*zoonotic diseases*

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*Review*

# A Review of the Molecular Understanding of the Mpox Virus (MPXV): Genomics, Immune Evasion, and Therapeutic Targets

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**Simple Summary:** Mpox is a viral disease that has gained global attention due to its ability to spread from animals to humans and between people. Recent outbreaks in endemic and previously unaffected areas underscore the importance of understanding the virus's mechanisms and evolution. This review explores the genetic structure of the Mpox virus (MPXV), how it interacts with human cells, and how it avoids the immune system. The review's aim is to provide researchers and public health experts with valuable insights into how the virus spreads, adapts, and causes disease. This research hopes to guide efforts to contain Mpox and improve preparedness for future outbreaks, benefiting scientists and public health policymakers.

**Abstract:** The Mpox virus (MPXV), a zoonotic pathogen from the *Orthopoxvirus* genus, has emerged as a significant global public health concern, especially after the unprecedented outbreak in 2022. This review synthesizes the MPXV's molecular features, focusing on its genomic structure, replication mechanisms, immune evasion strategies, and implications for diagnostics and therapeutics. The study examines the virus's genomic organization utilizing recent peer-reviewed literature, highlighting essential genes like OPG027 and D1L, which contribute to host adaptation, increased transmissibility, and immune evasion. Advances in molecular diagnostics, including real-time PCR and genome sequencing, are reviewed, emphasizing their critical role in outbreak monitoring and control. However, challenges persist, such as diagnostic limitations in resource-constrained settings and the lack of targeted vaccines and antivirals. This review discusses new antiviral candidates, confirmed through computational and in vitro techniques, identifying thymidine kinase and VP39 as key therapeutic targets. Emphasizing the need for genomic surveillance to track adaptive evolution, results show that particular mutations, such as in the OPG027 and D1L genes, increase the transmissibility and immune evasion of the MPXV. These molecular revelations highlight the urgent necessity for better diagnostics catered towards addressing present constraints and developing focused treatments that reduce the effect of the virus. This study emphasizes how these results underscore the need for combined public health plans to handle the changing MPXV epidemiology properly.

**Keywords:** MPXV; molecular biology; viral genomics; pathogenesis; immune evasion; antiviral targets; molecular diagnostics



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## 1. Introduction

Mpox is an infectious disease caused by the Mpox virus (MPXV), a virus of the genus *Orthopoxvirus*, belonging to the family Poxviridae [1–4]. This family includes other zoonotic

viruses specific to humans, such as smallpox, cowpox, camelpox, and molluscum contagiosum viruses [4]. The MPXV was first identified in 1958 in a colony of cynomolgus monkeys (*Macaca fascicularis*) that were being used for experimental purposes in Denmark [5]. The first human case was reported in 1970 in the Democratic Republic of Congo (DRC) in a 9-month-old baby [2]. Although its name suggests that monkeys are primary hosts of the MPXV, it is more common in small mammals from Africa. Mpox is often self-limiting, with symptoms lasting two to four weeks [4,6].

Mpox is a zoonotic disease [7–9], and though the definitive host has not been identified, rodents are the most probable reservoirs [3,7,8,10]. The MPXV is transmitted to humans through direct or indirect contact (e.g., through contaminated materials) with infected animals [8,9]. Consuming meat that is undercooked may pose a risk [3,8,10]. Human transmission occurs through bodily fluids, such as respiratory droplets, direct contact with cutaneous eruptions or crusts from infected people, or contaminated objects [5,9,11]. Close contact with infected people, particularly in domestic environments, increases the risk of transmission [12]. Recent evidence suggests that sexual transmission can also play a significant role in MPXV dissemination [1,4,7].

The disease is endemic in Central and West Africa [3,7]. The DRC has been the most affected country, with a significant incidence increase 30 years after the vaccination campaigns against smallpox [13,14]. Nigeria also presents recurrent outbreaks [5,7]. Mpox outbreaks have been rare outside Africa and are usually associated with travels to endemic countries or imports of infected animals [3,9]. However, the 2022 global outbreak caused by West Africa's B.1 lineage changed the MPXV epidemiological landscape [3,7]. Concerns persist about the MPXV's rapid spread in non-immune populations, especially among individuals unvaccinated against smallpox [1–3,11].

Understanding the MPXV's biological and pathogenic features is urgently needed due to the lack of commercially available antivirals and vaccines [7]. As the virus propagates globally with increasing cases, gaining insights into the MPXV's molecular evolution will be crucial for developing effective prevention and control strategies [15,16]. This narrative review seeks to comprehensively synthesize the current knowledge on the MPXV's molecular characteristics. The goal is to serve as a resource for researchers and decision-makers, offering actionable insights that can guide the development of diagnostics, therapeutics, and public health strategies to control the virus effectively.

## 2. Materials and Methods

The methodology for this review involved a systematic literature search and analysis process to compile relevant information on the molecular aspects of the MPXV's biology. A targeted search was conducted on Google Scholar using the keywords "molecular" and "Mpox," focusing on studies from 2022 to 2024. The year 2022 was chosen as the starting point due to significant global outbreaks that transformed our understanding of the MPXV's epidemiology and molecular biology. Inclusion criteria prioritized peer-reviewed articles with significant citations that addressed molecular detection, genomic annotation, evolution, or therapeutic developments. Key studies provided novel insights into the genomic structure, immune evasion mechanisms, and therapeutic implications of the MPXV. Articles failing to meet these criteria or focusing exclusively on clinical or epidemiological data were excluded to maintain the review's molecular focus.

Atlas.ti 9.1.7 [17] allowed for document organization and the systematic categorization of content into specific thematic codes, including viral structure, genome organization, replication mechanisms, immune evasion, and molecular diagnostics. This approach ensured that all critical aspects of Mpox's molecular biology were comprehensively covered in the review. During the data extraction, relevant information from each document was

organized under these codes, enabling a structured analysis across various facets of the MPXV's biology. Peer-reviewed articles were preferred, and preprints were excluded from the final selection to maintain academic rigor and credibility.

After categorizing and analyzing the documents, 17 articles were selected for in-depth review (Table 1). Most articles (70.6%) were from 2024, followed by 2023 (17.6%), 2022 (5.9%), and 2011 (5.9%). Even though the latter group of articles are from before 2022, they seemed relevant to include as the insights they provide are pivotal for understanding MPXV's molecular biology.

**Table 1.** List of the primary peer-reviewed sources used for this study.

| No. | Year | Area | Theme | Main Topic | Reference |
|---|---|---|---|---|---|
| 1 | 2011 | Global | Molecular Pathogenesis | Disease progression using molecular imaging | Dyall et al. [11] |
| 2 | 2022 | Global | Epidemiology and Evolution | Epidemiology, transmission, and molecular properties of Monkeypox | Kannan et al. [5] |
| 3 | 2023 | Global | Epidemiology and Evolution | Molecular evolution and genome analysis of the human Monkeypox virus | Wolf et al. [7] |
| 4 | 2023 | Brazil | Epidemiology and Evolution | Phylogenetic and molecular evolution of Monkeypox | Ferrareze et al. [1] |
| 5 | 2023 | Global | Therapeutics | Identification of flavonoid inhibitors targeting thymidine kinase | Abdizadeh [10] |
| 6 | 2024 | Global | Diagnostics | Validation of high-throughput molecular tests for Monkeypox detection | Anderson et al. [12] |
| 7 | 2024 | Nigeria | Diagnostics | Molecular detection of Monkeypox virus in wild rodents and humans | Abafi et al. [8] |
| 8 | 2024 | Global | Host-Virus Interactions | Host-virus interactions and immune response pathways | Tang et al. [6] |
| 9 | 2024 | Global | Host-Virus Interactions | Codon usage and protein evolution in Monkeypox virus | Shan et al. [15] |
| 10 | 2024 | Global | Therapeutics | Repositioning anti-infective compounds against cysteine proteinase | Rabaan et al. [18] |
| 11 | 2024 | China | Epidemiology and Evolution | Phylogeny and molecular evolution of Guangdong Monkeypox outbreak | Yu et al. [16] |
| 12 | 2024 | Global | Therapeutics | Potential inhibitors of envelope protein E8 using molecular simulations | Das et al. [19] |
| 13 | 2024 | Global | Therapeutics | Silencing E8L protein using siRNA for therapeutic purposes | Islam et al. [20] |
| 14 | 2024 | Global | Molecular Pathogenesis | Genetic, clinical, and therapeutic perspectives on Monkeypox | Wambani et al. [9] |
| 15 | 2024 | Global | Therapeutics | Pharmacophore modeling and drug discovery targeting thymidylate kinase | Charles et al. [21] |
| 16 | 2024 | Global | Therapeutics | Drug repurposing against Monkeypox virus RNA polymerase | Khan et al. [4] |
| 17 | 2024 | Africa | Epidemiology and Evolution | Monkeypox evolution and host interactions through computational studies | Abafi et al. [8] |

This selection included peer-reviewed studies that offered significant molecular insights into the MPXV's structure, pathogenic mechanisms, and potential therapeutic targets, aligning with the study's objective to provide an informative synthesis for the scientific community. Thematic analysis revealed that therapeutics dominates with 35.3% of the entries; followed by epidemiology and evolution at 29.4%; and diagnostics, host–virus interactions, and molecular pathogenesis, each at 11.8%. This thematic distribution strongly focuses on understanding the MPXV's therapeutic interventions, genetic diversity, and transmission dynamics, which are critical for outbreak preparedness and control. How-

ever, the lower representation of diagnostics and host–virus interactions suggest potential gaps in addressing rapid detection methods and the intricate mechanisms of virus–host dynamics. This imbalance may have influenced the review's depth in these areas.

As the review was written, other articles were included when needed. Furthermore, the author used the phyloT (version 2.0) tool developed by Ivica Letunic [22] to create phylogenetic trees based on NCBI taxonomy identifiers, contextualizing the molecular and evolutionary relationships within the MPXV. A focused subset of viral taxa, including species and isolates relevant to the MPXV, was selected using specific NCBI [23,24] tree elements for Entomopoxvirinae, the *Centapoxvirus*, the *Vaccinia virus*, the *Raccoonpox virus*, the *Orthopoxvirus akhmetapox*, the *Camelpox virus*, the MPXV, the *Cowpox virus*, and the *Ectromelia virus*, previously verified by Maluquer de Motes [25] as relevant taxa to consider. The generated tree was exported in the Newick format for further refinement and visualization on the Interactive Tree of Life (iTOL) platform developed by Letunic and Bork [26].

## 3. The MPXV—Its Genome and Genetics

Ndodo et al. [27] mentioned the existence of two main MPXV phylogenetic lineages: (1) the Central Africa lineage, also known as the Congo Bay lineage, and (2) the West Africa lineage (see Table 2, adapted from Okwor et al. [28], with notes on transmission and public health implications).

**Table 2.** The MPXV's main clades.

| Clade | Primary Region | Distinctive Feature | Transmission | Public Health Implications |
|---|---|---|---|---|
| I | Central Africa | High virulence, higher case fatality rate than Clade II. | Primarily zoonotic with limited human-to-human transmission. | High fatality rates require focused surveillance in endemic regions and access to effective treatments. |
| IIa | West Africa, Global cases traced to zoonotic origins | Less virulent than Clade I, endemic in West Africa. | Primarily zoonotic, lower human-to-human transmission. | This zoonotic transmission underscores the need for enhanced wildlife monitoring and public education. |
| IIb | Multiple global regions, no known zoonotic link for 2022 PHEIC | Sequence clustering from 2017–2019, distinct global transmission. | Exclusively human-to-human transmission through close contact. | Increased transmissibility necessitates global surveillance and targeted vaccine deployment. |

The MPXV's genome is 197 kb long [9], coding approximately 200 genes [15], with a highly conserved central region containing genes involved in essential functions, such as transcription, replication, and virion production [1,19]. While this central conservation is well-documented, debates persist over the role of terminal variability in shaping host range and pathogenesis, highlighting the need for studies that link specific gene variations to clinical outcomes. The genome's terminal regions are variable and contain genes that code proteins to determine the host range and pathogenesis [1]. The MPXV's genome contains inverted terminal repeats (ITRs), each carrying a gene copy, which adds to its complexity [1,2]. The Central and West African strains have unique genes associated with distinct disease presentations [1,9]. Further genome analysis has revealed synteny breaks between the different strains, indicating genomic rearrangements during the MPXV's evolution [1]. The evolutionary relationship of the MPXV within the *Orthopoxvirus* genus is shown in Figure 1, highlighting its divergence from closely related viruses such as Variola and Vaccinia. This phylogenetic insight underscores the need for robust genomic

surveillance to monitor adaptive mutations that may enhance the MPXV's transmissibility or immune evasion.

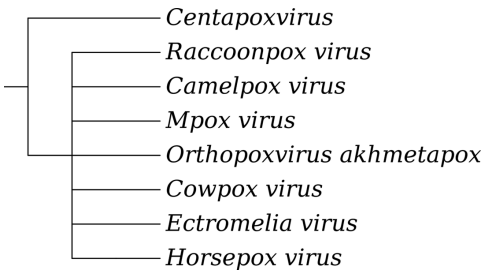


**Figure 1.** Phylogenetic tree of the Chordopoxvirinae subfamily (Poxviridae family). Within the subfamily, there are the *Centapoxvirus* and *Orthopoxvirus* genera; Mpox belongs to the latter of which. The evolutionary origin of Chordopoxvirinae is associated with the acquisition of the poxin gene by rodent poxviruses, facilitating immune evasion. The diversification of *Orthopoxvirus* may involve genetic adaptations, including interactions or acquisitions of immune-modulatory genes, such as poxin and Schlafen-related elements, though the specific mechanism of fusion requires further investigation. Image generated through phyloT [25] and edited on iTOL [26] and Microsoft Paint version 11.2410.39.0 [29].

Notable evolutionary events include acquiring host-adaptive genes, highlighting the importance of genomic surveillance in tracking the MPXV's evolution and implications for transmission and pathogenesis. The West African strain, for instance, is associated with milder disease, reduced lethality, and a lower reproduction rate than the Central African strain [7].

Several genes and proteins are crucial in the MPXV's replication and pathogenesis (Table 3), and slight genetic differences due to mutations are enough to originate distinct pathogenicity and reproduction rates [5]. However, the functional significance of specific mutations remains controversial, with conflicting evidence on whether these changes primarily enhance immune evasion or host adaptation. In addition, viral genome editing caused by APOBEC3, a host enzyme, can introduce C>T and G>A mutations, accelerating viral evolution [15,16]. Such mutations and others (e.g., the ones Tang et al. [6] mentioned in the D1L gene) may have contributed to the greater transmissibility of the virus between humans.

Recent studies have provided compelling evidence to suggest that specific mutations in the OPG027 and D1L genes, while enhancing the MPXV's transmissibility, may also affect therapeutic efficacy. These mutations, particularly those altering protein structures such as thymidine kinase (TK) and VP39 methyltransferase, can potentially reduce the effectiveness of key antivirals, including those targeting these enzymes [10,15]. For instance, mutations in TK, essential for viral DNA synthesis, and VP39, crucial for mRNA capping, may compromise the efficacy of drugs like tecovirimat and brincidofovir, necessitating the exploration of alternative inhibitors [10,21].

Integrating genomic surveillance into public health strategies becomes critical for the early detection of resistance-associated mutations. Monitoring these genomic changes allows researchers to anticipate shifts in the virus's drug susceptibility and informs the iterative development of targeted therapies [6,15]. By linking genomic data with phenotypic outcomes, such as drug resistance, this approach can guide the creation of more robust antiviral strategies and ensure the sustainability of therapeutic interventions amid the evolving epidemiology of the MPXV [18].

**Table 3.** Genes and proteins associated with the MPXV virulence and host adaptation. It is important to note that, although these genes and proteins are crucial, a complete understanding of the replication and pathogenesis of the MPXV requires further research.

| Gene | Protein Coded | Main Role | Source |
|---|---|---|---|
| OPG027 | C7L | Host range determination and antiviral activity inhibition | Shan et al. [15] |
| D1L | Ankyrin repeat protein | Adaptation to human host and person-to-person transmission | Tang et al. [6] |
| IFIT1/2 | IFIT1/2 | Antiviral activity by inhibiting mRNA translation initiation | |
| Cysteine protease | Cysteine protease | Viral replication by cleaving precursor polyproteins | Rabaan et al. [18] |
| Ankyrin-like/ Kelch-like | Ankyrin-like/ Kelch-like proteins | Immunomodulation and potential influence on host range | Ferrareze et al. [1] |
| BR-203, BR-209, COP-C3L | BR-203, BR-209, COP-C3L proteins | Variations in virulence between Central and Western African strains | Kannan et al. [5]; Tang et al. [6]; Ferrareze et al. [1] |
| D14R | MOPICE | Virulence factor, absent in Western African strains | Shan et al. [15]; Ferrareze et al. [1]; Wambani et al. [9] |

## 4. Molecular Mechanisms of the MPXV's Replication, Pathogenesis, and Immune Evasion

The MPXV's lifecycle, as depicted in Figure 2, involves adhesion, entry, replication, assembly, and release, illustrating the intricate molecular processes that enable the virus to propagate within the host. However, the molecular mechanisms governing the transition from replication to assembly remain unclear, and the role of host-specific factors in these stages warrants further investigation.

Adhesion is mediated by the E8L protein, which binds with glycosaminoglycans (GAGs) on host cell surfaces, a critical step for viral entry [9,30–32]. This highlights a potential therapeutic target, as disrupting this interaction could inhibit viral attachment. Inside the host cytoplasm, the MPXV replicates its DNA genome using a DNA polymerase holoenzyme complex comprising the F8 polymerase and processivity factors A22 and E4, with replication factories forming "mini nuclei" to protect the genome from immune detection [33,34].

These replication factories highlight the virus's ability to exploit host cellular machinery, complicating the design of interventions that target the virus without harming host cells. The figure emphasizes the complexity of viral assembly, which involves distinct stages—crescent formation, immature virions, mature virions, and wrapped virions—relying on various organelles, including melanosomes, particularly in skin lesions. This highlights the difficulty of targeting the assembly process, which involves multiple cellular pathways. The production of both intracellular mature virions (IMV) and extracellular enveloped virions (EEV) differ in their release mechanisms. Neutralization sensitivities [34–37] further complicate therapeutic strategies, as different virion forms may require distinct approaches to inhibition. While these insights provide a detailed understanding of the MPXV's lifecycle, several questions remain unanswered. For example, the exact molecular mechanisms underlying the transition from replication to assembly and how specific mutations in viral genes influence these processes require further investigation. Additionally, the role of host-specific factors in modulating replication efficiency and immune avoidance tactics needs to

be explored. Addressing these gaps will be critical for developing targeted therapies that disrupt the MPXV's replication without triggering unintended effects on host systems.

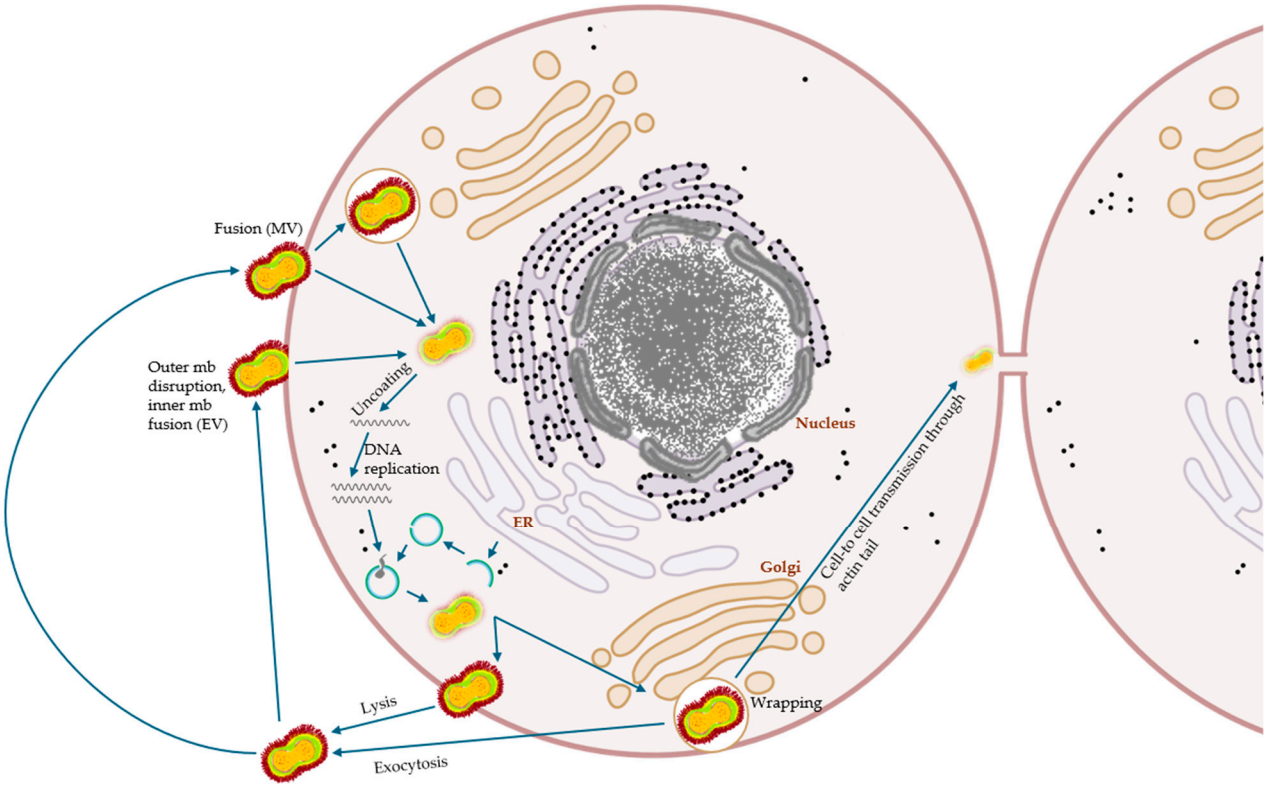


**Figure 2.** The MPXV's lifecycle—invading a cell, replicating its genetic material, forming new virions, and evading to the environment or other cells. Notes: EV = Enveloped virion; MV = Mature virion; ER = Endopasmatic reticulum.

Table 4 provides a comprehensive overview of the molecular interactions between the MPXV and host systems, emphasizing its immune suppression pathways and potential therapeutic targets.

**Table 4.** Molecular interactions between the MPXV and host cells.

| Interaction Type | Description | References |
| --- | --- | --- |
| Immune Response Inhibition | The MPXV developed strategies to suppress the host's immune response. The OPG027 gene also inhibits the host's antiviral activity and modulates the immune response of T cells, suppressing the activation of host T-cell receptors. | Wolf et al. [7], Kannan et al. [5], and Shan et al. [15] |
| Interaction with Host Proteins | The MPXV C7L gene, an ortholog of OPG027 in the Vaccinia virus, encodes a protein interacting with human SAMD9 protein, which is crucial for viral growth in human cells and SAMD9 binding. Mutations like F79L can impact transmission, pathogenicity, or immune evasion of the Clade I MPXV. | Shan et al. [15] |
| mRNA Translation Inhibition | Host genes IFIT1 and IFIT2 inhibit mRNA translation by binding to the eukaryotic initiation factor 3 (eIF3). The MPXV evolved to inhibit the expression of these genes, evading antiviral effects. | Tang et al. [6] |

**Table 4.** *Cont.*

| Interaction Type | Description | References |
| --- | --- | --- |
| Targets for Antiviral Therapies | Understanding molecular interactions between the MPXV and the host aids in developing antiviral therapy, focusing on viral proteins. | Abdizadeh [10], Charles et al. [21], Rabaan et al. [18], Das et al. [19], and Alharbi et al. [38], |
| Thymidine Kinase (TK) | Crucial for viral DNA synthesis. TK inhibition is a promising strategy for antiviral development, with natural flavonoids showing potential as TK inhibitors. | Abdizadeh [10], Yousaf et al. [3], and Charles et al. [21] |
| Nuclear Cysteine Protease | Essential for viral replication, making it a viable therapeutic target. Anti-infective compounds like tetracycline show potential as inhibitors. | Rabaan et al. [18] |
| VP39 Methyltransferase | The key to mRNA "capping" is to protect viral mRNA from degradation and ensure efficient translation. Inhibiting VP39 could impair viral replication and immune evasion, with marine fungi-derived compounds showing potential as inhibitors. | Alharbi et al. [38] and Shan et al. [15] |
| RNA Polymerase A6R Subunit | Crucial for viral genome transcription. FDA-approved drugs like Nilotinib, Conivaptan, and Ponatinib show potential as inhibitors. | Khan et al. [4] |
| Envelope Protein E8 | Crucial for viral binding to host cells. Inhibiting E8 could block viral entry. Natural compounds like punicalagin show potential as inhibitors. | Das et al. [19] and Islam et al. [20] |
| Mutations & Adaptation | Mutations in viral genes (e.g., D1L, OPG027, and A49R) may affect viral interaction with host cells, influencing virulence and transmission. APOBEC3-induced genome editing can accelerate viral adaptation to new hosts. | Tang et al. [6], Shan et al. [15], and Yu et al. [16] |

The MPXV exhibits complex interactions with host immunity to evade antiviral responses. It encodes proteins like OPG027 and C7L, which inhibit T-cell activation and modulate immune pathways such as TNF, IL-17, and NF-κB. These actions suppress host antiviral activity and allow immune evasion [6,39,40]. Additionally, the virus suppresses the expression of antiviral genes such as IFIT1 and IFIT2, which inhibit viral mRNA translation, thereby counteracting host defenses [6]. Proteins like A49R are essential for replication and transcription, with specific mutations (e.g., C151626T) influencing virulence and transmission [4]. The MPXV also encodes inhibitors of complement enzymes, altering the adaptive immune response. Deleting complement enzyme inhibitor loci could affect immune responses, highlighting the virus's genomic plasticity [6,15].

The MPXV adapts to human hosts through mutations in genes like F8L, D1L, and G9R, which can enhance viral replication, drug sensitivity, and host receptor binding. The positive selection of genes such as MPXVgp004, 010, and 012 and large genomic deletions illustrate the virus's adaptation capacity. The 2022 outbreak strains exhibited mutations that increased transmission potential and host interaction efficiency [39,41–43].

Despite replicating in the cytoplasm, the MPXV relies on host machinery, including ATP, $Mg^{2+}$, ribosomes, and tRNA, to synthesize viral proteins. By reorganizing host cellular structures and encoding proteins like ankyrins and Bcl-2-like proteins, the MPXV ensures efficient replication and immune evasion. These intricate molecular interactions make the MPXV a resilient pathogen and highlight the need for targeted therapeutic strategies to disrupt its replication and immune evasion mechanisms [3,4,6,19,21,44].

## 5. Pathogenesis and Immune Evasion

The MPXV causes a zoonotic disease characterized by skin lesions and systemic symptoms [45]. According to Wang and Lun [46], the virus displays tropism for skin epithelial cells, leading to characteristic rashes progressing through macules, papules, vesicles, and pustules. The authors added that the MPXV primarily spreads through direct skin contact, with respiratory transmission possible during prolonged close contact. MPXV infection in human skin organoids reveals productive keratinocyte replication and four stages of intracellular virus assembly [36]. Electron microscopy of clinical specimens shows typical brick-shaped virions with surface protrusions [35]. Animal studies suggest African rope squirrels are potential reservoirs that exhibit high viremia and prolonged viral shedding [47]. In cynomolgus monkeys, aerosolized MPXV causes fatal bronchopneumonia with systemic spread [48]. The antiviral tecovirimat inhibits MPXV replication in skin organoids [36].

Mpox severity varies based on host and viral factors. Host factors include: (1) immune status, with advanced HIV patients experiencing more severe disease; (2) age, with children under five at higher risk for severe outcomes [49]; and (3) prior viral infections in terms of disease presentation, as seen in patients with HCV and HIV co-infections [50]. Viral factors include the infecting strain, with Clade I causing more severe disease than Clades IIa and IIb [49], as mentioned. Cytokine modulation correlates with disease severity, with overproduction of specific cytokines observed in severe cases [51]. It is important also to mention that all molecular interactions between MPXV and the host cells mentioned in Table 4 can impact the disease severity, but this has already been discussed.

Studies in non-human primates (NHPs) have shown that the route of MPXV inoculation affects disease severity. Intrabronchial (IB) and aerosol administration, mimicking natural transmission, result in severe lung inflammation and pneumonia [52–54]. IB inoculation leads to delayed onset of clinical symptoms compared to intravenous (IV) administration [52]. IV inoculation causes lymphadenopathy and immune activation in axillary lymph nodes [11]. Aerosol exposure models in cynomolgus macaques closely resembles human Mpox, with lymphadenopathy, fever, and skin lesions [48,53]. The virus primarily targets lower airway epithelium and the mononuclear phagocyte/dendritic cell system in lymphoid tissues [48]. These NHP models are valuable for studying MPXV pathogenesis and evaluating medical countermeasures [55,56].

## 6. Molecular Diagnostic Tools and Their Importance

### *6.1. Current Methods*

Molecular methods have become essential tools for the accurate and rapid detection of the MPXV. Table 5 describes various techniques used to identify and characterize MPXV, offering valuable information on its epidemiology, evolution, and pathogenic potential. Real-time PCR is considered the gold standard for the MPXV detection [57,58]. Other techniques include digital PCR, genome sequencing, electron microscopy, and virus isolation [57]. Immunological methods and serological tests can complement molecular techniques for comprehensive diagnosis and epidemiological studies [57,58].

**Table 5.** Molecular methods for detecting the MPXV.

| Method | Principle | Advantages | Disadvantages | References |
| --- | --- | --- | --- | --- |
| Real-Time PCR (qPCR) | Amplifies specific viral DNA sequences to detect the MPXV, even in low viral load samples. | High sensitivity and specificity; improves assay reliability by using dual targets. | It requires specialized equipment and expertise and is prone to mutation-related resistance. | Kannan et al. [5], Anderson et al. [12], Dyall et al. [11], and Luciani et al. [59] |
| Transcriptome Sequencing | Analyzes gene expression in MPXV-infected cells to understand molecular pathways in infection and immune evasion. | Reveals differentially expressed genes, aiding in antiviral therapy development. | Requires advanced sequencing facilities; analysis can be time-consuming. | Tang et al. [6] |
| Phylogenetic Analysis | Sequences the MPXV's genome to classify isolates, track outbreaks, and detect mutations. | Tracks viral evolution identifies outbreak origins, and detects resistance-related mutations. | Dependent on access to diverse genomic data; costly and computationally intensive. | Ferrareze et al. [1] and Yu et al. [16] |

The choice of detection method depends on factors such as resource availability, response time, and test purpose [60,61]. Common target genes for MPXV detection include hemagglutinin and the A-type inclusion protein [2]. Combining different molecular techniques can improve the reliability and accuracy of MPXV diagnosis, contributing to epidemiological surveillance and disease control [62].

Molecular diagnostics are essential for tracking Mpox outbreaks, monitoring disease spread, and informing public health interventions [2]. However, challenges remain in designing assays that differentiate the MPXV from closely related orthopoxviruses, particularly in low-resource settings. This limitation has sparked debate over whether improved multiplex PCR methods or genome sequencing should be prioritized for surveillance efforts. PCR-based methods are the gold standard for MPXV detection, with over 90 primer/probe sets targeting various genes [2,63]. Genome sequencing enables detailed characterization of the MPXV, identifying variants, mutations, and specific markers [64,65]. This information is vital for tracing outbreak origins, identifying changes in virulence or transmissibility, and guiding vaccine development [63,66]. The recent global spread of Mpox has highlighted the need for rapid and efficient diagnostic technologies, particularly in non-endemic countries [67].

Recent phylogenomic analyses of the 2022 MPXV outbreak have revealed important insights into its evolution and spread. However, contrasting interpretations exist regarding the role of APOBEC3-mediated mutations in driving these changes, with some studies suggesting accelerated evolution while others point to a limited impact on transmissibility. Resolving these discrepancies is critical for understanding the virus's adaptive mechanisms. The outbreak strain belongs to Clade 3 [68] and shows accelerated evolution, possibly due to APOBEC3 activity [68,69]. Phylogenetic studies indicate a single origin for the outbreak [68] and the emergence of a new lineage B.1 [70,71]. This lineage exhibits unique gene mutations related to immune evasion, virulence, and host recognition [71]. The virus has an estimated evolutionary rate of $7.75 \times 10^{-5}$ substitutions/site/year [72]. Understanding the virus's phylogeny and host–pathogen interactions, particularly involving proteins like E3 and CrmB, is essential for developing effective control measures [40,73]. Various genes are targeted for detection, with hemagglutinin and the A-type inclusion protein being the most common [2].

### *6.2. Challenges and Limitations*

According to Ropp et al. [74], molecular detection of the MPXV faces several challenges, particularly in selecting appropriate genetic targets due to the central region of the MPXV genome closely resembling that of the smallpox virus, making differentiation difficult. The authors also noted that various genes and primers, including those targeting hemagglutinin (HA) and A-type inclusion proteins, are commonly used for orthopoxvirus detection. While the HA gene remains a popular target due to its effectiveness in several assays, the challenges of accurate differentiation and diagnostic reliability persist, particularly in overlapping genomic features with other orthopoxviruses. Real-time PCR assays targeting specific genes, such as the DNA polymerase and envelope protein genes, have been developed for accurate diagnosis [75]. The choice of genetic target is crucial, with hemagglutinin and A-type inclusion proteins being common targets [2]. Rapid detection methods like recombinase polymerase amplification have been developed, offering high sensitivity and specificity [76]. However, mutations in viral genes can lead to false-negative results [77]. Clinical samples for testing include lesion swabs, crusts, and bodily fluids [78,79]. Proper specimen collection, handling, and biosafety measures are essential for accurate diagnosis [61]. Validated assays have shown high sensitivity and specificity in detecting the MPXV during recent outbreaks [80].

The genetic similarity between orthopoxviruses poses challenges for specific MPXV detection using PCR-based assays. According to Stadhouders et al. [81], mismatches in primer and probe sequences used in molecular diagnostics, such as real-time PCR, can lead to underestimations of viral load and reduced sensitivity in detecting the MPXV. They stated that over 90 primer/probe sets target various genes for poxvirus detection, with hemagglutinin and A-type inclusion proteins being common targets, emphasizing the importance of precise primer/probe design to ensure accurate viral load quantification. This reference pertains specifically to molecular diagnostic methods, not rapid antigen detection (RAT). Multiplex PCR can improve diagnostic capacity by detecting multiple targets simultaneously [82]. Real-time PCR assays have been developed to distinguish between the MPXV West African and Congo Basin strains [83]. Validation studies have shown high specificity and sensitivity for MPXV detection using orthopoxvirus-specific assays [80]. Proper specimen collection, handling, and biosafety measures are crucial for accurate diagnoses [61]. Genetic sequencing provides additional information on viral origin and epidemic characteristics [78].

High false-negative rates for the MPXV in low-viral-load samples, especially with rapid antigen tests, remain concerning [2]. While skin lesions provide the highest viral loads and positivity rates [84], alternative sampling methods have shown promise. Saliva samples demonstrated higher sensitivity (88%) compared to oropharyngeal swabs (64%) and plasma (80%) [85]. Viral culture positivity correlates with PCR cycle threshold values, with 50% positivity at Ct 34.1 [85]. Asymptomatic infections have been detected in men who have sex with men, raising concerns about silent transmission [86]. Improving diagnostic techniques, especially for low viral load samples, remains a critical challenge in controlling the MPXV's spread [60].

While numerous molecular tests target various MPXV genes, challenges remain in primer design and differentiation between the clades [2]. Validation studies have demonstrated good analytical performance for both commercial and laboratory-developed tests [62,87]. However, the necessity for national technologies and increased testing capacity is evident, particularly in resource-limited settings [2]. Continued research and development of molecular diagnostic methods are crucial for effective MPXV surveillance and outbreak control.

## 7. Therapeutic Targets and Vaccine Development

Recent research has identified potential treatments for MPXV infection. According to Tang et al. [6], transcriptome analysis revealed that the MPXV inhibits antiviral genes IFIT1 and IFIT2, suggesting that drugs promoting their expression could be effective treatments; they also stated that AP-26113 and itraconazole were identified as candidates for counteracting this inhibition. While tecovirimat and brincidofovir are FDA-approved for smallpox treatment and recommended for the MPXV [88], other potential antivirals have been explored through in silico methods. Candidates include batefenterol, burixafor, eluxadoline, and plant metabolites, such as amentoflavone and pseudohypericin [89,90]. In vitro studies have also identified several compounds effective against the MPXV, including mycophenolic acid, AVN-944, and brequinar [91]. Despite these advancements, the MPXV can evade host immune responses by suppressing pro-inflammatory gene expression [92], highlighting the need for continued research into effective treatments.

Several promising inhibitors target the Mpox virus's thymidine kinase (TK) protein. Natural flavonoids and their derivatives have shown potential as TK inhibitors. Fisetin and its derivatives have demonstrated a solid binding affinity and favorable ADMET profiles against Mpox targets [93]. Other natural compounds, including amentoflavone and pseudohypericin, exhibited high binding efficiency and stability in molecular dynamics simulations [90]. FDA-approved drugs, such as zidovudine and fludarabine and their structural analogs, have also demonstrated strong interactions with viral proteins [94]. Pinocembrin derivatives also displayed promising results in silico, with some compounds exhibiting exceptional binding affinities [4,93]. Additionally, compounds from traditional Chinese medicines and other natural product databases were identified as potential thymidylate kinase inhibitors, a crucial replication protein in Mpox [95].

For Mpox treatment, the viral methyltransferase VP39 has been validated as a viable target, with several inhibitors showing antiviral activity against the MPXV in cellular assays [96,97]. Computational approaches have led to the discovery of potential inhibitors for various MPXV proteins, including DNA polymerase [98], DNA-dependent RNA polymerase [99], viral core cysteine proteinase [100], and the A42R profilin-like protein [101]. These studies employed virtual screening, molecular docking, and molecular dynamics simulations to identify compounds with high binding affinity and stability. Natural products derived from marine fungi have shown promise as potential MPXV inhibitors [102,103].

While most MPXV genes are under purifying selection, several genes involved in immunomodulation and host adaptation show evidence of positive selection [39,104]. These include genes encoding ankyrin and Bcl-2-like proteins, which are crucial for host range determination [39]. An analysis of core orthopoxvirus genes found positively selected sites in genes involved in viral morphogenesis, transcription/replication, and immune evasion [104]. Genomic and proteomic approaches have identified highly conserved, druggable MPXV proteins as potential therapeutic targets [89]. The virus continues to evolve through point mutations, with some genes showing codon usage bias and improved human adaptation [105]. Understanding these evolutionary mechanisms and their impact on virus–host interactions is crucial for developing effective antiviral strategies [106–108].

Though repurposed from smallpox treatments, such as tecovirimat and brincidofovir, FDA-approved drugs lack comprehensive clinical efficacy data specific to the MPXV [6,10]. Additionally, the emergence of mutations in critical therapeutic targets, including VP39 methyltransferase and thymidine kinase, raises concerns about reduced drug efficacy due to resistance [15,21]. These challenges underscore the need for innovative drug design strategies focused on highly conserved viral regions with lower mutational rates.

## 8. Future Directions in Mpox Molecular Research

Exploring virus–host interactions and immune evasion mechanisms remains pivotal for antiviral development. Research emphasizes understanding the MPXV's lifecycle, from entry to replication and assembly [109,110]. One theory suggests that the MPXV's capacity to reorganize host cellular machinery, such as transforming the endoplasmic reticulum into replication factories, may enhance its ability to evade immune responses and inhibit apoptosis, offering unique therapeutic targets [109,111]. High-throughput screening methods continue to advance our understanding of host factors hijacked by viruses and antiviral responses [112,113], while studies explore the MPXV's structural and pathogenetic mechanisms, replication processes, and interactions with innate immunity [114,115]. The host immune response, particularly involving natural killer cells and T cells, is crucial for combating the MPXV, yet gaps persist in understanding how the MPXV suppresses these responses through specific molecular pathways [116]. Investigating the immunomodulatory genes involved in the MPXV's pathogenesis may uncover the selective pressures that can guide the development of targeted drugs [1,9].

The evolution of the MPXV warrants further exploration. The emergence of new lineages, such as the IIb C.1 lineage identified in Guangdong, China, suggests that recombination events may drive the virus's genetic diversity and adaptive evolution [16]. This raises questions about whether such genetic shifts contribute to increased transmissibility and immune evasion. Selective pressure on genes like OPG027 offers critical insights into how the virus adapts to human hosts [15]. Further investigation of these evolutionary patterns will be essential for predicting the virus's trajectory and informing intervention strategies.

Research into the genetic differences between the West African and Congo Basin strains is significant for developing effective therapies and vaccines [2,9]. Differences in key viral proteins, such as thymidine kinase, VP39, and E8L, may explain variations in pathogenicity and transmissibility, suggesting these proteins as high-priority therapeutic targets [3,10,20,38]. Immunomodulatory genes may also play a dual role in shaping the host immune response while enhancing viral fitness, highlighting a critical area for further study [1,9].

Understanding the MPXV's genetic diversity and molecular mechanisms is fundamental to implementing effective prevention and control strategies. Continuous genomic surveillance is crucial for tracking genetic changes and identifying mutations that enhance transmissibility or immune evasion [16]. For example, APOBEC3-driven mutations may accelerate the MPXV's adaptation to human hosts, providing challenges and opportunities for targeted interventions [15,16]. The development of rapid, sensitive, and specific molecular diagnostic tools, such as real-time PCR tests targeting highly conserved viral genes, is essential for early detection. Early identification of cases can enable timely public health measures, such as isolation and contact tracing, to limit viral spread [11,12]. Finally, further research into the MPXV's replication, virus–host interactions, and immune evasion mechanisms can uncover actionable targets for antiviral development. Essential viral proteins like thymidine kinase, VP39, and E8L offer promising avenues for disrupting viral processes and mitigating disease spread [6,10,38]. These areas of study represent critical pathways for developing effective countermeasures against the MPXV.

Future research efforts must prioritize a deeper understanding of the interplay between genomic mutations and the MPXV's resistance to therapeutic agents. Specifically, studies elucidating structural and functional impact of mutations on therapeutic targets like VP39 methyltransferase and thymidine kinase can inform the design of resistance-proof drugs [15,18]. Computational models predicting resistance-associated mutations should be integrated into drug development pipelines to ensure long-term therapeutic efficacy. In diagnostics,

refining assays capable of detecting resistance-associated mutations in real-time will be critical for proactive outbreak management. Tools leveraging CRISPR-based diagnostics or biosensor technologies could enable rapid, point-of-care testing with high sensitivity and specificity. Moreover, expanding genomic surveillance frameworks to monitor mutations across diverse geographic regions will enhance the ability to anticipate and respond to emerging MPXV variants.

Integrating these advancements into global public health frameworks is vital to strengthening MPXV surveillance and intervention strategies. Collaborative efforts between academia, industry, and policymakers should focus on developing scalable solutions tailored to resource-constrained settings. By addressing these gaps, the global scientific community can significantly mitigate the MPXV's impact on vulnerable populations, ensuring preparedness for future outbreaks.

## 9. Conclusions

The MPXV demonstrates remarkable adaptive capacity, resulting in global outbreaks. This review outlines its molecular characteristics, including genomic architecture, replication, and immune evasion, to identify critical vulnerabilities for containment. Mutations in genes such as OPG027 and D1L illustrate the adaptive evolution of the virus, contributing to increased transmissibility and immune evasion. These findings highlight that the MPXV's dependence on host cellular machinery for replication and immune modulation offers distinct opportunities for therapeutic intervention relative to other viruses. Diagnostic challenges, including the differentiation of the MPXV from other orthopoxviruses and the detection of low viral loads, necessitate the creation of targeted diagnostic assays utilizing molecular markers such as OPG027. Improving the accessibility and accuracy of these tools in resource-limited settings should be prioritized. Therapeutic research advances should prioritize validating antiviral candidates identified in silico and in vitro while accelerating vaccine development efforts. Addressing these gaps can mitigate the virus's impact and improve preparedness for future outbreaks. This study offers actionable insights for researchers and policymakers to address current challenges in MPXV diagnosis, treatment, and containment.

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