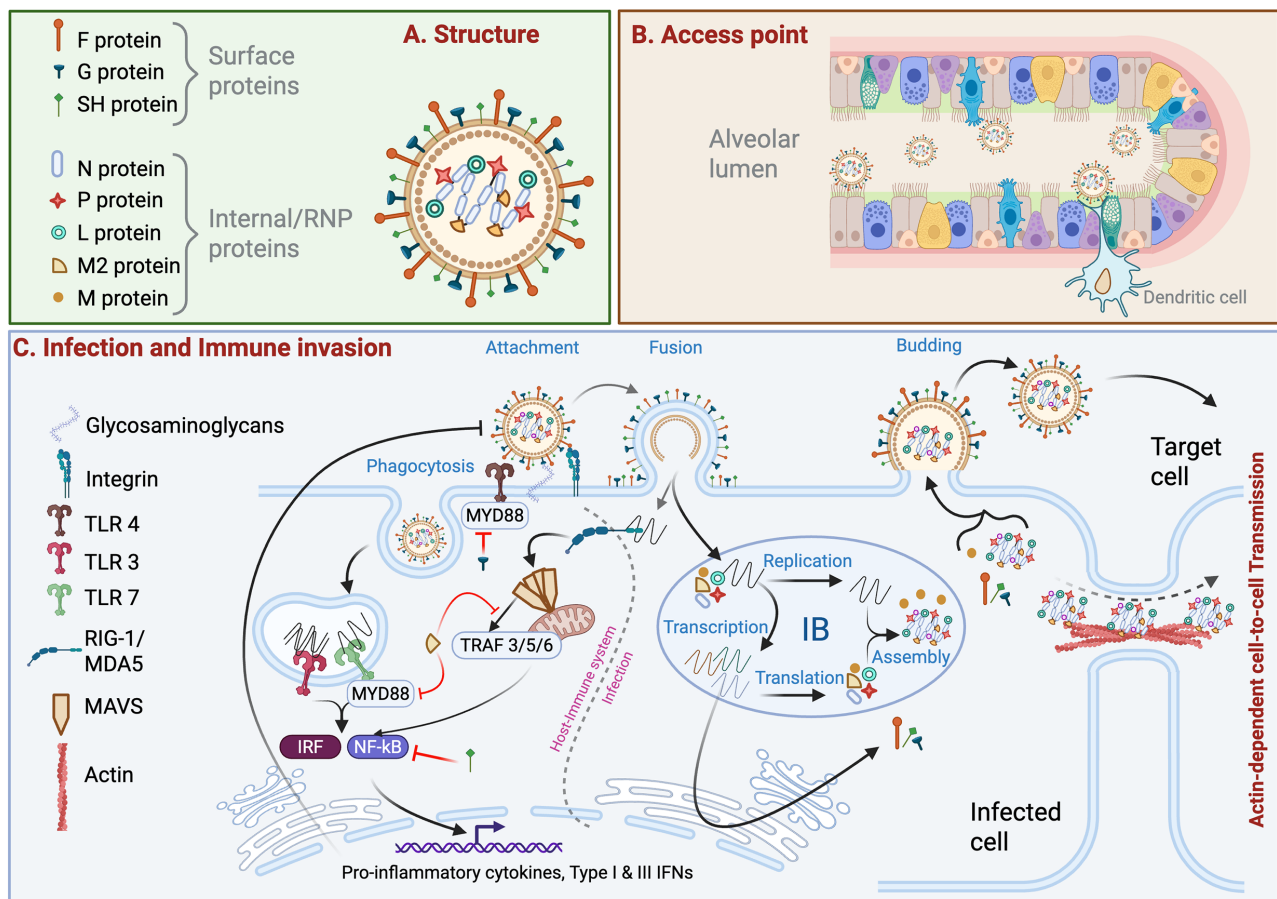


Fig. 2. HMPV Structure and mode of infection. (A) Structure: hMPV is an enveloped, non-segmented, negative-sense RNA virus. Its surface glycoproteins include F, G, and SH. At the same time, its internal proteins make up the RNP, including N, P, L, M2, and M. (B) Access point: hMPV infection begins at the respiratory epithelium, where the G protein binds to the host glycosaminoglycans, and the F protein mediates membrane fusion via $\alpha V\beta 1$ integrins, for viral entry into the host. Lung-resident antigen-presenting cells, such as dendritic cells, can phagocytose the virus and elicit a host immune response. (C) (i) Infection and Host Immune Evasion: Following membrane fusion, viral replication occurs in cytoplasmic IBs, where transcription and translation take place. Host PRRs- including TLR3/4/7, RIG-I, and MDA5 detect viral RNA and trigger MAVS-mediated activation of TRAF3/5/6 and downstream IRF and NF- κ B signaling, inducing interferons (type I and III) and proinflammatory cytokines. hMPV evades immunity by inhibiting these pathways via SH-mediated NF- κ B and STAT1 suppression, G protein inhibition of MyD88/TLR4 signaling, and M2-2 disruption of MAVS-TRAF recruitment. (ii) Cell-to-Cell Transmission: hMPV spreads between cells via actin-based intercellular extensions, bypassing classical syncytia formation. Viral proteins associate with actin filaments to form filopodia-like structures, facilitating direct transfer of viral components to adjacent cells, enhancing rapid viral replication, and immune evasion. This figure was created by the authors using BioRender (BioRender Inc., Toronto, ON, Canada; <https://www.biorender.com/>). F, fusion; G, attachment; SH, small hydrophobic; $\alpha V\beta 1$, integrin alpha-V beta-1; IBs, inclusion bodies; PRRs, pattern recognition receptors; TLR3, Toll-Like Receptor 3; RIG-I, *Retinoic acid-inducible gene 1*; MDA5, *melanoma differentiation-associated gene 5*; MAVS, mitochondrial antiviral signaling protein; TRAF3, TNF receptor-associated factor 3; IRF, interferon Regulatory Factor; NF- κ B, Nuclear factor kappa-light-chain-enhancer of activated B cells; STAT1, Signal Transducer and Activator of Transcription 1; MyD88, myeloid differentiation primary response 88; N, nucleoprotein; P, phosphoprotein; L, large RNA-dependent RNA polymerase.

of exposure, and host immune competence. Progression of infection may involve the bronchi, bronchioles, and alveoli, where hMPV can induce bronchiolitis, pneumonia, and other lower respiratory tract manifestations (Fig. 2B).

Virus Replication and Host Immune Evasion

Following attachment and fusion with ciliated respiratory epithelial cells, the hMPV ribonucleoprotein (RNP) complex is released into the host-cell cytoplasm. This RNP complex, consisting of the nucleoprotein (N), phosphoprotein (P), and large RNA-dependent RNA polymerase (L)

protein, encapsulates and protects the viral genome from host immune recognition while facilitating the early stages of viral replication and transcription. Subsequently, the N, P, and L proteins assemble into a functional polymerase complex at the 3' end of the viral genome, initiating sequential transcription of viral mRNAs. The P serves as a critical scaffold that recruits and coordinates N protein interactions, promoting the formation of cytoplasmic inclusion bodies (IBs) (Fig. 2C). These membrane-less viral replication factories concentrate viral and host factors required for efficient genome replication, transcription, and virion production, thereby enhancing viral propagation within infected cells [50,51]. Two matrix proteins, M2-1 and M2-2, are encoded by the *M2* gene and serve pivotal functions in coordinating viral transcription and replication during the hMPV life cycle.

M2-1 serves as a critical transcription elongation factor that enhances viral mRNA synthesis, thereby supporting efficient viral replication and promoting disease pathogenesis. Within the host cytoplasm, *M2-2* plays a critical role in coordinating the balance between viral gene transcription and genome replication, thereby optimizing viral propagation [52,53]. As viral RNA replication progresses, protein synthesis occurs concurrently to generate essential structural and regulatory proteins. Newly synthesized N, P, L, and M2 proteins associate with progeny viral genomes to form nucleocapsid complexes. These nucleocapsids are then encapsulated by the matrix (M) protein, which acts as a bridge between the nucleocapsid and the viral envelope, coordinating virion assembly, membrane deformation, and the budding of mature infectious particles from the host cell surface [54]. In parallel with nucleocapsid assembly, the viral surface glycoproteins F, G, and SH undergo maturation and glycosylation through the secretory pathway before trafficking to the host-cell membrane. At sites of virion assembly, these glycoproteins are incorporated into the viral envelope, facilitating the formation, budding, and release of mature infectious hMPV particles [55].

The host responds to hMPV infection through the activation of pattern recognition receptors (PRRs), particularly MDA5 and RIG-I, which detect viral RNA in the cytoplasm. Recognition of viral RNA by these sensors initiates downstream signaling cascades that promote the production of type I and type III interferons and other antiviral mediators, thereby limiting viral replication and spread (Fig. 2C). Following the recognition of viral RNA by melanoma differentiation-associated protein 5 (MDA5) and *retinoic acid-inducible gene 1* (*RIG-I*), mitochondrial antiviral-signaling protein (MAVS) is activated and serves as a central signaling hub for the recruitment of tumor necrosis factor receptor-associated factor 3 (TRAF3), TRAF5, and TRAF6. These adaptor proteins propagate downstream signaling events that culminate in the activation of interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), as well as

signal transducer and activator of transcription 1 (STAT1) phosphorylation. Together, these pathways stimulate the expression of interferons, *interferon-stimulated* genes, and proinflammatory cytokines that contribute to host antiviral defense [56]. As an important viral immune-modulatory protein, M2-2 inhibits MAVS-dependent signaling by preventing the recruitment of TRAF adaptor proteins. This inhibition dampens the activation of IRF3, NF- κ B, and interferon-dependent pathways, enabling hMPV to evade host antiviral defenses and establish productive infection [57,58,59]. Both the G and SH proteins have been shown *in vitro* to modulate host innate immunity by suppressing interferon responses. This immune evasion strategy involves inhibition of key antiviral signaling molecules, including IRF3, IRF7, and STAT1, thereby reducing the expression of interferons and *interferon-stimulated* genes and promoting viral persistence and replication [60,61].

Cell-to-Cell Transmission

Distinct from the classical dissemination pattern of many respiratory viruses, hMPV induces actin-dependent intercellular extensions that facilitate direct cell-to-cell spread of viral material (Fig. 2C). This transmission mechanism can occur despite the presence of neutralizing antibodies, suggesting that direct intercellular transfer may promote viral dissemination while limiting exposure to extracellular immune defenses [54]. Notably, this transmission strategy is mechanistically distinct from the extensive syncytium formation employed by measles virus and from the tunneling nanotube-mediated intercellular spread reported for influenza and parainfluenza viruses [54,62]. Within infected human airway epithelial cells, hMPV proteins associate with and reorganize host actin filaments, leading to the formation of elongated filopodia-like extensions that span neighboring cells. These actin-rich intercellular structures facilitate direct viral transmission while potentially limiting exposure to extracellular immune defenses [51,55]. The formation of these actin-based intercellular extensions enables direct delivery of viral genomes and proteins to neighboring cells, facilitating rapid local spread and allowing hMPV to partially evade extracellular immune surveillance mechanisms, such as neutralizing antibodies, complement factors, and other soluble antiviral mediators [54].

Self-assembly of the M and P proteins into higher-order macromolecular structures provides a scaffold for the formation of filamentous viral projections. The subsequent actin-dependent elongation and budding of these branched structures facilitate viral dissemination within infected tissues. By promoting rapid IB formation and localized intracellular amplification, this mechanism may enable hMPV to achieve efficient early replication before host innate immune responses are fully activated [50]. Like many RNA viruses, hMPV lacks a proofreading mechanism in its RNA-dependent RNA polymerase. As a result, each replication

cycle introduces mutations at an estimated frequency of 10^{-5} – 10^{-6} per nucleotide, leading to the continual generation of genetically diverse quasispecies populations. This intra-host diversity may enhance viral adaptability, immune evasion, and persistence under selective pressures [63,64].

Diagnosis

Diagnostic testing for hMPV has evolved considerably over the past two decades. While traditional approaches such as virus culture and immunoassays remain valuable, molecular techniques—including reverse transcription polymerase chain reaction (RT-PCR), other nucleic acid amplification assays, and melting curve analysis—have become the preferred methods due to their superior sensitivity and specificity. The major diagnostic platforms currently employed for hMPV detection are compared in Table 1.

Viral Culture

Prior to the widespread use of molecular diagnostics, isolation of hMPV in susceptible cell cultures was the principal method for laboratory diagnosis. Although laborious and slow, virus isolation continues to serve as an important reference standard for evaluating newer diagnostic assays [3,65]. Virus isolation remains a valuable diagnostic and research tool for hMPV. The method involves inoculating infectious clinical specimens onto appropriate cell lines and monitoring for cytopathic effects (CPEs) that typically develop within 7–10 days. A variety of cell lines, including Vero, human epithelial type 2 cells (HEp-2), human hepatocellular carcinoma cells (HepG2), 293, and Lilly Laboratories Cell-Monkey Kidney 2 (LLC-MK2) cells, have been employed to support hMPV replication and facilitate viral detection [3,66,67]. Comparative analyses of hMPV isolation methods have demonstrated significant differences in cell-line susceptibility. In one study examining 19 distinct cell lines, human Chang conjunctival cells and feline kidney Crandell-Rees feline kidney (CRFK) cells were found to be the most sensitive for hMPV isolation, highlighting their potential utility for virus cultivation and diagnostic applications [68]. Among the cell lines used for hMPV propagation, LLC-MK2 cells are regarded as particularly advantageous owing to their tolerance to trypsin supplementation, a factor that enhances viral growth and facilitates efficient virus isolation and cultivation [66].

Shell vial culture has emerged as a sensitive and time-efficient alternative to traditional viral culture techniques for hMPV diagnosis. The procedure involves inoculating clinical specimens onto a susceptible cell monolayer in a shell vial, followed by low-speed centrifugation to promote viral attachment and infection. After a relatively short incubation period of 24–48 hours, infected cells are stained with hMPV-specific antibodies, enabling detection of viral antigens by immunofluorescence microscopy and facil-

itating relatively rapid diagnosis compared with conventional culture methods [69]. Shell-vial culture has similar or higher sensitivity and a substantially faster turnaround time compared with conventional culture [70]. The development of R-Mix cell culture systems, composed of mink lung and human adenocarcinoma-derived cells, has substantially improved shell vial culture diagnostics. These cells offer rapid viral detection, high diagnostic sensitivity (97.1%), and cost-effective performance, making them a valuable tool for the identification of respiratory viral pathogens, including hMPV [71,72].

Fluorescent Immunoassay (FIA)

Before the widespread adoption of molecular diagnostic methods, hMPV detection was primarily accomplished using FIAs and virus neutralization assays [3]. FIA represents an important immunodiagnostic approach for hMPV detection, allowing the visualization of either viral antigens or anti-hMPV antibodies through fluorescence-based labeling techniques. The direct fluorescent antibody (DFA) assay remains one of the most widely utilized immunofluorescence methods in clinical settings, as it enables rapid detection of hMPV antigens using fluorescently labeled antibodies and provides results within a relatively short time frame [73,74]. Increasingly, diagnostic laboratories utilize a combination of conventional viral culture and shell vial culture to improve the detection of respiratory viruses. This complementary approach capitalizes on the strengths of each method, providing greater sensitivity, specificity, and diagnostic confidence than a single culture-based technique alone [75]. Recent advances in immunofluorescence-based diagnostics have led to the development of cytospin-enhanced DFA techniques. By concentrating clinical specimens onto microscope slides before staining, this approach improves cellular yield and antigen detection, resulting in enhanced sensitivity for hMPV diagnosis compared with conventional DFA methods [73,76].

Molecular Diagnostics (RT-PCR and Multiplex RT-PCR)

Among molecular diagnostic approaches, RT-PCR is the most utilized technique for hMPV detection in clinical practice. Viral RNA extracted from respiratory specimens is first reverse transcribed into complementary DNA (cDNA). This is followed by real-time PCR amplification and detection using gene-specific primers and fluorescent probes, allowing rapid, sensitive, and highly specific identification of hMPV in clinical samples [77]. The evolution of automated RT-PCR systems has substantially improved the robustness and reproducibility of hMPV detection. Advances in specimen processing, including automated RNA extraction technologies and commercially available extraction kits optimized for respiratory swabs and aspirates, have streamlined laboratory workflows. Moreover, the incorporation of standardized primers and highly specific flu-

Table 1. Comparative overview of diagnostic methods for human metapneumovirus (hMPV): Specimen types, performance characteristics, and clinical applications.

Diagnostic methods	Specimens/Target	Sensitivity	Expected time to Result	Advantages	Limitations	Clinical applicability
Viral culture	Respiratory specimens; live virus isolation in cell lines	Low to moderate sensitivity; specificity high when positive	Days to >1 week	Enables virus recovery for downstream studies, a traditional reference method	Slow, labor-intensive, requires cell culture facilities	Limited; mainly research or confirmatory testing
Shell vial culture	Respiratory specimens; cell monolayer with centrifugation and immunofluorescent detection	Better sensitivity than conventional culture; specificity high	24 to 48 h	Faster than standard culture	Less sensitive than molecular assays; specialized setup required	Limited clinical culture use
Direct fluorescent antigen assay (DFA/FIA)	Respiratory specimens; hMPV antigen	Moderate sensitivity: specificity increases with specimen quality	Same day	Rapid and relatively simple	Lower sensitivity than RT-PCR; operator-dependent	Screening or adjunct testing
RT-PCR/real-time RT-PCR	Respiratory swabs, aspirates, BAL; conserved viral targets, commonly <i>N</i> gene	High sensitivity and specificity	Same day to <1 day	Widely used, robust routine diagnostic method	Requires equipment, trained staff, quality control	Primary routine diagnostic method
Multiplex RT-PCR	Respiratory specimens; simultaneous detection of hMPV and other respiratory viruses	High sensitivity and specificity; assay-dependent	Same day to <1 day	Detects coinfections, useful in syndromic testing	Higher cost and assay complexity than single plex RT-PCR	Preferred method in hospital respiratory panels
Isothermal amplification (LAMP, RAA, RPA)	Respiratory specimens; nucleic acid amplification at constant temperature	High analytical sensitivity: specificity depends on primer design	Minutes to <1 h	Rapid, simpler instrumentation, suitable for resource-limited settings	Validation and standardization vary by platform	Point-of-care/decentralized testing
CRISPR-based assays	Respiratory specimens; usually <i>N</i> gene after pre-amplification	High specificity; based on emerging evidence	Rapid	Promising rapid molecular detection	Limited clinical validation and standardization	Research/early translational use
mNGS	Respiratory specimens; untargeted sequencing of nucleic acids	Moderate to high sensitivity, depends on workflow	Hours to days	Detects known, rare, and novel pathogens; useful in severe/unexplained disease	High cost, complex analysis, lower routine feasibility	Tertiary care, outbreak investigation
Nanopore sequencing	Respiratory specimens; real-time long-read sequencing	Moderate sensitivity; specificity high with bioinformatics support	Rapid, real time	Portable; useful for epidemiology and pathogen discovery	Lower sensitivity/accuracy than PCR in many settings	Specialized diagnostics and surveillance
Serology	Serum; host antibody response	Limited utility for acute infection	Days	Helpful for epidemiology and exposure studies	Poor for early diagnosis; timing dependent	Research/retrospective studies

orescent probes has further increased the sensitivity and accuracy of hMPV detection, establishing RT-PCR as the preferred diagnostic approach in modern clinical practice [78,79,80]. The *N* gene is one of the most frequently targeted regions in hMPV RT-PCR assays because of its high degree of sequence conservation among viral lineages. Primers designed against conserved *N*-gene sequences enable robust amplification and improve the sensitivity and reliability of hMPV detection in clinical samples [81,82].

TaqMan and molecular beacon technologies have become standard fluorescence-based detection systems in real-time RT-PCR assays for hMPV. TaqMan probes consist of oligonucleotide sequences labeled with a reporter fluorophore and a quencher molecule. Following hybridization of primers and probes to the target cDNA, DNA polymerase initiates strand elongation. As the enzyme encounters the bound probe, its exonuclease activity cleaves the probe, separating the reporter from the quencher and generating a fluorescent signal. The increase in fluorescence is monitored in real time and serves as a quantitative measure of viral nucleic acid amplification [83,84,85]. Unlike TaqMan probes, which generate fluorescence through probe cleavage, molecular beacon probes rely on a conformational change for signal generation. These probes possess a stem-loop (hairpin) structure that positions the fluorescent reporter and quencher adjacent to one another, suppressing fluorescence. Upon binding to the complementary target sequence, the hairpin structure unfolds, separating the reporter from the quencher and producing a fluorescent signal that enables real-time detection of the amplified product [86], making it preferred method for virus detection. A recent systematic review assessed 47 available diagnostic approaches for hMPV detection and conducted a meta-analysis of clinical studies published from 2017 to 2019 to compare their diagnostic performance [87]. Recent analyses indicate that multiplex PCR is the predominant molecular diagnostic platform for hMPV detection, being employed in 57.4% of reported studies, while real-time RT-PCR accounts for 38.3% of investigations. Multiplex PCR offers several advantages, including the simultaneous amplification of multiple target sequences in a single reaction with multiple primer pairs. Detection is accomplished using target-specific fluorescent probes labeled with different fluorophores, allowing the parallel identification of multiple respiratory pathogens or viral gene targets with high sensitivity, specificity, and diagnostic efficiency [88]. By enabling the concurrent detection of multiple respiratory pathogens and viral strains within a single reaction, multiplex PCR has become the diagnostic platform of choice in clinical laboratories [89,90].

Other Detection Methods

Serological assays were traditionally used for the diagnosis of hMPV infection and are predominantly based on enzyme immunoassay (EIA) methodologies. These tests

detect virus-specific humoral immune responses by measuring circulating anti-hMPV antibodies in patient samples [3,91]. In addition to enzyme immunoassays, virus-neutralization assays have historically been used for hMPV detection and serological characterization. These assays measure the ability of virus-specific antibodies to inhibit viral infection or replication in susceptible cell cultures [92,93]. Nucleic acid isothermal amplification (NAIA) methods have emerged as rapid and cost-effective alternatives to conventional PCR-based diagnostics. These techniques amplify target nucleic acids under isothermal conditions, eliminating the need for sophisticated thermocycling equipment. Widely used NAIA platforms include loop-mediated isothermal amplification (LAMP), recombinase-aided amplification (RAA), and recombinase polymerase amplification (RPA), each offering rapid amplification and high analytical sensitivity for viral detection [94,95,96]. All three nucleic acid isothermal amplification techniques—LAMP, RAA, and RPA—rely on the use of target-specific primers and probes designed to recognize conserved viral genomic regions. Amplification occurs under a constant temperature regime, often generating detectable products within 30–45 minutes. Depending on the assay format, amplification products may be visualized through fluorescent signals, colorimetric reactions, or gel electrophoresis. To further improve point-of-care applicability, RAA and RPA platforms are frequently coupled with lateral flow immunoassays (LFIAs), which provide rapid, sensitive, and visually interpretable results without the need for specialized laboratory equipment [97]. Although all three isothermal amplification technologies provide rapid and sensitive nucleic acid detection, their performance characteristics differ. LAMP is generally favored for applications requiring high analytical sensitivity and is well suited for use in resource-limited settings. RAA and RPA, however, operate at lower temperatures (32–42 °C) and typically produce results more quickly, while also requiring less complex primer designs. Furthermore, RPA exhibits greater adaptability to portable diagnostic platforms, making it particularly valuable for field-based and point-of-care viral detection.

Recent advances in CRISPR-based diagnostics have led to the development of Cas12a-mediated nucleic acid detection platforms for hMPV. Cas12a, a member of the CRISPR-associated nuclease family, can be programmed with guide RNAs to recognize specific viral sequences and, upon target recognition, exhibits collateral nuclease activity that generates a detectable signal. This technology has shown considerable promise for rapid, sensitive, and specific *in vitro* detection of hMPV and other respiratory viruses [98]. Using the CRISPR-Cas12a detection platform, the highly conserved *N* gene was selected as the target for amplification, enabling the successful and sensitive detection of hMPV nucleic acids [99]. Building on the same CRISPR-based detection strategy, a recent study targeted

the highly conserved hMPV *N* gene for one-step viral detection and demonstrated a 98.53% concordance rate with conventional real-time RT-PCR [96].

Recent developments in genomic technologies have expanded the use of next-generation sequencing (NGS)-based diagnostics for respiratory virus detection. Among these approaches, metagenomic next-generation sequencing (mNGS) has emerged as a powerful tool for hMPV screening because it enables unbiased sequencing of all nucleic acids within a clinical sample, allowing simultaneous detection of hMPV, coinfecting pathogens, and novel viral variants. Owing to its broad diagnostic scope and high analytical sensitivity, mNGS is increasingly being incorporated into clinical and epidemiological investigations of respiratory viral infections [100]. mNGS is an untargeted, shotgun-sequencing approach that analyzes all nucleic acids present in a clinical sample. Unlike targeted PCR-based assays, mNGS does not require specific primers and can detect a wide range of microorganisms, including rare, emerging, and novel pathogens. To improve sensitivity, host nucleic acids are reduced through depletion strategies and bioinformatic removal of human sequencing reads. Compared with conventional culture, serology, and targeted molecular assays, mNGS provides unbiased detection of all microorganisms present in a sample, making it particularly useful for identifying coinfections and previously unrecognized pathogens. Recent studies have also employed nanopore-based metagenomic sequencing for hMPV detection, demonstrating its potential as a rapid, real-time diagnostic tool for clinical and epidemiological investigations [101].

Nanopore sequencing detects nucleic acids by monitoring changes in ionic current as individual DNA or RNA molecules pass through biological nanopores embedded within a membrane. Because this technology enables direct sequencing without prior PCR amplification, it is particularly well suited for metagenomic applications requiring unbiased detection of multiple pathogens. Although its reported sensitivity (~80%) remains lower than that of conventional PCR-based assays, nanopore sequencing offers distinct advantages, including long-read sequencing capability and real-time data generation. These features make it a valuable tool for pathogen surveillance, outbreak investigations, and infection-control monitoring. Nevertheless, further improvements in sensitivity, sequencing accuracy, and bioinformatics workflows are needed to support its widespread clinical adoption.

Molecular diagnostic platforms have become the cornerstone of hMPV detection, largely superseding traditional culture- and serology-based approaches due to their enhanced sensitivity, specificity, and diagnostic efficiency. Among these technologies, next-generation sequencing (NGS) has markedly advanced both basic and translational research by enabling unbiased viral detection, comprehensive genomic analysis, and large-scale epidemiological

surveillance. However, despite the precision and analytical power of modern molecular diagnostics, important gaps remain in clinical management. Specifically, the absence of approved antiviral therapies and the lack of standardized diagnostic algorithms across healthcare systems continue to pose significant barriers to effective disease control, surveillance, and public health intervention efforts [102].

Therapeutics and Treatment Options

Despite advances in diagnostic technologies, the management of hMPV infection remains largely supportive, with treatment focused on symptom control, maintenance of adequate hydration, and respiratory support in severe cases. Recent reviews have emphasized that effective management of hMPV will require a more comprehensive framework that integrates clinical impact assessments with improved diagnostic algorithms, standardized management protocols, and the development of targeted antiviral therapies. Such efforts are expected to enhance patient care while strengthening public health preparedness for respiratory viral infections among older and high-risk adults [2]. Symptomatic treatment includes the use of antipyretic agents, such as acetaminophen and ibuprofen, to alleviate fever and improve patient comfort. Maintenance of adequate hydration is achieved through oral fluid intake or intravenous fluid therapy, depending on disease severity and the patient's ability to tolerate enteral hydration. For patients who develop acute respiratory distress or respiratory failure, oxygen supplementation may be provided through nasal cannula, high-flow oxygen delivery systems, noninvasive ventilation, or invasive mechanical ventilation in severe cases. These interventions are particularly crucial for individuals with chronic cardiopulmonary conditions, older adults, young children, and immunocompromised patients, who are more susceptible to severe lower respiratory tract disease and may require intensive respiratory support to maintain adequate oxygenation and pulmonary function.

Among available antiviral therapies, ribavirin has been most frequently used in high-risk populations, including immunocompromised individuals, owing to its broad-spectrum antiviral activity and reported efficacy against hMPV in experimental and clinical settings [103,104]. Although ribavirin has been employed as a therapeutic option its efficacy remains limited. A systematic review comparing treated and untreated immunocompromised cohorts found no significant improvement in clinical outcomes, including progression to lower respiratory tract infection and hMPV-associated mortality. These findings highlight the need for well-designed prospective studies and the development of more effective antiviral strategies for vulnerable patient populations [15]. The current evidence supporting hMPV therapeutics is derived primarily from case reports, case series, retrospective cohort studies, and preclinical investigations rather than randomized controlled trials. The

lack of consistent clinical benefit may be attributable to delayed treatment initiation, dose-limiting toxicities, and the predominance of retrospective and observational evidence in the published literature.

Among emerging antiviral strategies, neutralizing antibody-based therapies have shown considerable promise for the treatment and prevention of hMPV infection. Recent structural and immunological studies have identified rare, highly potent broadly neutralizing antibodies (bnAbs) that target conserved epitopes unique to the prefusion conformation of the hMPV F glycoprotein. By stabilizing or binding critical functional regions of the F protein, these antibodies can effectively block viral attachment and membrane fusion, thereby preventing infection. The discovery of such bnAbs has also provided important insights for the rational design of next-generation vaccines and antibody-based immunotherapies against hMPV [105,106]. Recent studies have also identified potent human monoclonal antibodies capable of cross-neutralizing both hMPV and respiratory syncytial virus (RSV), highlighting the potential for broadly protective antiviral immunotherapies [107]. RSV-199 represents a promising example of a broadly neutralizing monoclonal antibody with activity against both RSV and hMPV. This antibody targets antigenic site III located on the prefusion form of the viral F glycoprotein, a critical structure required for membrane fusion and viral entry. By binding this conserved epitope, RSV-199 effectively inhibits fusion between the viral envelope and host-cell membrane, thereby preventing infection. Structural analyses have demonstrated that the antibody possesses an extended complementarity-determining region 3 (CDR3), enabling recognition of conserved antigenic features shared by both pneumoviruses. Although these findings provide a strong foundation for the development of cross-protective antibody therapies, additional studies are required to optimize antibody design, evaluate long-term efficacy and safety, and establish its potential role in clinical management of RSV and hMPV infections [107,108,109].

Several research groups have investigated F protein-targeted antiviral strategies in experimental hMPV infection models. Peptide inhibitors derived from the heptad repeat A (HRA) and heptad repeat B (HRB) domains of the F protein have shown promising antiviral activity. Among these candidates, the HRA2 peptide demonstrated potent inhibitory activity against all major hMPV subgroups (A1, A2, B1, and B2). In mouse challenge studies, intranasal administration of HRA2 provided protection against clinical disease and mortality following lethal hMPV infection and reduced lung viral loads to undetectable levels [18,110]. Beyond fusion-targeting peptides and monoclonal antibodies, naturally derived antiviral compounds have also shown promise against hMPV. In a study originally focused on RSV, two plant- and lichen-derived molecules—fumarprotocetraric acid and geraniin—were found to possess significant anti-hMPV activity [111]. Fumarprotocetraric acid, a lichen secondary metabolite, and geraniin, a bioactive polyphenol isolated from geranium species, inhibited viral replication across multiple experimental systems, including HEp-2 cells, differentiated air–liquid interface human airway epithelial (ALI-HAE) cultures, and LLC-MK2 rhesus monkey kidney epithelial cells. The broad antiviral activity observed in physiologically relevant airway epithelial models underscores the therapeutic potential of natural products as a source of novel antivirals for hMPV and related respiratory viruses [111].

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Vaccine Strategies

Currently, no licensed vaccine is available for the prevention of hMPV infection. Nevertheless, substantial progress has been made in vaccine development, and several vaccine candidates are undergoing preclinical evaluation in non-human primate models, with encouraging results in terms of immunogenicity and protective efficacy [112,113]. Despite considerable progress in hMPV vaccine research, several early vaccine candidates have failed to advance because of inadequate attenuation or insufficient protective efficacy. These challenges have shifted attention toward structure-based vaccine design strategies targeting the viral F glycoprotein. The F protein is a critical mediator of viral attachment and membrane fusion and is prominently displayed on the viral surface, making it readily accessible to neutralizing antibodies. Importantly, the F protein exhibits a high degree of conservation among hMPV genotypes and subgroups, increasing the likelihood of eliciting broadly protective immunity. Consequently, F protein-based vaccine platforms have become the leading candidates in current hMPV vaccine development efforts and continue to show promise in preclinical studies [114]. In addition to the F glycoprotein, the G and SH envelope proteins contribute to immune modulation and viral replication. Consequently, multivalent vaccine strategies that target G and SH alongside the F protein may enhance protective immunity and provide broader protection against hMPV infection.

Among emerging vaccine candidates, mRNA-1653 represents a promising mRNA-based approach designed to confer immunity against both human metapneumovirus (hMPV) and parainfluenza virus type 3 (PIV3). The vaccine comprises two nucleoside-modified mRNA molecules encoding the full-length membrane-anchored F glycoproteins of hMPV and PIV3, encapsulated within a lipid nanoparticle formulation to enhance cellular delivery and antigen expression. Early clinical evaluation in phase 1a and phase 1b trials involving healthy adults and seropositive pediatric participants demonstrated a favorable safety and tolerability profile. Furthermore, vaccination induced substantial increases in neutralizing antibody titers against hMPV-A, hMPV-B, and PIV3, with measurable responses observed approximately one month after administration of a single dose. These findings support the continued devel-

opment of mRNA-1653 as a potential strategy for preventing respiratory infections caused by multiple pediatric viral pathogens [16,17].

Following the success of the SARS-CoV-2 vaccine [115], an emerging strategy for hMPV vaccine development involves a rationally designed multi-epitope mRNA platform incorporating antigenic determinants from the F, G, SH, M, M2-1, and M2-2 proteins. The construct was engineered to induce broad immune responses encompassing cytotoxic T lymphocytes, helper T lymphocytes, and B-cell-mediated immunity. Immunoinformatics-guided selection identified 15 CTL epitopes, 4 HTL epitopes, and 9 linear B-cell epitopes, which were combined with immune-enhancing adjuvant elements. Computational analyses predicted high antigenicity, strong immunogenicity, and an absence of toxicity, while also suggesting the potential induction of IFN- γ , IL-4, and IL-10 responses. To further augment immunogenicity, TLR4 and TLR2 agonists were incorporated at the terminal regions of the construct, and a PADRE sequence was included to promote robust adaptive immune activation. Although codon optimization supported efficient translational potential, experimental validation in relevant *in vitro* and animal models remains essential to determine the feasibility of this approach for future clinical application [116].

Recent advances in structural virology and immunology have facilitated the identification of several hMPV epitopes with vaccine potential. Among these, epitopes located on the viral F glycoprotein have received the greatest attention because the F protein is essential for viral entry and is the dominant target of neutralizing antibody responses. Antigenic sites III, IV, and V have been shown to elicit potent neutralizing antibodies and are structurally conserved between hMPV and RSV. The ability of these epitopes to be recognized by cross-reactive monoclonal antibodies highlights their importance as conserved immunological targets and supports their inclusion in the design of next-generation vaccines aimed at providing broad protection against diverse hMPV strains and related pneumoviruses. A unique antigenic epitope on the hMPV F protein recognized by the human monoclonal antibody DS7 has been structurally characterized and represents an attractive target for vaccine design. In addition to humoral immune targets, several T-cell epitopes have been identified throughout the hMPV proteome. To date, nine cytotoxic T-lymphocyte (CTL) epitopes restricted by common human leukocyte antigen (HLA) alleles have been reported. Among these, six HLA-B7-restricted CD8⁺ T-cell epitopes were mapped to the F, M, and N proteins, with the M195–203 epitope inducing the most robust cellular immune response [117]. In addition to F protein-specific epitopes, highly conserved antigenic regions within the N and M2-2 proteins have been identified as promising targets for vaccine development. Their high degree of conservation across diverse hMPV lineages makes them attractive

candidates for eliciting broad protective immunity. Consequently, these conserved epitopes have been incorporated into multiepitope vaccine constructs to expand strain coverage and enhance overall vaccine efficacy [116,118].

Epitope Immunogenicity and Vaccine Development

The incorporation of conserved B-cell and T-cell epitopes into vaccine platforms has yielded encouraging results in experimental models of hMPV infection. Virus-like particle (VLP) vaccines containing epitopes derived from the F, M, and N proteins have been shown to induce strong neutralizing antibody responses together with robust cytotoxic T-lymphocyte (CTL) activity in mice, demonstrating the ability to stimulate both arms of the adaptive immune system. In parallel, multiepitope mRNA vaccine approaches encoding carefully selected antigenic determinants have shown promising immunogenicity and favorable safety profiles in preclinical studies and early clinical testing. Collectively, these findings suggest that epitope-focused vaccine strategies may offer broad protection against diverse hMPV strains while promoting balanced humoral and cellular immunity, supporting their continued advancement toward clinical development [16,118,119,120].

Comprehensive immunoinformatic analyses have demonstrated that these epitopes exhibit strong antigenic potential and favorable immunogenic characteristics while maintaining a low probability of toxicity, allergenicity, or other undesirable biological effects. These findings support their suitability as key components of rationally designed vaccine platforms, including multiepitope, virus-like particle, and mRNA-based vaccines. Consequently, these epitopes provide a robust foundation for the development of safe, effective, and broadly protective next-generation vaccines against hMPV [121].

In summary, vaccine development efforts continue to focus on several highly promising antigenic targets, including neutralizing epitopes located at antigenic sites III, IV, and V of the F glycoprotein, immunogenic CTL epitopes derived from the F, M, and N proteins, and conserved regions within the N and M2-2 proteins. The incorporation of these epitopes into multicomponent vaccine platforms may facilitate the development of broadly protective and durable vaccines against diverse hMPV strains (as shown in Table 2) [16,106,117,119,122].

Perspectives and Future Directions to Prevent and Control the spread of the disease

More than a quarter-century after the identification of human metapneumovirus, important aspects of its pathogenesis remain unresolved, particularly the mechanisms governing host immune evasion and the determinants of disease severity. In the absence of licensed vaccines and effective antiviral therapies, prevention strategies continue to depend largely on established public health interven-

Table 2. Specific epitopes suitable for the hMPV vaccine candidate.

Epitope type	Protein source	Specific epitopes/Antigenic sites	Importance
Neutralizing antibody	F protein	Antigenic sites III, IV, V; DS7 epitope	Primary target for neutralizing antibodies
CTL epitopes	F, M, N proteins	HLA-B7-restricted six epitopes, including M195–203	Induces strong CD8+ T cell responses
Conserved epitopes	N, M2-2 proteins	Conserved peptides across hMPV types	Essential for broad vaccine coverage

tions, including rigorous hand hygiene, appropriate mask use, and physical distancing measures. At the same time, advances in multiplex molecular diagnostics are expanding the capacity to detect hMPV in conjunction with other respiratory pathogens, thereby improving clinical recognition, epidemiological surveillance, and outbreak investigation. Looking forward, sustained efforts to accelerate vaccine development and the clinical evaluation of emerging vaccine candidates will be critical for reducing the global burden of hMPV and improving long-term disease prevention and control.

Future research efforts should focus on several critical priorities to advance understanding of hMPV epidemiology, pathogenesis, and disease burden. First, standardized surveillance programs are needed to better define testing practices, quantify case detection rates, and determine whether hMPV positivity varies according to geographic region, season, age group, or underlying risk factors. Second, additional epidemiological studies are required to clarify whether the relatively low number of reported hMPV cases reflects limited diagnostic testing, a genuinely lower incidence of infection, or partial immunity within certain populations. Third, more comprehensive data are needed to elucidate the relationships among infection timing, asymptomatic carriage, viral shedding, and progression to severe disease.

Equally important is the identification of host factors that influence susceptibility to infection, viral immune evasion, and adverse clinical outcomes. A deeper understanding of the immunological and host-related determinants of disease severity will help identify individuals at greatest risk and inform more targeted prevention and management strategies. Addressing these knowledge gaps will strengthen public health surveillance, improve risk stratification, and support the development of more effective measures for the prevention and control of hMPV transmission.

Conclusion

More than 25 years after its discovery, hMPV continues to be a significant cause of respiratory illness worldwide, imposing a substantial clinical burden across all age groups, particularly among young children, older adults, and immunocompromised individuals. Considerable progress has been made in elucidating the epidemiology, molecular biology, pathogenesis, and transmission dynamics of hMPV, while advances in molecular diagnos-

tics have markedly improved the accuracy and timeliness of viral detection. In parallel, promising therapeutic and preventive strategies—including monoclonal antibodies, fusion inhibitors, mRNA-based vaccines, and multiepitope vaccine platforms—have emerged as potential tools for disease control.

Despite these advances, important challenges remain. The mechanisms underlying viral immune evasion, determinants of disease severity, and factors governing transmission are not yet fully understood. Furthermore, the absence of licensed antiviral therapies and approved vaccines continues to limit effective clinical management and prevention efforts. Addressing these gaps will require enhanced surveillance programs, standardized diagnostic approaches, deeper characterization of host and viral factors associated with severe disease, and continued investment in translational research aimed at developing effective therapeutics and vaccines.

Looking forward, the integration of advanced diagnostic technologies, improved epidemiological surveillance, and next-generation vaccine platforms offers a promising path toward reducing the global burden of hMPV infection. Continued multidisciplinary research efforts will be essential to advance prevention strategies, optimize patient care, and ultimately achieve more effective control of this important respiratory pathogen.

Availability of Data and Materials

Not applicable.

Author Contributions

PKD: Conceptualization, Writing-Original Draft. RR: Visualization, Writing-Original Draft. LYP: Literature review, Writing-Review & Editing. RTS: Literature review, Figure Generation, Writing-Review & Editing. All authors have read and approved the final version of the paper. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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