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Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: Epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines

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Published in
Frontiers in Pharmacology
Frontiers in Tropical Diseases
Frontiers in Public Health

ISSN 1664-8714
ISBN 978-2-8325-3603-2
DOI 10.3389/978-2-8325-3603-2

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Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: Epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines

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Citation

Mohapatra, R. K., Seidel, V., Kandi, V., Rabaan, A. A., eds. (2023). *Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: Epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3603-2

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frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 18 September 2023
DOI 10.3389/fphar.2023.1265803

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 23 July 2023
ACCEPTED 31 July 2023
PUBLISHED 18 September 2023

CITATION
Mohapatra RK, Kandi V, Seidel V and Rabaan AA (2023), Editorial: Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines. *Front. Pharmacol.* 14:1265803. doi: 10.3389/fphar.2023.1265803

Editorial: Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines

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KEYWORDS

NTDs, neglected tropical diseases, epidemiology, transmission, mitigation strategy, vaccines

Editorial on the Research Topic
Re-emergence of neglected tropical diseases amid the COVID-19 pandemic: epidemiology, transmission, mitigation strategies, and recent advances in chemotherapy and vaccines

Neglected tropical diseases (NTDs) are a group of infectious diseases that are common in the tropical regions of the world that include landmasses surrounding the equator such as North America, South America, Africa, Asia, and Australia. NTDs are caused by different microorganisms including bacteria, viruses, fungi, and parasites. Many NTDs involve specific environmental conditions, vectors, and animal reservoirs that favor the survival of microorganisms with complex life cycles. The vast majority of NTDs are caused by parasites followed by bacterial species, fungi, and viruses. Additionally, vector-borne arthropods like mites causing scabies and other ectoparasites can cause NTDs. A list of NTDs, as currently listed by the World Health Organization (WHO), is provided in Figure 1A.

During the 73rd World Health Assembly, the WHO proposed a road map for the elimination of NTDs by 2030. This mainly aims to control, prevent, and/or eliminate the WHO-listed NTDs (WHO, 2021). Given the emergence, and re-emergence, of novel and existing microbes, respectively, it is obvious that the list of NTDs proposed by the WHO is not exhaustive. Indeed, many other diseases may fall under the WHO criteria for NTDs (Figure 1B), including diseases affecting people living in poverty, and those residing in the

tropical and sub-tropical areas of the world. In view of the rising incidence of NTDs and their influence on the social, economic, physiological, and psychological wellbeing of people, the WHO decided that 30 January 2022 be designated as the first World Neglected Tropical Diseases Day (WNTDD) (World Neglected Tropical Diseases Day, 2022).

During the COVID-19 pandemic, several cases of NTDs re-emerged in various countries worldwide. Mohapatra et al. reported a cluster of legionellosis (severe pneumonia) cases, including four deaths, in Argentina. *Legionella pneumophila* is a bacterium that commonly lives in the environment. In COVID-19 patients who suffered from debilitating post-disease syndromes, this opportunistic microbe was found to cause lung infections, especially in hospital settings, leading to nosocomial infections that further deteriorated the patients' quality of life.

The ongoing COVID-19 pandemic not only contributed to the worsening of people's health, but also affected health-related services that were in place for the management of existing diseases like tuberculosis (caused by *Mycobacterium tuberculosis*), malaria, dengue, measles, and diseases caused by the human immunodeficiency virus (HIV) among others. Additionally, preventive measures such as mass drug administration against filariasis and other parasitic infections were severely affected during the pandemic. Moreover, it was observed that the co-infection of COVID-19 among tuberculosis patients worsened health outcomes and contributed to an increase in morbidity and mortality (Satapathy et al.).

Further, Eastern equine encephalitis virus (EEEV) is a zoonotic virus belonging to the family Togaviridae and causes life-threatening encephalitis. It is a vector-borne/arboviral disease that is generally common in the equine population. There is evidence of accidental infections among humans and other vertebrate hosts. It has a high mortality rate, and more than half of infected persons suffer from the sequelae. The first human infection caused by EEEV was reported in Massachusetts, United States in 1938. Since then, sporadic cases of EEEV have been reported from various states confirming their existence in the environment and a re-emergence that accounted for a mortality rate greater than 40%. Despite being confined to the North American region, the virus may spread to non-endemic regions owing to cross-border animal transport and increased globalization (Sah et al.).

A re-emergence of the Marburg virus (MARV), the causative agent of viral hemorrhagic fever, was also noticed during the prevailing COVID-19 pandemic. MARV belongs to the same virus family group (Filovirus) as the Ebola virus. MARV is a highly pathogenic risk-group-4 virus that results in high mortality (approximately 90%) among infected persons. MARV infection has recently been reported in Ghana. Its re-emergence may be inevitable and could only be controlled with the discovery of an acceptable vaccine and therapeutic drugs which require further research (Islam et al.).

The recent outbreak of Ebolavirus (EBV) disease in Uganda appears to be the best example of how improved viral disease tracking capabilities allow health administrators and scientists to predict the spread and other clinical and epidemiological characteristic features (Branda et al.). This enables better preparedness among authorities, minimizes disease transmissibility, and contributes to better control.

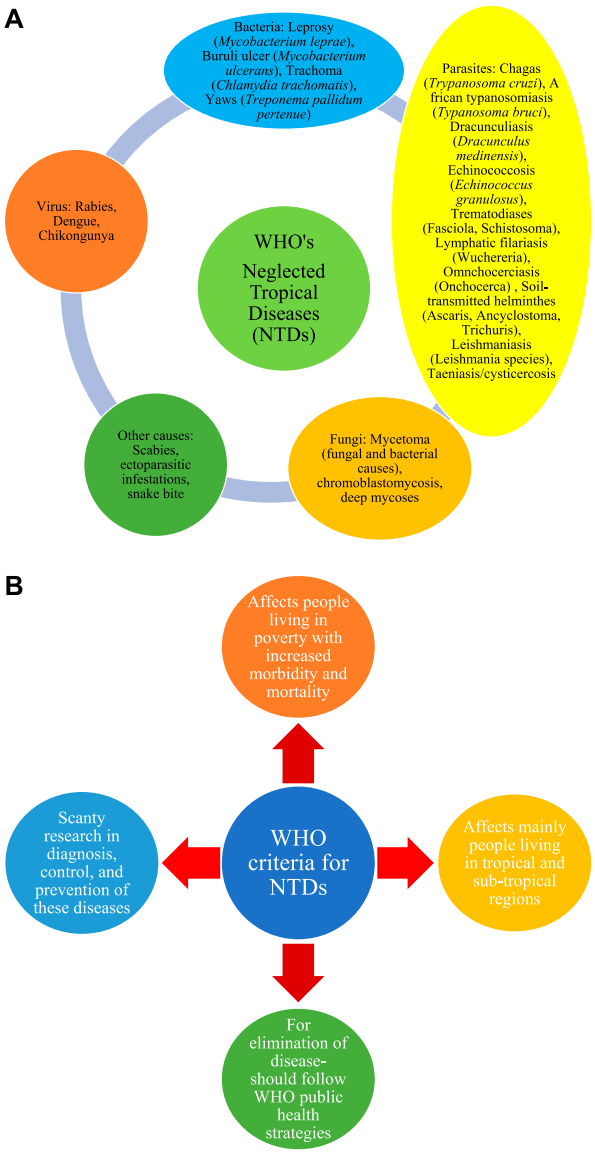

FIGURE 1
(A) List of NTDs as declared by WHO. **(B)** WHO criteria for NTDs.

Given that most diseases globally prevalent can be endemic to certain geographical regions, it is essential to understand their etiology, pathophysiology, epidemiology, diagnosis, management, control, and prevention. The same applies to SARS-CoV-2, which has been continuously mutating and evolving into several viral variants including the more pathogenic Delta and Omicron variants (Islam et al.). Therefore, public health administrators must use genomic surveillance methodologies to understand the viral evolution that could facilitate better preparedness to tackle future pandemic-like situations. Further, vaccine equity (availability to all, rich and poor) is an Research Topic that needs immediate attention along with improved vaccine that includes/covers recent variants of the virus and booster immunization doses for persons who are prone to re-infections (Rana et al.).

In a recent observation from Haryana, North India, hundreds of people reportedly presented to the hospitals with acute febrile illness

(AFI). Later, diagnostic work-up among 58 of them revealed that the majority of patients were infected with Dengue virus (77.58%), followed by Chikungunya virus (3.44%), Japanese B Encephalitis virus (3.44%), and some had dual infections (2.23%). This study found none of the AFI patients had West Nile fever, scrub typhus, or leptospirosis. The results of this study emphasize the role of laboratory diagnostic methods in identifying the causative microbes during an outbreak or any similar health emergency (Satapathy et al.).

Despite being endemic to Africa, the Monkeypox virus (MPXV) was recently reported in non-endemic regions including the Americas, Europe, and other countries. Interestingly, the current MPXV outbreak was noted to be transmitted majorly through sexual routes. Additionally, the prevailing outbreak of MPXV has been noticed increasingly among HIV seropositive patients probably owing to their abnormal sexual activities (Yuan et al.). It was also observed that knowledge of MPXV was low (45%) among healthcare workers (HCWs). The vast majority (82%) of the HCWs believed that there is a need to learn more about the virus. Among the people who had suffered from COVID-19, many were afraid of MPXV compared to those who did not suffer from the disease (Swed et al.).

Among the various strategies that can be employed to tackle emerging, re-emerging, and other NTDs, vaccination and therapeutic drugs assume increased significance. A recent *in silico* study evaluated the efficacy of modified coptisine derivatives as a therapeutic alternative to treat infections with *Rhizomucor miehei* (fungus), *Mycolicibacterium smegmatis* (Mycobacteria), MPXV, and MARV (Akash et al.). Drugs that have been used to treat MPXV infection include tecovirimat, brincidofovir, cidofovir, vaccinia immune globulin, and trifluridine (Shamim et al.). Although not currently approved, there are two candidate vaccines in the pipeline against MPXV infection. This Research Topic provides up-to-date knowledge on the re-emergence of NTDs in the context of the COVID-19 pandemic and discusses how such diseases are transmitted and what mitigation strategies should be put in place to control their spread.

Author contributions

RM: Conceptualization, Writing–original draft. VK: Writing–original draft. VS: Writing–review and editing. AR: Writing–original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Opinion
PUBLISHED 08 November 2022
DOI 10.3389/fphar.2022.1063237

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 06 October 2022
ACCEPTED 18 October 2022
PUBLISHED 08 November 2022

CITATION
Mohapatra RK, Mahal A, Kandi V, Kutikuppala LVS, Sarangi AK and Mishra S (2022), Emerging pneumonia-like illness "legionellosis" in Argentina in the COVID-19 era: Cause to panic? *Front. Pharmacol.* 13:1063237. doi: 10.3389/fphar.2022.1063237

Emerging pneumonia-like illness "legionellosis" in Argentina in the COVID-19 era: Cause to panic?

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KEYWORDS

legionellosis, epidemiology, complications, transmission route, prevention and control measures

Introduction

A cluster of eleven cases (seven males and four females of 45 years median age) of legionellosis (severe pneumonia) including four deaths in the Tucuman province of Argentina were reported recently on 3 September 2022 (MPH, 2022a; MPH, 2022b). Legionellosis shows pneumonia-like symptoms, varying from mild febrile to serious illness and sometimes even being fatal. These cases were epidemiologically traced to a private healthcare facility. Out of all the cases, eight were healthcare workers of that facility itself, and three of the four deaths were of these workers. All cases presented similar clinical symptoms like fever, myalgia, bilateral pneumonia, abdominal pain and dyspnea (WHO, 2022). In four cases, *Legionella* sp. was medically identified as the causative organism. Ten cases including the four deaths had underlying comorbidity and severe disease risk (WHO, 2022). As on 3 September 2022, four cases were still hospitalised. Although contacts of these cases are under follow-up surveillance, preliminary investigations revealed no secondary cases, albeit sporadic legionellosis outbreaks earlier are documented in Argentina. Health authorities of the province are coordinating cluster investigation to search for source(s) of infection, identify additional active cases, contact tracing and public health measures to limit further spread.

Initially hospitalised for unrelated reasons, all the eleven reported cases were were shifted to intensive care units after developing pneumonia. Preliminary investigation reports of their blood, respiratory and tissues samples in the local laboratory were negative for respiratory viruses and other suspected bacterial, viral and fungal agents. Samples sent for additional testing to the National Reference Laboratory (the Administration of National Laboratories and Health Institutes; ANLIS) were negative for COVID-19, influenza, hantavirus, *Yersinia pestis*, histoplasma, leptospirosis and a group of

12 respiratory viruses. Additional highly sensitive whole genome sequencing (metagenomics) and bioinformatics analyses of two bronchoalveolar lavage samples revealed similarities with *Legionella* sp. (AMH, 2022). The results of the amplified products of the 16S ribosomal sequences for *Legionella* sp. hinted at similarities with *Legionella pneumophila*, as documented by ANLIS (AMH, 2022). Routine blood culture and serological test were performed to validate the diagnosis of *Legionella* infections. For early and quick diagnosis of suspected *Legionella* case, urine antigen testing and sputum culture are suggested. Urine antigen test is the only tests for *Legionella pneumophila* sero-type whereas sputum culture identifies other serotypes (Brady and Sundareshan, 2022).

Legionellosis by *Legionella* sp. is manifested by pneumonia and related clinical symptoms, typically after 2–10 days incubation although up to 16 days have been recorded in some cases. *Legionella* enters the cell by binding to alveolar macrophages and respiratory epithelial cells, and promotes proliferation by inhibiting the fusion of phagosome and lysosome. Legionellae histopathologic lesions have been noticed in intestinal linings, polymorphonuclear cells and macrophages (Brady and Sundareshan, 2022). With initial mild cough, fever, headache, malaise, loss of appetite and lethargy symptoms, patients could also experience diarrhoea, muscle pain and confusion. Acute respiratory failure, shock, endocarditis, neurological deficits, coma, rhabdomyolysis, renal failure, multiple organ failure, sepsis and death are other complications (CDC, 2022). Usually, if untreated, the disease worsens in the first week. The overall death rate usually is 5%–10% although it may be up to 40%–80% if untreated or if the patient is immunocompromised (WHO, 2022). Medically fit healthy individual exposed to *Legionella* does not fall sick, but individuals of more than 50 years, smokers, the immunocompromised, with chronic lung disease, cancer, diabetes, and kidney or liver failure are at bigger risk (CDC, 2022). This uncommon but important cause of community- and hospital-acquired pneumonia is of public health significance and may cause outbreaks. Rifampin, fluoroquinolones and macrolides are few recommended antibiotics classes, and need to be chosen carefully for effective treatment.

General route of legionellosis transmission is inhaling infective aerosol from contaminated water sources. Infection could occur in vulnerable hospital patients by aspiring contaminated water or ice. As chlorine decomposes at high temperature and *Legionella* is fairly chlorine resistant, hyperchlorination of potable water is futile (Brady and Sundareshan, 2022). Ultraviolet light and copper-silver ionisation unit could be effective against *Legionella* on a sustained basis. No report of direct human-to-human transmission exists yet (WHO, 2022), and legionellosis cases in travellers to Argentina are not reported either.

Legionellae is waterborne and poor water management and the global climate changes could potentially increase the risk of its survival, growth and transmission (Herwaldt and Marra, 2018). The population with respiratory complications like chronic obstructive pulmonary disease are on the rise, attributed to the polluted air that could potentially increase the risk of such individuals to legionellosis (Brady and Sundareshan, 2022). *Legionella* infection could be community-acquired or nosocomial. The corticosteroids and other immunosuppressive therapeutic drugs to alleviate immune reconstitution inflammatory syndrome in the COVID-19 pandemic may also predispose people to legionellosis (Azoulay et al., 2020). In light of this, the predisposed population to legionellosis as post-COVID-19 health complications is rising. The data available in the literature about *Legionella* is limited due to the difficulties in culturing it. Outbreaks in future could be effectively managed through improved water management that includes regular cleaning and maintenance to avoid rusting and biofilm formation, and increased surveillance especially in hospital settings. Air conditioners and water cooling systems may be cleaned and disinfected with biocides to limit microbial growth. There is an urgent need for genomic analysis of the environmental strains of *Legionella* to track and find out virulence genes and assess the associated threats.

Health facilities need to assess risks during healthcare. Health agencies may extend seamless communication strategies to healthcare workers and contiguous community. Global support measures to investigate outbreaks, manage hospitals, sampling, environmental assessment, and infection control and prevention are urgent. Infection prevention and control (IPC) measures may be reinforced and upgraded in the face of COVID-19 pandemic to prevent or reduce healthcare-associated nosocomial transmissions (WHO, 2022). Robust surveillance to locate active and passive cases and isolating them may be done. Environmental sampling, laboratory test and metagenomics to trace and define the source, and implementing effective control measures are highly recommended urgently.

Author contributions

RKM: conceptualised, wrote the first draft and edited. LVSK and AM: teamed up during the first draft. VK and AKS: updated the manuscript. SM: teamed up during drafting and edited. All authors have critically reviewed and approved the final draft.

Conflict of interest

AM was employed by Guangzhou HC Pharmaceutical Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Opinion
PUBLISHED 16 November 2022
DOI 10.3389/fphar.2022.1065124

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 09 October 2022
ACCEPTED 03 November 2022
PUBLISHED 16 November 2022

CITATION
Satapathy P, Ratho RK and Sethi S (2022), Immunopathogenesis in SARS-CoV-2 and *Mycobacterium tuberculosis:* The danger of overlapping crises.
Front. Pharmacol. 13:1065124.
doi: 10.3389/fphar.2022.1065124

Immunopathogenesis in SARS-CoV-2 and *Mycobacterium tuberculosis:* The danger of overlapping crises

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KEYWORDS

***Mycobacterium tuberculosis* (*M Tuberculosis*), COVID, coinfections, biomarkers (MESH D015415), treatment regimens**

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the genus Betacoronavirus, was initially reported in Wuhan city, Hubei Province, China in late December 2019 (Gralinski and Menachery, 2020; Zhu et al., 2020). As SARS-CoV-2 spreads rapidly across the world, the World Health Organization (WHO) declared it a pandemic and public health emergency of international concern on 11 March 2020. The COVID-19 pandemic has severely impacted global public health activities, the economy, and curative services. It worsened the elimination program and adherence to treatment of TB, HIV (human immunodeficiency virus), malaria, measles, dengue fever, and neglected tropical diseases (NTDs) like lymphatic filariasis, soil-transmitted helminths, schistosomiasis, onchocerciasis, and trachoma (Mohan et al., 2021; Roberts, 2021; Toor et al., 2021; Aborode et al., 2022) As per 2021, WHO global survey report, 44% countries had disruption of NTD activities (World Health Organization (WHO), 2021a). Many ongoing NTD activities like mass administration campaigns of drugs and vaccines, case detection and vector control were postponed during the pandemic to avoid the additional transmission of SARS-COV-2 which ultimately leads to increased burden of NTDs in high transmission area (Toor et al., 2021).

TB is an infectious disease caused by *Mycobacterium tuberculosis*, is transmitted by aerosol affecting the lungs. It is a key public health concern due to mortality in low and middle-income countries. The majority of people exposed to MTB during childhood are asymptomatic and remain in latent form, whereas 5–10% of those exposed turn up with active disease (Dheda et al., 2017). National lockdown adversely affected TB care access, thereby leading to disease progression in many cases (Shariq et al., 2022). According to

Abbreviations: ACE, Angiotensin-converting enzyme; CD, Clusters of differentiation; FC receptor, Fragment crystallizable receptor; G-CSF, Filgrastim and granulocyte colony-stimulating factor; IL, Interleukin; INF, Interferons; NK cells, Natural Killer cells; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; T cells, T lymphocytes; TNF, Tumor necrosis factor.

WHO, in 2020 death from *tuberculosis* increased from 1.4 million to 1.5 million with a 18% decline of new cases globally. Philippines (37%), Indonesia (31%), South Africa (26%) and India (25%) account for major declined of case detection globally (World Health Organization (WHO), 2021b). This requires further intervention to elucidate the risk factors in both SARS COV-2 and TB coinfection in terms of improvisation in case detection and management of TB in endemic countries.

Clinical presentation of SARS COV-2 and MTB

Lungs are the common platform for both SARS-COV-2 and MTB, where both the pathogen replicate in alveolar macrophages and ciliated mucus-secreting epithelial type-2 pneumocytes. MTB utilises various pattern recognition receptors i.e., FCγ receptors, toll-like receptors, mannose receptors, complement receptors, nod- like receptors, dendritic cell-specific intercellular adhesion molecule grabbing nonintegrin, CD14 receptors, and scavenger receptors either singly or in combination (Russell, 2001). MTB induces the expression of ACE2 receptors for cell entry, which interestingly serves for the entry of SARS-COV-2 (Rosas Mejia et al., 2022), also thereby, sharing the common cell entry pathways. Patients with severe COVID-19 reported to have elevated levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α and G-CSF cytokines and chemokines in comparison to mild cases (Huang et al., 2020; Lee et al., 2021). Following alveolar entry, MTB infects type II pneumocytes, alveolar macrophages, and alveolar epithelial cells to release TNF-α, IL-1α. IL-1β, IFN-γ chemokines mediating inflammatory pathway (Etna et al., 2014). MTB favours intracellular survival through downgrading nitric oxide production, phagosomal maturation, and blocking IFN-γ signalling pathway in macrophages (Abdalla et al., 2016). Similarly Influenzae viruses also aggravate TB through elevated IL-10 in co-infected patients (Ring et al., 2019). There is every possibility that severe COVID-19 might reactivate the latent *tuberculosis* (LTBI) with in the patients.

T-cell-mediated immunity plays pivotal role in controlling disease progression. The frequencies of CD4 + T cells, CD8 + T cells, and NK cells reported to be low in COVID-19 patients associated with lymphopenia (Chen et al., 2020a; Tan et al., 2020), which possibly triggers the reactivation of LTBI (Amelio et al., 2019; Leonso et al., 2022). The mouse coronavirus model also reflects the reactivation of dormant TB by virus that triggering type-1 interferon signalling and activation of mesenchymal stem cell-based defence (Singh et al., 2020). Further studies have reported the reactivation of LTBI during corticosteroid (CST) therapy in COVID-19 patients, due to generalised immunosuppression (Gopalaswamy and Subbian, 2021; Friedman and DeGeorge, 2022).

COVID-19 and tuberculosis coinfection

Table 1 shows a list of studies that reported coinfection of COVID-19 and TB and its severity. COVID-19 in TB patients is more commonly observed in high TB burden countries like India, China, and Vietnam (Dong et al., 2020). MTB infection in patients with COVID-19 was more commonly found than other comorbidities like diabetes, hypertension, and coronary heart disease (Guan et al., 2020). When comparing patients with TB and COVID-19 with pneumonia, 22% of the patients had mild clinical disease, while 78% of COVID-19 had increased severity (Chen et al., 2020b; Guan et al., 2020). Co-infection with SARS CoV-2 and MTB is of concern as the diagnosis of *tuberculosis* is more likely to be missed due to nonspecific presentation and a lack of typical radiological findings. Pre-existing TB and underlying lung comorbidities aggravate the disease in COVID-19 (Tadolini et al., 2020) possibly through

TABLE 1 Studies reported COVID-19 and TB Coinfection.

Study	Country	COVID-19 and TB coinfection
Crowder et al. (2021) (Crowder et al., 2021)	Philippines	Two times higher risk with mortality and 25% lower recovery in COVID-19-TB co-infected patients in comparison to COVID-19 patients without TB. Further in Philippines there was an increase of 56.3% TB associated death due to health service disruption of TB care during COVID-19 pandemic.
Sereda et al. (2022) (Sereda et al., 2022)	Belarus	Reported 5.6% of active TB coinfection in hospitalised COVID-19 patients.
Boulle et al. (2022) (Boulle et al., 2021)	South Africa	South Africa with high TB and HIV burden has experienced surge of COVID-19 cases due to Omicron variant, 10% of COVID-19 patients of Western Cape Province had either history of TB or active TB (Boulle et al., 2021).
Kumar et al. (2021) (Kumar et al., 2021) Mathur et al. (2022) (Mathur et al., 2022)	India	Kerala reported 15.2% deaths in active TB-COVID-19 coinfection (Kumar et al., 2021), further a tertiary care hospital in India also showed association of TB (10%) in paediatric COVID-19 patients (Mathur et al., 2022).
TB/COVID-19 Global Study Group (2022) (TB/COVID-19 Global Study Group, 2022)	Multi-country study	A cohort study involving 34 countries reported, 12% mortality of coinfected patients associated with male and older age group (TB/COVID-19 Global Study Group, 2022).

alteration in metabolic pathways. A metabolomic analysis reveals low levels of metabolic biomarkers (Branch chain amino acids, Betaine and its derivatives) as a consequence of post TB infections, are associated with COVID-19 severity (Diboun et al., 2022).

Chen Y, et al., reported MTB and SARS-COV-2 coinfection induces disease progression and severity in hospitalized COVID-19 patients in China (Chen et al., 2020b). A modelling study by Hogan AB, et al., assumed that COVID-19 pandemic response could increase TB mortality up to 20% with in 2020 and 2025 (Hogan et al., 2020). The disruption epidemiological surveillance and reduction in *tuberculosis* tests due to COVID-19 pandemic might lead to increase in *tuberculosis* mortality. In addition to mortality treatment adherence and follow up of TB patients have been negatively affected.

Even though MTB is an apparent risk factor for COVID-19 aggravation, features like alcohol consumption, smoking, HIV and other viral, bacterial and fungal co-infections might have associated risk. Thus, clinical details and social determinants of coinfected patients needs to be assessed for the risk of morbidity and mortality. Early diagnosis of the disease or co-infections makes it mandatory for at risk and compromised patient groups for better management.

Management of coinfection

Despite mass vaccination breakthrough COVID-19 infections have been reported in TB endemic countries, because of emergence of new variants of the SARS-CoV-2 that can escape the host's immune response (Hacisuleyman et al., 2021; Prévost and Finzi, 2021; Cascella et al., 2022). A study demonstrated that in countries vaccinated with BCG, the frequency of the S 614G variant was associated with the highest mortality rate related to COVID-19 (Toyoshima et al., 2020).

According to the World Health Organization, exacerbation of TB appeared as the consequences of the COVID-19 epidemic. The possible key factors are: The emergence of COVID-19 pandemic has exerted high pressure on existing health system, weakened many national programmes including national TB elimination programme as well as the intricate association between the two pathogen within the host (Visca et al., 2021). This problem still needs a better evaluation of the coinfection of patients with TB and COVID-19.

Simultaneous testing for TB and COVID-19 may help in detecting new TB cases that missed public services in the context of COVID-19 (MacLean et al., 2022). Some of the strategies adopted to control COVID-19 pandemic may be implemented towards strengthening TB control programme like, teleconsultation, virtually support for self-administration of therapy to avoid delay in treatment, contact tracing and community awareness about any changes in health services etc. Hotspot mapping for active cases could help to identify the undiagnosed TB cases during the pandemic. Further social distancing to be implemented with MDR TB patients living overcrowded location with poor sanitation.

Way forward

The rapid spread of the new variants of SARS-CoV-2 and drug resistance MTB has warned the public health system and requires active molecular and genomic surveillance of disease transmission and pathogenicity. It is important to recuperate in massive screening, case finding, including targeting high risk groups and allocation of more resources to find the missed TB cases during the COVID-19 pandemic to achieve the end goal of TB. Other chronic diseases, especially those spread through close contacts, should not be ignored in pandemic times and utmost care must be taken to avoid mortality from coinfection and inaccessibility of timely treatment.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Opinion
PUBLISHED 05 January 2023
DOI 10.3389/fphar.2022.1109928

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 28 November 2022
ACCEPTED 23 December 2022
PUBLISHED 05 January 2023

CITATION
Yuan S, Jiang S-C, Zhang Z-W, Fu Y-F, Yang X-Y, Li Z-L and Hu J (2023), How and when does monkeypox (mpox) transmit: Implications for prevention and treatments.
Front. Pharmacol. 13:1109928.
doi: 10.3389/fphar.2022.1109928

How and when does monkeypox (mpox) transmit: Implications for prevention and treatments

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KEYWORDS

monkeypox virus, mild-symptomatic patients, asymptomatic patients, seminal transmission, viral load

Introduction

In this year, more than 70,000 mpox cases in non-endemic countries around the world have been reported, most of which were in American and Europe. Monkeypox virus (MPXV) is mainly transmitted by direct contact, including close contacts with skin lesions, respiratory secretions, or contaminated items of infected patients or animals (Bunge et al., 2022; Perez Duque et al., 2022). MPXV infection outbreak usually has a central point, and the original patient should have travelled to epidemic areas or have a clear history of exposure to infectious sources (such as some animals; Bunge et al., 2022). However, the current outbreak occurs in several non-endemic countries simultaneously, and the most reported cases have neither contacted with wild animals directly nor been to the endemic countries in Africa (Perez Duque et al., 2022; Saied et al., 2022). Moreover, after the Corona Virus Disease 2019 (COVID-19), people's social distance increases, and the probability of contact transmission was decreased. It is difficult to explain the current mpox epidemic with the common transmission pathways (Saied et al., 2022).

In our previous study (Yuan et al., 2022), through cluster analysis of MPXV based on relative synonymous codon usage (RSCU) bias, we concluded that the current mpox outbreak in American and Europe may have at least three origins: Sudan 2005—Nigeria 2017 cluster, Sierra Leone 2004 cluster, and Libya 1970 cluster. The geographical distribution of viral clusters was in cross, implying that they were multi-originated and the transmission paths might be very complex (Yuan et al., 2022).

Before this year, mpox was not listed as a sexually transmitted disease (STD). For the current outbreak, most mpox patients were gay, bisexual, and other men who have sex with men (MSM) with sex tourisms (Thornhill et al., 2022a; Thornhill et al., 2022b; Patel et al., 2022). However, for a contagious STD, the median incubation period was only about 7–9 days (Thornhill et al., 2022a; Guzzetta et al., 2022; Miura et al., 2022; Ward et al., 2022), which may be too short to cause a large-scale transmission (the incubation period of HIV was about 10 years; Román-Montoya et al., 2013). The unexpected and sudden appearance of MPXV concurrently in several non-endemic areas indicates that there may be some unnoticed transmission in some unknown duration of time followed by recent amplifier events (Alakunle and Okeke, 2022).

High ratio of mpox-HIV co-infection

A large number of mpox patients had concomitant HIV infection with a ratio of 42.2% (78/185; Català et al., 2022), 35.9% (70/195; Patel et al., 2022) or 41.3% (218/528; Thornhill et al.,

2022a) respectively. Although most mpox patients were MSM (Thornhill et al., 2022a; Thornhill et al., 2022b; Patel et al., 2022), the ratios of mpox-HIV co-infection were much higher than the usual percentage of HIV diagnoses in MSM (<2%; Rao et al., 2016).

We noticed that only 8% of the patients showed detectable HIV viral loads (Català et al., 2022). Other two reports also demonstrated that 78.6% (55/70; Patel et al., 2022) or 97.4% (185/190; Thornhill et al., 2022a) patients with mpox-HIV co-infection had low HIV viral loads (<200 copies/mL). All these data suggested that HIV-positive population in mpox patients showed very good HIV control. Therefore, they were individuals living with HIV infection (but not HIV clinics with symptoms) and more likely to have high-risk sexual behaviors.

Secondly, in HIV patients, some clinical characteristics of mpox might be different from non-those in non-HIV patients (Amorosa and Isaacs, 2003; Saied et al., 2022). Although in general, well-controlled HIV was not associated with severity of the symptoms, HIV-positive patients were more likely to have fevers (60% of HIV patients vs. 50% of non-HIV patients; Català et al., 2022). And the HIV-positive patients tended to show larger numbers of lesions or affected areas (Català et al., 2022). In non-HIV infected cases, the patients usually present with generalized skin rash. For the HIV infected cases, there might be the more skin lesion at genital or perinatal areas (Hammerschlag et al., 2022; Mungmunpuntipantip and Wiwanitkit, 2022). In a retrospective review of hospital records of 40 human mpox cases from Nigeria, the HIV type 1-coinfected cases showed more prolonged illness, larger lesions, and higher rates of both secondary bacterial skin infections and genital ulcers (Ogoina et al., 2020). Severe symptoms after poxvirus infections may develop in immuno-compromised individuals (Amorosa and Isaacs, 2003). So HIV-positive patients were more likely to go to the hospital, although they might seek dermatovenerologic diagnosis prior to visiting other specialists (Hammerschlag et al., 2022). A study reported that, of 20 participants admitted to hospital for clinical reasons, 15 (75.0%) had HIV co-infection (Patel et al., 2022).

The role of mild-symptomatic patients in unnoticed mpox transmission

Thornhill et al. (2022a) demonstrated that the median incubation period of mpox was about 7 (3–20) days. However, longer mean incubation periods have also been reported, which were estimated to be 7.6–7.8 days (95% credible interval 6.5 to 9.9; Ward et al., 2022), 8.5 days (95% credible interval 4.2 to 17.3; Miura et al., 2022) or 9.1 days (95% credible interval 6.5 to 10.9; Guzzetta et al., 2022). The difference in incubation period may be attributed into different definition to the symptom onset. Usually, the definition of symptom onset describes the date that an individual first noticed their symptoms. However, the initial appearance after mpox virus (MPXV) infection may be just atypical (mild) genital and peri-anal rashes without severe pain (Thornhill et al., 2022a; Thornhill et al., 2022b; Patel et al., 2022; Tarín-Vicente et al., 2022). Thus, the true date of symptom onset may be earlier but not detected.

Ward et al. (2022) found that short serial intervals were more common than short incubation periods, therefore suggesting a considerable pre-symptomatic transmission. Nevertheless, the genital or rectal lesion swabs obtained from mpox patients only became positive for MPXV DNA until after 3–5 days post symptom onset (Table 1). In other words, most pre-symptomatic patients may be not infectious. The term "pre-symptomatic transmission" may be inaccurate and should be interpreted as "mild-symptomatic transmission."

The mild-symptomatic patients may play a key role in the early unnoticed transmission, because that the individuals may still be engaged in high-risk sexual behaviors in the first few days post symptom onset. The genital and peri-anal rashes may be rubbed raw during the sexual intercourse and the virus would be released. Then MPXV may get into the blood stream directly, if anal bleeding occurs. A case study reported a MPXV transmission to a healthcare worker through a needlestick injury, confirming a possibility of direct blood transmission (Carvalho et al., 2022).

Possible seminal transmission of MPXV

MSM are prone to have condomless sexual intercourse and leave the seminal fluid inside the body. Before this year, mpox was not known as a sexually transmitted disease. MSM usually adopt HIV pre-exposure prophylaxis (PrEP; Hodges-Mameletzis et al., 2019; Atim et al., 2020; Thornhill et al., 2022a). However, use of PrEP may be a risk factor for MPXV infection, because that MSM with PrEP do not often use condoms (Torster et al., 2022). WHO recommended PrEP since 2015 (Hodges-Mameletzis et al., 2019; Atim et al., 2020). Thus, the current correlation between sexual behaviors and MPXV infections found in this year might be explained.

The available literatures showed increasing concerns about possible seminal transmission of MPXV (Hornuss et al., 2022; Lapa et al., 2022; Noe et al., 2022; Peiró-Mestres et al., 2022; Raccagni et al., 2022; Reda et al., 2022; Reda et al., 2023). Detection of viruses in the testes is commonly secondary to viraemia because the blood–testis barrier may be liable to viruses, especially when systemic or local inflammation occurs. Viral persistence through the tract is also likely, no matter of its capability to replicate, because the testis can be an immunological-favored site for viruses (Li et al., 2012; Annandale et al., 2014; Mead et al., 2018). Interestingly, culturing MPXV was successful in two out of four patients included in two studies (Lapa et al., 2022; Noe et al., 2022), suggesting a replication competence of MPXV detected in seminal specimens.

A clinical study reported positive MPXV results in the seminal fluid obtained from mpox patients at the time closest (5–7 days) to symptoms onset with a Ct range from 27 to 30 (Antinori et al., 2022); when the symptoms may be mild. Though in a low viral load, seminal MPXV may be still contagious. Alternatively, seminal MPXV may get into the blood stream directly, if anal bleeding occurs.

Asymptomatic patients might transmit the virus through seminal fluids

Asymptomatic mpox infections may be observed in both smallpox vaccinated and unvaccinated individuals (Karem et al., 2007; Guagliardo et al., 2020). Ferré et al. (2022) detected MPXV in anorectal swabs from asymptomatic MSM. Among 200 participants who were subjected to MPXV PCR tests, they reported 13 MPXV-positive participants who were initially asymptomatic (two of them showed mild symptoms 7–9 days later). However, asymptomatic patients do not develop rashes or

TABLE 1 Timeline of PCR results from mpox cases in 2022.

Time of the first positive PCR result	Sampling site	PCR Ct value	Sample size (*n*)	References
5 (2–20) days after symptom onset (dso)	Skin or anogenital lesion (97%)	≤40	528	Thornhill et al. (2022a)
	Nose or throat swab (26%)			
	Blood (7%)			
	Urine (3%)			
	Semen (5%)			
5 dso	Serum	29.7	4	Antinori et al. (2022)
5 dso	Plasma	30.2		
3–5 dso	Genital or rectal lesions	14.7–17.5		
3–5 dso	Nasopharyngeal swab	27.6–30.4		
3–5 dso	Skin lesions	17.6–30.4		
5–9 dso	Seminal fluid	27.7–43.2		
5 dso	Scab	13.1–20.0		
3–6 dso	Faeces	22.6–26.1		
3 dso	Saliva	27.1		
Asymptomatic stage (-7—-9 dso)	Anal swabs	20.7–38.2	2	Ferré et al. (2022)
Presymptomatic patients	Anorectal swab	17.16–26.69	3	De Baetselier et al., 2022; Van Dijck et al., 2022
7.0 (5.0–10.0) dso	Skin swab (99%)	23	180	Tarín-Vicente et al. (2022)
	Throat swab (70%)	32	117	
	Anal swab (78%)	27	55	
4–16 dso	Saliva	20.3–37.9	22	Peiró-Mestres et al. (2022)
4–14 dso	Rectal swab	17.6–38.4	23	
4–14 dso	Nasopharyngeal swab	25.4–40.0	23	
1–14 dso	Semen	22.7–40.0	16	
1–16 dso	Urine	24.4–40.0	23	
4–16 dso	Faeces	19.9–31.4	22	
3–6 dso	Skin lesions	17–27	4	Hornuss et al. (2022)
3–9 dso	Nasopharyngeal swab	28–35		
4–9 dso	Anal mucosa	23–31		
3–11 dso	Blood	30–39		
4–9 dso	Urine	34–38		
14	Seminal fluid	33-38		

skin lesions, where the viral loads are the highest (about 10,000 times higher than in serum; Table 1). Therefore they are believed to be of little or no epidemiologic importance. Nevertheless, Ferré et al. (2022) also found a high viral load in a patient during the asymptomatic stage with a very low Ct value of 20.7. And serology confirmed that MPXV isolated from two presymptomatic cases can be cultured (De Baetselier et al., 2022; Van Dijck et al., 2022). Whether a high viral load in seminal fluid obtained from some asymptomatic patients could be detected needs further investigations. There might be a possibility that asymptomatic patients transmit the virus through seminal fluids.

Condom, vaccines and drugs

The condom could prevent direct contact with anogenital lesions, where the viral loads are the highest (Table 1). Although the actual protection rate of condoms against mpox infection is unclear, compared with the vaccines and drugs, use of condoms may be the most effective and convenient way to control the current epidemic.

Given that in most cases, the viral load peaks after 3–5 days post symptom onset (Table 1), vaccination and/or drug treatments before this time-point may show good therapeutic effects. All

highly-susceptible populations should be subjected to viral tests and priority treatments, no matter in symptomatic or asymptomatic, especially for those are too young to receive childhood smallpox vaccination, whose viral loads may be higher than unvaccinated people. However a large part of them had concomitant HIV infection (Thornhill et al., 2022a; Català et al., 2022; Patel et al., 2022). Previous studies suggested that HIV-positive individuals with CD4 cell counts of <300 cells/mm^3 may develop severe complications after vaccinia virus vaccination (Amorosa and Isaacs, 2003). Thus, for those with low CD4 cell counts, the decision whether or not to vaccinate must be made within the context and circumstances of the mpox outbreak. Alternatively, the immuno-compromised people or the patients with atopic dermatitis should receive a third-generation non-replicating vaccine that was made based on modified vaccinia Ankara (MVA) (Saied et al., 2022). It is interesting to note that some MVA vaccine may be considered for post-exposure prophylaxis, ideally within 4 days of high-grade exposure (Vaughan et al., 2020).

The mainstay of clinical treatments for MPXV infections are supportive and/or symptomatic managements (Reynolds et al., 2017). Although there are a few antiviral drugs have been prescribed for mpox patients, such as Cidofovir, Brincidofovir, and Tecovirimat (Adler et al., 2022; Thornhill et al., 2022a; Rizk et al., 2022; Saied et al., 2022), no prophylactic drug has been approved. Whether some drugs could be considered in mpox pre-exposure prophylaxis needs further investigations. Besides above vaccines and drugs, Saied et al. (2022) further suggested that vaccinia immune globulin intravenous (VIGIV) or vaccine immune globulin (VIG) may be used for mpox treatments, and especially helpful to the immuno-compromised people, pregnant women, or the patients with complicated lesions.

Author contributions

SY conceived the project. S-CJ, Z-WZ, Y-FF, and X-YY performed the literature search. SY wrote the manuscript with input from S-CJ, Z-WZ, Y-FF, X-YY, Z-LL, and JH. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Sichuan Province Youth Science and Technology Innovation Team (20CXTD0062) to SY and the Applied Basic Research Program of Sichuan Province (2020YJ0410) to Z-WZ.

Conflict of interest

S-CJ was employed by the Haisco Pharmaceutical Group Comp., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Tropical Diseases

TYPE Review
PUBLISHED 05 January 2023
DOI 10.3389/fitd.2022.1068364

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neglected Tropical Diseases, a section of the journal Frontiers in Tropical Diseases

RECEIVED 12 October 2022
ACCEPTED 25 November 2022
PUBLISHED 05 January 2023

CITATION
Islam MA, Adeiza SS, Amin MR, Kaifa FH, Lorenzo JM, Bhattacharya P and Dhama K (2023) A bibliometric study on Marburg virus research with prevention and control strategies. *Front. Trop. Dis.* 3:1068364. doi: 10.3389/fitd.2022.1068364

A bibliometric study on Marburg virus research with prevention and control strategies

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Marburg virus (MARV) is a pathogenic zoonotic RNA virus etiologic for Marburg virus disease (MVD), a severe hemorrhagic fever. This is a rare disease, with a high fatality rate, that spreads *via* infected blood or body fluids or indirectly *via* fomites (contaminated objects and substances such as clothed, beds, personal protective equipment, or medical equipments). A few vaccines to protect against MARV are undergoing clinical trials, but there is not yet an approved vaccine against this disease. Eventually, prevention and control guidelines should be adhered to rigorously to alleviate this infection. This bibliometric analysis aimed to harness narrative evaluation, emphasizing the significance of quantitative approaches and delineating the most thought-provoking concerns for researchers using VOSviewer software (Centre for Science and Technology Studies, Leiden University, the Netherlands). "Marburg Virus" OR "MARV" AND "Diseases" search criteria were used for the analysis of articles published between 1962 and 2022. Co-occurrence analysis was carried out, which characterized different thematic clusters. From this analysis, we found that 1688 published articles, and the number of publications increased across that period annually, with a growth rate of 8.78%. It is also conspicuous that the number of publications in the United States reached its acme during this period (i.e., 714 publications, accounting for 42.29% of the total), and the United States Army Medical Research Institute of Infectious Diseases published the most literature (i.e., 146 papers). Our study found that the three pre-eminent authors of Marburg virus papers were "FELDMANN, HEINZ" of the National Institute of Allergy and Infectious Diseases, United States, "BECKER, STEPHAN" of the Philipps University of Marburg, Germany, and "GEISBERT, THOMAS W" of the University of Texas Medical Branch, United States. In this study we found that "JOURNAL OF VIROLOGY" has published the most pertinent literature, totaling 88 articles, followed by "The journal of Infectious Diseases", which published 76 relevant papers, and "VIRUSES", which published 52 corresponding papers. The most cited paper on the Marburg virus was published in *Nature Medicine*, with

522 total citations and 29 citations/year. Studies of the changing epidemiology and evolving nature of the virus and its ecological niche are required; breakthrough and implementation of the efficacious vaccine candidate(s), prophylaxis and therapeutic alternatives and supervision strategies, unveiling awareness-raising programs, and developing apposite and timely preparedness, prevention, and proactive control strategies are of utmost importance.

KEYWORDS

marburg virus (MARV), marburg virus disease (MVD), hemorrhagic fever, bibliometric analysis, prevention and control, re-emerging zoonotic disease, vaccine, prevention and treatment

1 Introduction

Marburg virus (MARV) is a deadly zoonotic virus and the WHO identified it as a risk group 4 pathogen (WHO 2022) (https://www.who.int/news-room/fact-sheets/detail/marburg-virus-disease). It is a leading member of the family that includes *Ebolavirus*, i.e., Filoviridae, it belongs to the genus *Marburgvirus* and species *Marburg arburgvirus*, and it causes diseases in both humans and non-human primates (1). Other viruses of the same family as *Marburgvirus*—Filoviridae—are *Ebolavirus*, *Cuevavirus*, *Striavirus*, and *Thamnovirus* (2). MARV is enveloped and pleomorphic, consisting of filamentous, non-segmented, rod-shaped, cobra-like, circular/ring-like, and branch-shaped particles of uniform diameter but variable length (≈ 19.1 kb). The viral genome, negative-sense single-stranded RNA(−ssRNA), contains seven Open Reading Fragments (ORFs): (1) nucleoprotein (NP); (2) virion protein 35 (VP35); (3) VP40; (4) VP40; (5) VP24; (6) glycoprotein (GP); and (7) large-viral polymerase (L) (3, 4). In 1967, in Germany (Marburg and Frankfurt) and Serbia (Belgrade), MARV was first detected in laboratories. Recently, two MARV-confirmed deaths were reported from the southern Ashanti region of Ghana (1). Since the first detection of Marburg virus disease (MVD), outbreaks have occurred around the world (5). In 2008, cases were recorded in the USA and the Netherlands; the Democratic Republic of Congo (DRC), Uganda, Kenya, and South Africa also confirmed MARV cases, and the largest outbreak was in Angola in 2005 [374 positive tests and 329 deaths, with an 88% care fatality rate (CFR)] (Figure 1 and Supplementary Table 1) (6, 7). According to previous research, the incubation time of MARV is 3–21 days (typically 5–10 days) from exposure (8, 9). The range was reported as 3–13 days for filoviruses (*Zaire ebolavirus* and Marburg virus) in one study (10) and 2–26 days in another (11). After virus entry to the host cell through damaged skin and mucosal surfaces, it infects the immune cells (e.g., monocytes, macrophages, and dendritic cells). Viral entry into the host body includes three distinct steps—attachment, macropinocytosis, and fusion—and early replication disseminates to the hepatocytes, endothelial cells, fibroblasts, and epithelial cells, targeting the spleen, liver, and secondary lymphoid organs, with inhibition of type-I interferon (IFN-1) synthesis (12). Among a few entry mechanisms, with subsequent MRV through cellular attachment or fusion, endocytosis (clathrin mediated) and macropinocytosis are notably reported in previous studies, involving GP, tyrosine kinase, and C-like lectins in receptor binding sites (12). Sometimes the severity of MARV is higher than Ebola virus (EBOV), while the most modifications are affiliated with immune cells and gene expression against the viral cell. Phagocytes, monocytes, macrophages, Kupffer cells, dendritic cells, and endothelial cells are decimated by this virus (13). Various organs, such as the liver, kidney, stomach, heart, brain, spleen, and lymph nodes, are conspicuous in swelling during infection, with hemorrhage and necrosis. Predominantly, hepatic, lymphatic, and liver tissue, testes, and ovaries are affected more severely, with a paucity of lymphocytes (2).

Clinical symptom onset is sudden, with high fever, fatigue, chills, headache, diarrhea, and myalgia. Moreover, a maculopapular rash is noticeable after 5–6 days and severe hemorrhagic symptoms within 7 days (14). The disease is categorized into three phases: an initial phase, with flu-like symptoms, persisting for 0–4 days; the organ phase, lasting 5–13 days, with neurological symptoms; and the convalescence phase, from day 13 onward (1). Multiorgan failure, pharyngitis, nausea, vomiting, chest pain, a sore throat, and abdominal pain may appear, and sometimes jaundice, inflammation of the pancreas, and severe weight loss are also conspicuous. In fatal cases death occurs after the onset of the clinical symptoms (15). Contact with positive patients' saliva, sweat, stool, urine, tears, or breast milk can result in direct human-to-human transmission, and contaminated objects and substances can result in indirect transmission.

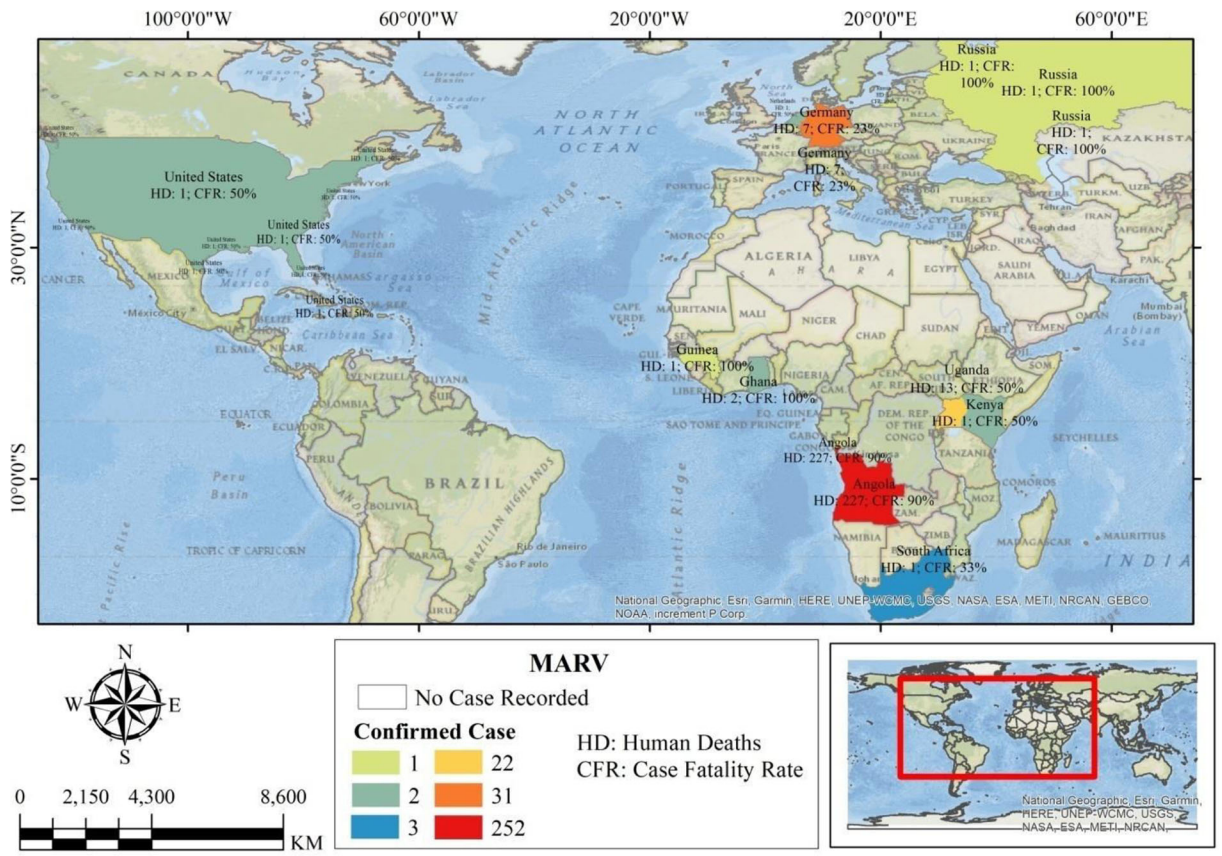

FIGURE 1
The global map indicating the epidemiology of Marburg virus, showing Angola as a highly affected area.

MARV can be introduced to human populations through inhalation of the contaminated excreta from bats, with occasional secondary human-to-human transmission (16). It can also persist in immune-privileged sites in individuals who have recovered from the disease. Mines or caves were the main sources of spreading this disease initially, usually where *Rousettus* bats were found. Direct human-to-human transmission is also possible through damaged/broken skin, secretions, body fluids (e.g., vaginal secretions, amniotic fluid, semen, and vomit), blood, and contaminated objects and substances such as beds, clothes, needles, and medical equipment (2). However, as *Marburgvirus* and *Ebolavirus* belong to the same family, it can be predicted that MARV persists in a patient's body fluids. One study reported the highest level of MARV shedding in oral secretions from a bat (13). Interestingly, (17) detected MARV RNA in the urine of infected Egyptian rousette bats (*Rousettus aegyptiacus*), which indicated that infectious urine may be another route of transmission. Public health workers are a major risk group for MVD because of direct contact with patients. Moreover, people who are involved with burial ceremonies need to follow precautions to avoid direct contact with the body (18).

To fulfill sensitivity and quantification of viral titer, qRT-PCR using nucleocapsid protein can be used to detect MARV, whereas blood serum, plasma, or whole blood can be used to detect viral antibodies or antigens using the ELISA approach (19). In addition, patients' tissues, serum, and plasma are preferable for the conventional polymerase chain reaction (PCR) method where RT-PCR is not available. It is recommended to collect blood samples without anticoagulant agents such as heparin or EDTA (20). Microscopy can predict viral morphology from tissue specimens that have been previously fixed in formalin.

To date, there is no approved treatment or vaccine available against MARV. As larger outbreaks are rare, available clinical data are often insufficient for finding out appropriate treatment regimens. Therefore, the treatment is solely based on supportive care, which needs to be given without delay. This encompasses rehydration with oral or intravenous fluids, regulating oxygen status and blood pressure, reinstating lost blood, rendering treatment as per the patient's symptoms, and scrutinizing

complicating infections, which could help in improving the survival of MVD patients. To delineate current knowledge, knowledge gaps, and hidden research trends, a bibliometric analysis is necessary. The central targets of the current research are to identify the outbreak effects of MVD and focus on the essential lessons that can be learned about this deadly disease. This study is designed to identify research trends, the number of published articles, highly cited articles, and the pre-eminent countries, institutions, and authors of Marburg virus papers.

2 Main objectives of the study

The first stage primarily focuses on identifying and categorizing the current literature. The overall objective involved five sub-categories aiming to:

- clarify the attributes and interrelatedness of appropriate investigations
- constitute a well-structured organization of related studies concepts and findings
- validate the restrictions and gaps in current information, thus establishing the scope of potential areas of the study
- develop a conceptual mindset on the environmental issues along with the effect of the Marburg virus situation
- pursue the pathway of hot themes and potential future study trends.

3 Methods

The bibliographic data gathered from published research articles was parsed using bibliometric analysis in order to investigate patterns and trends in MARV literature and identify the core scientific networks (21). Previous research has employed bibliometric approaches to investigate the mutuality, subject classification, and future research directions of scientific disciplines (22).

The trend, which is the critical informative statistics of the appropriate form of knowledge, its effects, and the evolutionary process of the topic's high recurrence rate, with a discernible analysis of co-occurrence, could be illustrated by bibliometrics and literature systematization. The bibliometric analysis makes some facilities for the researcher, such as obtaining true data from several scientific publications and the suppliers of this information and the publications.

3.1 Inclusion and exclusion criteria

Two researchers (AI and SSA) reviewed all titles and abstracts to choose publications that met the inclusion criteria (Figure 2). Before reaching a consensus, no concerns were discussed. Once the following criteria were met, data were retrieved from the literature: (a) the primary topic had to be linked to the study; (b) the literature could take any form (such as an article, review, editorial piece, or meeting abstract); and (c) the literature had to cover other topics that were also connected to the study. Duplicate entries, titles, abstracts of papers unrelated to the topic, and inaccessible abstracts were the exclusion criteria.

3.2 Citation data collection

The Dimensions database is ranked among the top repositories for bibliometric and patentometric analysis because of its thorough coverage of millions of research works, grants, clinical trials, data sets, policy documents, and patents (23, 24). A title and abstract search for the terms "Marburg Virus" OR "MARV" AND "Diseases" was used to search for articles published in between 1962 and 2022. To account for changes in publication and citation count, all metadata were gathered and evaluated by two researchers in one sitting. Bibliometrix and ggplot2 R packages (25) were used to examine, analyze, and visualize data gathered from the Dimension database for bibliometric pointers. Bibliometric indicators included authors, affiliated countries and institutions, keywords, and citation counts. Bradford's law of publication scattering was used to evaluate the distribution of literature in preferred journals.

3.3 Data visualization analysis

VOSviewer software (Centre for Science and Technology Studies, Leiden University, the Netherlands) was used to create networks and visualize links of metadata (*VOS viewer*). The size of the circle reflects the degree of connection between documents. Coupling analysis was applied to view the country and institutional co-operation. Co-occurrence clustering was used to visualize research themes and identify research trends and current hot spots (26).

Significant challenges in the purification process were the ambiguity in papers' titles, keywords, abstracts, and their topic incorporation. However, some addressed the Marburg virus and included "MARV" or its synonyms. The situation was assigned to a temporal relationship with the MARV crisis rather than evaluating its environmental impacts. During data cleanup, some generic terms, such as human, humans, article, and study, were removed.

3.4 Methods describing and systemizing the literature

The selected review papers were read in detail to delineate the essential data for the evaluations, especially the critical lessons presented in the following section. The papers were divided into several clusters based on commonalities. Top-cited papers were

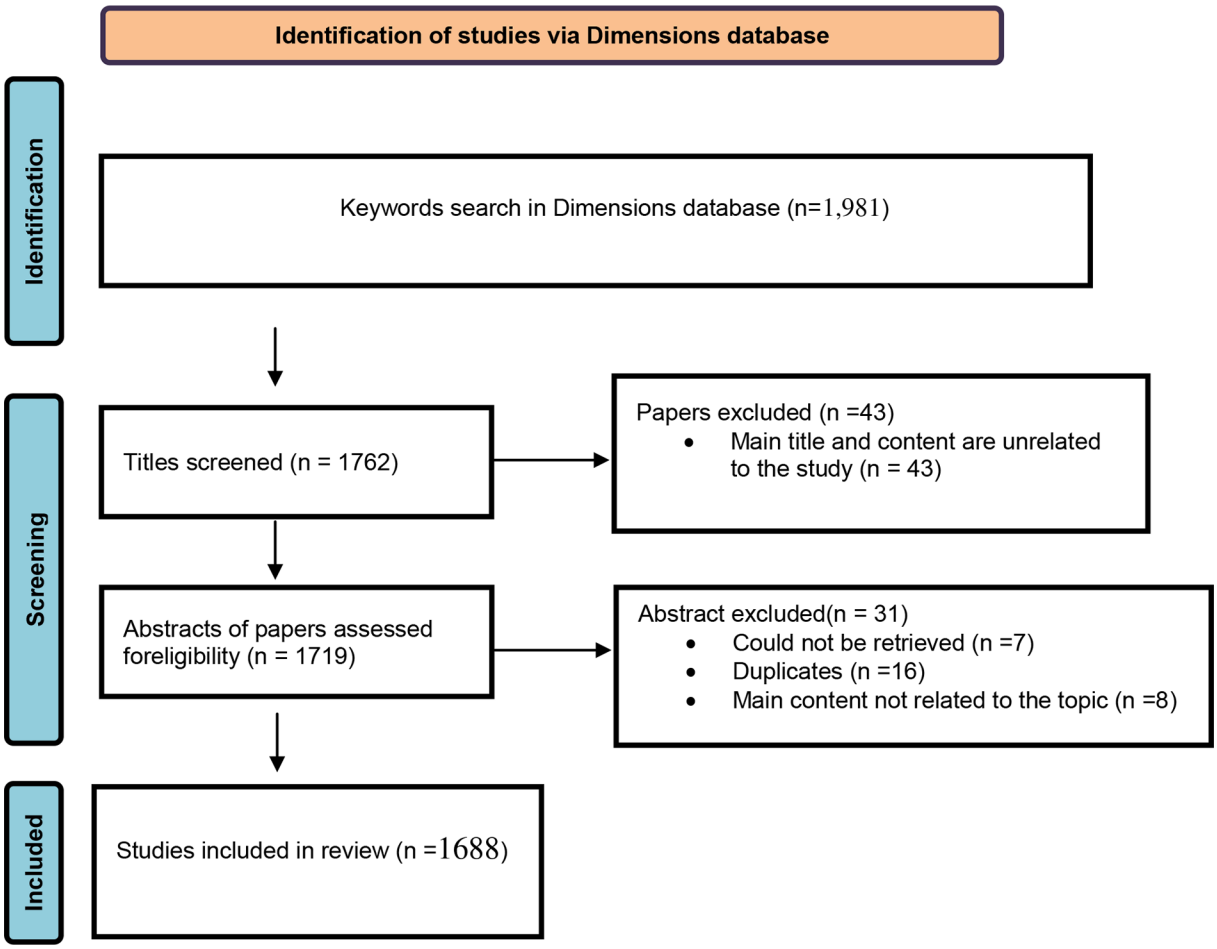

FIGURE 2
Selection of studies for inclusion.

selected after examining the papers' titles and abstracts and assigned to various clusters dependent on their thematic aims. This stage ended by refining the categorization by combining analogous clusters.

4 Results

4.1 Bibliometric analysis

A total of 1688 documents were included, and the global volume of publications peaked at an annual growth rate of 8.78%. The years 2015 (112 documents) and 2020 (108 documents) marked the peaks of global publications, which accounted for 6.635% and 6.398% of the total number of publications, respectively. (Figure 3A). The highest number of average citations per year (6.5) occurred in 2011 (Figure 3B). The included document types were article (n=1,463, 86.61%), chapter (n=176, 10.43%), edited book (n=4, 0.25%), monograph (n=1, 0.06%), preprint (n=40, 2.4%), and conference proceeding (n=4, 0.25%).

4.2 Countries contributing to global publications

Figure 3C shows that, out of the 1688 documents, the United States published the most documents (714, accounting for 42.299%), followed by Germany (183, accounting for 10.841%) and the United Kingdom (85, accounting for 5.036%).

4.3 Spread of organizations paying attention to Marburg virus

Figure 3D shows the top 20 institutions engaging in Marburg virus studies in terms of the volume of publications worldwide. Among them, the United States Army Medical Research Institute of Infectious Diseases has published the most literature (146 papers), the National Institute of Allergy and Infectious Diseases ranked second (108 papers), and the University of Texas Medical Branch at Galveston ranked third (80 papers).

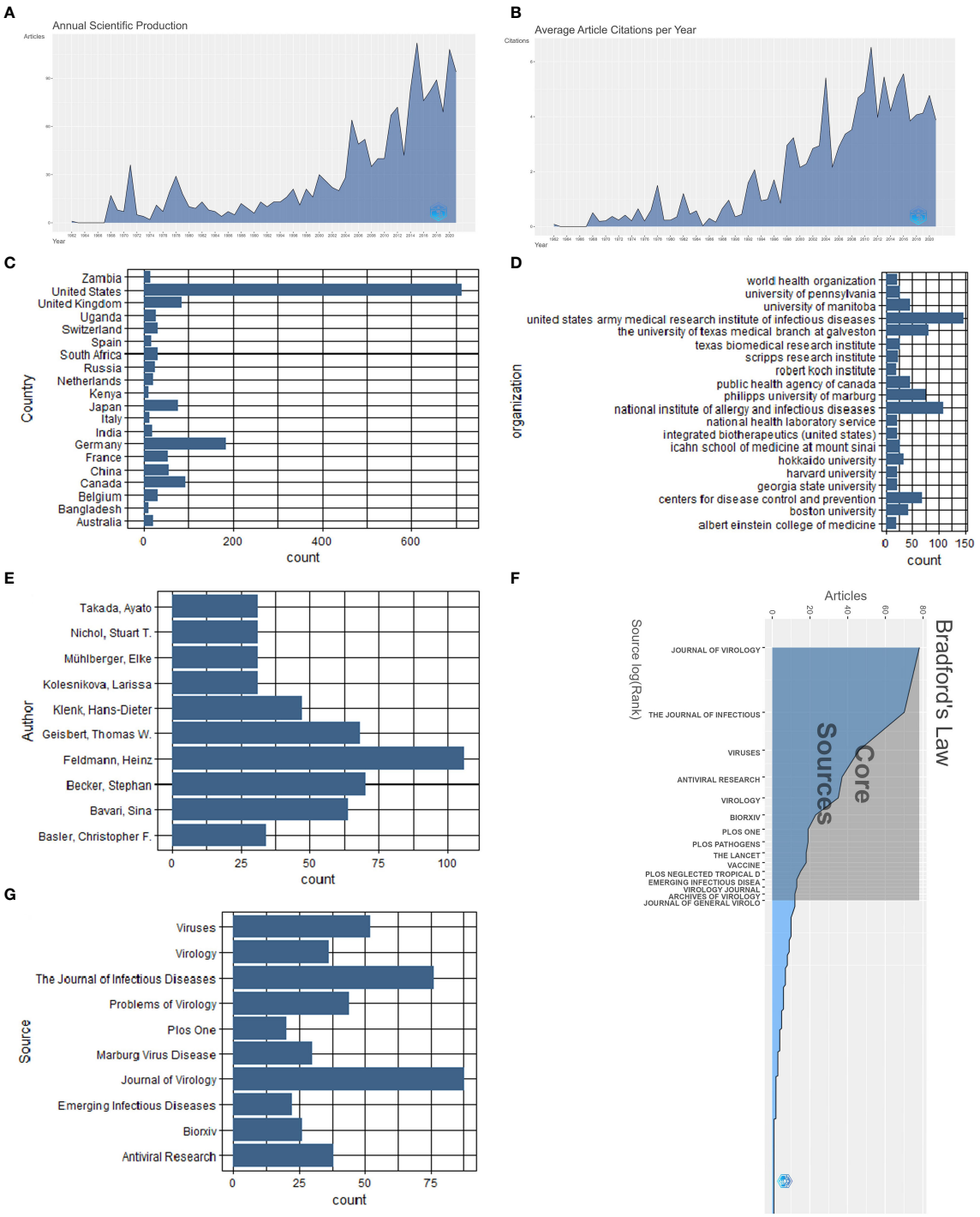

FIGURE 3
Marburg virus-related publications: **(A)** Annual scientific production; **(B)** Average citation per year; **(C)** Top 20 countries by publication volume; **(D)** Top 20 institutions by publication volume; **(E)** Top 10 authors by publication volume; **(F)** Bradford's law of publication; **(G)** Top 10 journals by publication volume.

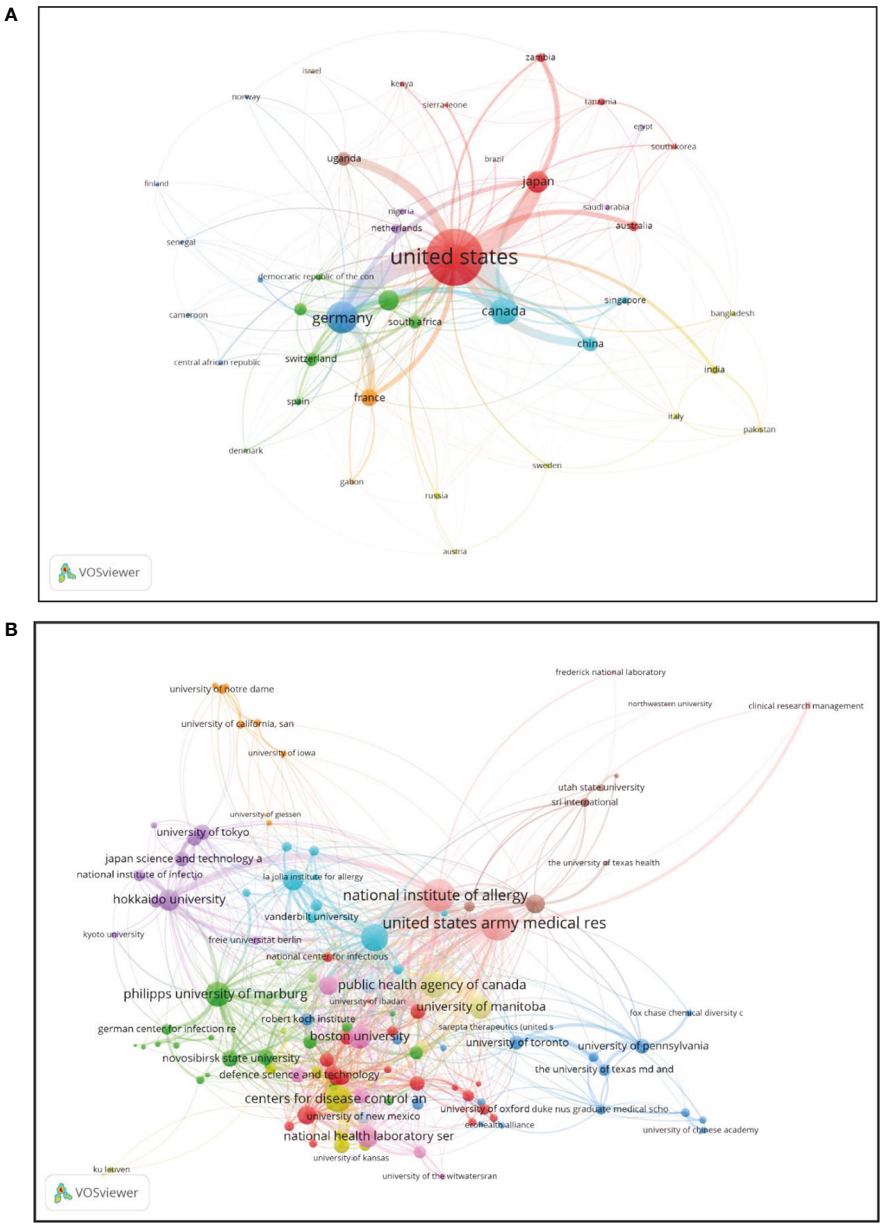

FIGURE 4
Coupling analysis: The size of the bubbles reflects the weight of the literature, and the color signifies clustering. The broadness of the lines is consistent with the intensity connection. **(A)** Country coupling analysis; **(B)** Institutional coupling analysis.

4.4 Contribution of authors to global publications

Figure 3E shows the top 10 authors in this field. The top three authors of the papers were "FELDMANN, HEINZ" of the National Institute of Allergy and Infectious Diseases, United States, "BECKER, STEPHAN" of the Philipps University of Marburg, Germany, and "GEISBERT, THOMAS W" of the University of Texas Medical Branch at Galveston, the United States, publishing 106, 70, and 68 articles, respectively. The majority of Marburg virus-related publications were published in 15 core sources in accordance with Bradford's law (Figure 3F).

4.5 Flux of published journals

In Marburg virus research, "JOURNAL OF VIROLOGY" has published the most pertinent literature, totaling 88 articles,

followed by "THE JOURNAL OF INFECTIOUS DISEASES", which published 76 relevant papers, and "VIRUSES" published 52 related papers. Figure 3G shows the top 10 journals in terms of the number of publications on Marburg virus research worldwide.

4.6 Coupled visual analysis of Marburg virus research

4.6.1 Country coupling analysis

A total of 81 countries were included by the criterion of at least five study records (Figure 4A). The top three countries in terms of the coupling strength of the literature in this field were the United States, whose total link strength (TLS) was 224, Germany (92), and Japan (52).

4.6.2 Organization coupling analysis

An analysis of all the materials included covered a total of 875 institutions, and 127 institutions according to the criterion of being mentioned at least five times in the literature (Figure 4B). The top three institutions in terms of coupling strength in the literature in this field were the United States Army Medical Research Institute of Infectious Diseases (TLS 216), National Institute of Allergy and Infectious Diseases (TLS 201), and University of Texas Medical Branch (TLS 144).

4.7 Co-occurrence analysis of Marburg virus research keywords

4.7.1 Research direction

Research works relevant to the Marburg virus can be divided into three categories based on the results of keyword clustering: epidemiology and public health, pathogenicity and vaccine development, and immunology and molecular biology (Figures 5A, B). In epidemiology and public health (red circle), frequently used keywords were "Marburg", "Virus", "Ebola Disease", "Outbreak", "Case", "Patient", "Transmission", "Pathogen", "Viral hemorrhagic fever", and "Bat". Pathogenicity and vaccine development (blue circle) used "Infection", "Treatment", "Vaccine", "Non human primate", "Therapeutic", "Response", "Antibody", "Protection", and "Human" keywords very often. The frequent keywords applied by immunology and molecular biology (green circles) include "Marburg virus", "EBV", "Proteins", "VP24", "VP40", "Expression", "Mechanism", "Genome", "Domain", "Inhibitor", and "Cell".

4.7.2 Research hot-spots and development trends

By dividing different keywords in terms of time, a distribution map of research priorities in different time periods was generated. Figure 5B shows that "Small molecule inhibitor", "Protein analysis", and "Immunogenicity" are future hot spots in the Marburg virus research field.

4.7.3 Highly cited papers on Marburg virus

Table 1 lists 10 highly cited papers on the Marburg virus. The first paper was published in "NATURE MEDICINE" in 2005, and was titled "LIVE ATTENUATED RECOMBINANT VACCINE PROTECTS NONHUMAN PRIMATES AGAINST EBOLA AND MARBURG VIRUSES" (27). The article assessed the efficacy of replication-competent vaccines against EBOV and MARV based on attenuated recombinant vesicular stomatitis virus vectors expressing either the EBOV glycoprotein or MARV glycoprotein in nonhuman primates. "The study data suggested that the vaccine candidates were safe and highly efficacious in a relevant animal model because the EBOV vaccine induced humoral and apparent cellular immune responses in all vaccinated monkeys, but the MARV vaccine induced a stronger humoral than cellular immune response." Published in the "JOURNAL OF CLINICAL MICROBIOLOGY" in 2002, the second study looked at "RAPID DETECTION AND QUANTIFICATION OF RNA OF EBOLA AND MARBURG VIRUSES, LASSA VIRUS, CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS, RIFT VALLEY FEVER VIRUS, DENGUE VIRUS, AND YELLOW FEVER VIRUS BY REAL-TIME REVERSE TRANSCRIPTION-PCR". The study showed "six one-step, real-time reverse transcription-PCR assays for viral pathogen detection based on the SuperScript™ reverse transcriptase-platinum *Taq* polymerase enzyme mixture". "The suitability of the assays was demonstrated by detection and of viral RNA in serum samples of VHF patients by the ≥95% detection limits observed" (28). The authors of the third quantification study published in "PLOS PATHOGENS" in 2011 focused on "DISTINCT PATTERNS OF IFITM-MEDIATED RESTRICTION OF FILOVIRUSES, SARS CORONAVIRUS, AND INFLUENZA A VIRUS". The study showed that "IFITM proteins restricted infection mediated by the entry of glycoproteins (GP (1, 2) of Marburg and Ebola Filoviruses (MARV, EBOV)". "The data indicated that IFITM-mediated restriction is localized to a late stage in the endocytic pathway." "The results also showed, IFITM proteins differentially restrict the entry of a broad range of enveloped viruses, and modulate cellular tropism independently of viral receptor expression" (29).

5 Discussion

A quick understanding of each area's front lines is crucial, and the papers from pioneer authors and/or groups were tracked. To find out the themes of interest in paper data based on the Marburg virus (MARV) and Marburg virus disease (MVD), two approaches were employed to reach more reliable outcomes: title, keyword, and abstract based (30). From the start

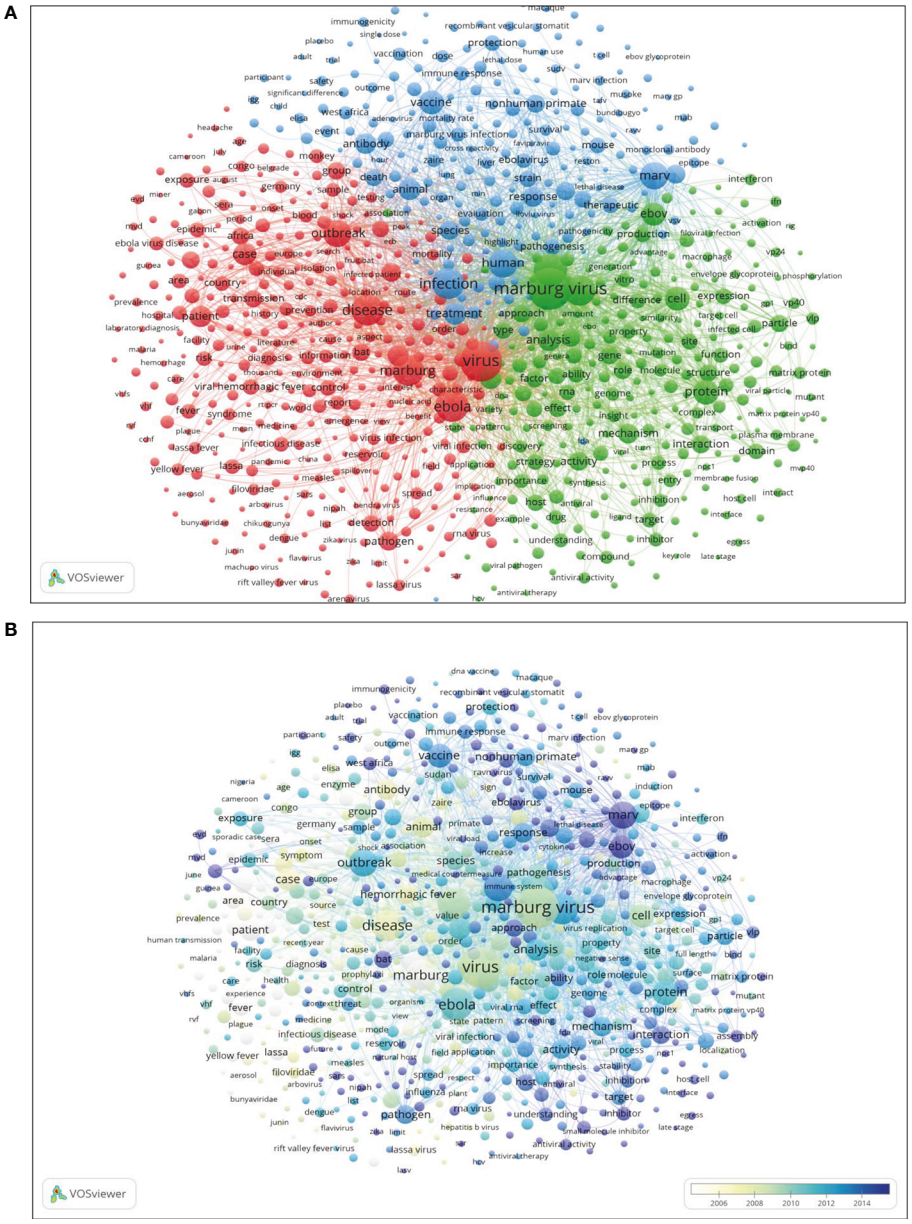

FIGURE 5
Text mining: **(A)** Research theme distribution map; **(B)** Breakdown of research hot spot trends. Temporal change in research hot spot is shown by the color gradient of white (early trends) to dark purple (future trends).

of the study to its end in 2021, 1688 articles were published in various scientific journals.

The MARV was largely ignored for many years, and a reasonable proportion of scientific papers have been published in the decades since. Controlling MARV rapidly and effectively appears to be a challenge. This is owing in part to an incomplete knowledge map of MARV research and a lack of understanding of current research status, hot spots, and development trends. This can lead to a considerable amount of repetitive, unimportant, or inefficient research, which can impede proper virus research and further hinder the development of targeted prevention and surveillance methods.

In consideration of transmission scale, MARV is less contagious than COVID-19, owing to the pathogen's limited infectivity and protracted asymptomatic incubation (31). Hence, a mapping of MARV research was created through bibliometric and visualization techniques by selecting and evaluating over 1688 MARV-related scientific articles. The annual number of

TABLE 1 Top 10 highly cited papers.

Paper	DOI	TC	ATC
Jones SM, 2005, "NATURE MEDICINE"	10.1038/NM1258	522	29
Drosten C, 2002, "JOURNAL OF CLINICAL MICROBIOLOGY"	10.1128/JCM.40.7.2323-2330.2002	498	23.71
Huang I, 2011, "PLOS PATHOGENS"	10.1371/JOURNAL.PPAT.1001258	453	37.75
Towner JS, 2009, "PLOS PATHOGENS"	10.1371/JOURNAL.PPAT.1000536	446	31.86
Warren TK, 2014, "NATURE"	10.1038/NATURE13027	420	46.67
Mühlberger E, 1999, "JOURNAL OF VIROLOGY"	10.1128/JVI.73.3.2333-2342.1999	381	15.88
Marzi A, 2004, "JOURNAL OF VIROLOGY"	10.1128/JVI.78.21.12090-12095.2004	304	16
Towner JS, 2007, "PLOS ONE"	10.1371/JOURNAL.PONE.0000764	283	17.69
Amman BR, 2012, "PLOS PATHOGENS"	10.1371/JOURNAL.PPAT.1002877	260	23.64
Gear JS, 1975, "BMJ"	10.1136/BMJ.4.5995.489	255	5.31

Total number of citations = TC
Average number of total citations per year = ATC

papers and cumulative citations of publications on MARV demonstrate the areas of interest and development over time. Between 1962 and 2022, the number of publications was modest and constant. Surprisingly, dramatic growth peaks occurred separately in 2015 and 2021, coinciding with the breakouts of MARV. This rapid increase demonstrates the substantial impact of growing MVD on public health. The number of publications is related to the size and spread of the infectious disease outbreak (32).

The United States, the United Kingdom, Canada, Germany, and Japan were the most active countries at the forefront of MARV research. Excluding Japan, our findings are consistent with other findings that the United States, the United Kingdom, Germany, and other European countries are typically the most active in scientific research (33).

The prominent contributors' different backgrounds (scholars, clinicians, research experts, etc.) represent the interdisciplinary nature of MARV research. A unified approach to responding to and controlling new viral disease outbreaks is required; therefore, scientists, clinicians, and CDC experts must communicate and exchange information even though it is critical (34).

In this study, only a few journals accounted for the majority of MARV research publications. This is consistent with Bradford's law. This reflects the authority of these journals, as well as their high level of interest in MARV research. Popular journals and their research trends in a specific topic provide dependable references for researchers. Furthermore, key journals facilitate faster search routes for scholars and can act as an important publication guide (33).

Keyword co-occurrence analysis can yield a wealth of useful information, allowing the identification of hot spots and trends and directing researchers to similar topics in their field (35). Three distinct MARV research topics can be identified, which mostly involve issues of epidemiology and public health, pathogenicity and vaccine development, and immunology and molecular biology, and commensurate effort by research scientists is compulsory for effective MVD prevention and control.

The highest number of confirmed cases of MARV was observed between 2004 and 2005, when the fatality rate was 90% (confirmed cases 252, deaths 227) in Angola. After that, various outbreaks were reported in Uganda from 2007 to 2017; the highest number of deaths recorded was 18 in 2012 and 100% CFR was documented in 2014 (Supplementary Table 1). The software used to evaluate the co-occurrence, bibliographic coupling, and clusters was VOSviewer version 1.6.18 (36). Standard weight features employed in this study are described as "Links attribute" and "Total link strength attribute" (37). With the increase of significance, the number of neighboring elements and the distance between these elements and the point of interest became smaller, and the elements' density was higher. Furthermore, as the more significant the neighboring elements' weight, the higher was the element density (36).

(28), established a new diagnosis protocol for detecting hemorrhagic fevers (VHFs) related to MARV with acute infections. (29), published an article in "PLOS PATHOGENS" reporting interferon-inducible transmembrane proteins (IFITM) originated from the MARV. (38), isolated Marburg viruses from Egyptian fruit bats (31/611, 5.1%), suggesting that they are a natural reservoir and source of the Marburg virus. One published article first reported that BCX4430 fights against MVD, acting on the RNA polymerase of the virus (39). From the country-based coupling analysis of MARV it can be concluded that the top 10 highest articles were published in the United States, Germany, Canada, Japan, France, South Africa, China, Switzerland, Uganda, and Spain. It also observed that among the top 10 highest institutes were the National Institute of Allergy, United States Army Medical Center for Disease Control, Philips University, National Health Laboratory, Boston University, Hokkaido University, University of New Mexico, University of Toronto, University

of Pennsylvania. The availability of Marburg virus samples, case numbers, or research interest might be reasons behind this scenario. From research theme distribution map analysis, we detected several clusters where the Marburg virus, Ebola, Marburg, infection, human, outbreak, disease, MARV, and vaccine are notably observed. The first paper of MARV was published in 1962 and the top-cited article was accepted by "NATURE MEDICINE" in 2005, which discussed vaccination against two deadly viruses, Ebola and Marburg. Vaccine candidates formulated with either the EBOV or MARV glycoproteins expressed in attenuated recombinant virus vectors were safe and highly effective when tested in non-human primates. Although the database search was thorough, it is conceivable that some items were missed.

6 Study gaps and future research

As an infectious disease, MVD with high CFR is able to create devastating outbreaks around the world at any time (40–45). It is high time for conducting research studies based on previous outbreaks and data regarding the virus (19). According to previous studies, it can be found that the clinical investigations of this disease are not inadequate (5, 46). In addition to that, there is no detailed knowledge about host and virus interaction for designing vaccines or therapeutic drugs to prevent and control MVD (47).

It is important to enhance awareness programs about MVD, educating people at greater risk, including healthcare workers. Proactive planning and highly collaborative efforts involving researchers, experts in public health, policymakers, and biologists are necessary to design suitable strategies to counteract MVD (46). Surveillance and monitoring need to be upgraded, along with the strengthening of rapid and confirmatory diagnosis of MVD cases and contact tracing and tracking in affected regions employing wastewater-based surveillance, with serological and molecular epidemiological investigations (48–50). Numerous publications show that circumspection, wastewater monitoring, and prognosis of the outbreak are important as well for this type of virus (51–53).

Indisputably, further analyses are necessary to inquire about the relationship between MARV and the environment (54–56). The public health sector should act on this knowledge gap to empower the community, supplying educational materials for epidemic preparedness in the future, using communication channels proposed by the communities. One Health rules implementation, the upgrade of Biosafety Level 4 Laboratories (BSL-4s), and conducting multidisciplinary research is necessary. The development of effective vaccines, antivirals, and other therapies, and adopting apposite mitigation strategies, are the current priorities in combating MARV as it poses a drastic global health concern and could cause a deadly pandemic due to its intense lethality (5, 50, 57).

7 Prevention and control

Given the recently reported MVD cases in Ghana, along with last year's report in Guinea and outbreaks in preceding years, and feasible subsequent threats, there is an utmost need for discovering an effective vaccine and therapies for this devastating disease (58). As MARV can only be handled in BSL-4s, very few laboratories have the capability for basic and applied research for developing prophylactics and therapeutics against this lethal virus. Hence, strengthening research facilities with maximum containment laboratories is critical for handling MARV. From the SARS-CoV-2 pandemic situation, everyone should understand the lesson of One Health, which is that three major things (environment, animals, and humans) are involved in the prevention and inspection of zoonotic diseases such as MVD (59–62). Swift, multidisciplinary action is required to incorporate and inspect such incidences of MVD before an unwanted quick spread of MARV occurs in other regions and countries amid the ongoing COVID-19 pandemic and current global health concern that is the burgeoning cases of monkeypox (63, 64).

Although, there is no specific treatment (i.e., vaccines/antiviral drugs) for this disease, cardiac glycosides, antipyretics, and steroids have previously been prescribed (65). Currently, various drugs are being investigated; a recent study suggested that remdesivir may be effective against the Marburg virus in cynomolgus macaque models (66), and another research study identified that cholesterol-conjugated fusion inhibitors are efficacious against this virus (67). 4-(aminomethyl)benzamide, BCX4430, favipiravir, aloperine small molecules, monoclonal antibodies, and cytokines are feasible against MARV infection (15, 68). Several attempts have been made to develop a vaccine and appropriate treatment regimen to counteract MVD (69). The GP and VP40 matrix proteins have been identified as the most antigenic viral proteins to develop a new chimeric subunit vaccine (70). An inhibitor compound, FC-10696, has recently been discovered to suppress the egress of MARV (71). Also, AVI-7288 has been indicated to exhibit potential as post-exposure prophylaxis against MARV (72). Numerous experiments were methodized on rodent and Non Human Primates (NHP) models for testing vaccine efficacies against MARV. To date, some vaccines have been trialed for human use. Among them, the cAd3 vaccine, also known as chimpanzee adenovirus serotype 3 vectors, encoded with wild-type GP from MARV, is the subject of a Phase 1 clinical trial for human use (73). To maintain the patient's electrolytes, fluids, oxygen status, and blood pressure, as well as to replace lost blood and clotting factors, and treatment for any complicating infections, supportive hospital therapy should be utilized (63, 64, 74).

8 Conclusion

In the present bibliometric study, we attempted to analyze studies published on MARV to identify the most cited published papers, delineate some topics for researchers for further research and identify knowledge gaps. To understand more about MARV and control outbreaks, analysis of published research articles plays a significant role in revealing the epidemiology, genomics, and signs and symptoms; this bibliometric study provides wider knowledge about the previously published article areas, authors, citations, and institutions related to this research. Among the countries that are potential clusters based on the review, the USA is the most prevalent, followed by Germany, Canada, and Japan. However, there is no balanced reporting of these clusters. This is likely to be due to data set availability for MARV research. Actions taken almost immediately are vital to avoid outbreaks of MARV in the future and determine whether or not the post-outbreak situation will be managed sustainably. By tackling this challenge, cities will be able to foster cycling culture and shift short-term cyclists into long-term ones.

Author contributions

MI: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Roles/Writing - original draft, Writing - review & editing. SA: Data curation, Formal analysis, Investigation, Methodology, Roles/Writing. MA: Data curation, Investigation, Visualization, Writing - review & editing, Funding. FK: review & editing. JL: Review & editing, Investigation, Visualization. PB: Conceptualization, Funding, Roles/Writing - original draft, Writing - review & editing, Supervision, Funding. KD: Review & editing, Investigation, Visualization, Supervision. All authors critically scrutinisedand approved the final version of the manuscript. The corresponding authors are responsible for confirming that the descriptions are accurate and agreed by all authors.

Funding

This study was supported by President Abdul Hamid Medical College, Noakhali Science and Technology University, Bangladesh and KTH Royal Institute of Technology, Sweden.

Acknowledgments

We acknowledge all the authors institutions. Authors of this manuscript also acknowledge handling editors and reviewer of this manuscript. We also thankful to Tahmid Anam from SUST for supporting map in this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2022.1068364/full#supplementary-material

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frontiers | Frontiers in Tropical Diseases

TYPE Opinion
PUBLISHED 30 January 2023
DOI 10.3389/fitd.2022.1077962

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Neglected Tropical Diseases, a section of the journal Frontiers in Tropical Diseases

RECEIVED 24 October 2022
ACCEPTED 30 December 2022
PUBLISHED 30 January 2023

CITATION
Sah R, Siddiq A, Al-Ahdal T, Maulud SQ, Mohanty A, Padhi BK, El-Shall NA, Chandran D, Emran TB, Hussein NR, Dhama K and Satapathy P (2023) The emerging scenario for the Eastern equine encephalitis virus and mitigation strategies to counteract this deadly mosquito-borne zoonotic virus, the cause of the most severe arboviral encephalitis in humans—an update. *Front. Trop. Dis.* 3:1077962. doi: 10.3389/fitd.2022.1077962

The emerging scenario for the Eastern equine encephalitis virus and mitigation strategies to counteract this deadly mosquito-borne zoonotic virus, the cause of the most severe arboviral encephalitis in humans—an update

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KEYWORDS

Eastern equine encephalitis, EEE, diagnosis, treatment, vaccine

Introduction

Eastern equine encephalitis (EEE), caused by the mosquito-borne Eastern equine encephalitis virus (EEEV), is an important, high-mortality disease affecting equines, humans, and other vertebrate hosts (1, 2). EEE is one of the most severe forms of arboviral encephalitis in the USA, with a mortality rate of 30%–40%, and neurological

sequelae are observed in 50% of survivors (3). The enzootic cycle between *Culiseta melanura* (Coquillett) and passerine birds is crucial to the maintenance of EEEV. EEEV causes intermittent outbreaks in the east and midwest of the USA, and has the highest recorded case fatality rate (CFR) among arboviruses in the Americas (4). It is an uncommon vector-borne disease, and approximately 6–8 cases, on average, are reported annually in the USA. There has been a rise in virus activity over the past decade, with major outbreaks in both human and equine populations. It is anticipated that the range of mosquitoes in the Americas, especially vectors of EEEV, may be impacted by predicted climate change, which may modify disease risk and constitute a public health problem (5). The consistent rise in incidence, seen across a wider region and population, demonstrates that EEE is an emerging disease. Notably, EEEV is also considered a potential bioterrorism weapon owing to its airborne transmissibility. This article presents an overview of EEEV and EEE, the current emerging scenario of increasing incidence, and salient prevention and control measures.

Virology

Eastern equine encephalitis virus (EEEV) is a single-stranded positive-sense RNA virus that belongs to the *Alphavirus* genus of the *Togaviridae* family. Its genetic structure has two main parts, responsible for the structural and non-structural proteins, respectively: the 5′ end is responsible for four non-structural proteins (i.e., nsP1, nsP2, nsP3, and nsP4) and the 3′ tail for three structural proteins, comprising the capsid and E1 and E2 glycoproteins (6). EEEV is considered the most pathogenic among viruses in the same genus, which was formerly named the South American EEE (Madariaga) virus and was changed by the new classification of the virus. The CFR of EEE ranges from 30% to 70% in humans and from 75% to 90% in equines (7–9). EEEV has been divided into two types, North American and South American, based on its antigenic properties, and, with the new classification in 2010, it was divided into four lineages. Lineage 1 is mainly present in North America and the Caribbean, and lineages 2–4 are present in Central and South America (9).

Routes of viral transmission

The transmission cycle of EEEV is similar to that of other arboviruses. It depends on the presence of enzootic vectors (*C. melanura*) that feed on the amplifying hosts, commonly bird species, and then with the aid of bridge vectors, such as *Aedes albopictus*, *Ochlerotatus japonicus japonicus*, *Coquillettidia perturbans*, and *Culex erraticus*, transmit the disease to the end hosts of humans and horses, as shown in Figure 1. Humans and horses are considered dead-end hosts, as they do not form a high viral load, which in turn facilitates disease transmission to other hosts; birds, in contrast, do form a high viral load (4, 10). The North American type of the disease is associated with the typical cycle of mosquitoes and birds, leading to sporadic cases in humans, equines, and other animals (9).

The South American variant of EEEV, Madariaga virus (MADV), differs from the North American type in that it is found almost exclusively in animals. However, in an outbreak in Panama in 2010, a dozen cases of encephalitis in humans were recorded. And, in another serological study, in Panama and the Peruvian Amazon, it was found that 2%–5% of the general population had detectable serological evidence of the virus, which means that they had been exposed to an asymptomatic or mild infection in the past. Eight cases in children were reported in the period between 2015 and 2016, which shows the difference in the nature of infection between the two types of the virus (11).

The transmission cycle of the disease is considered the basis for the difference in the number of reported cases and in disease severity and mortality, as different enzootic vectors are associated with different numbers of dead-end host cases. Differences in bridge

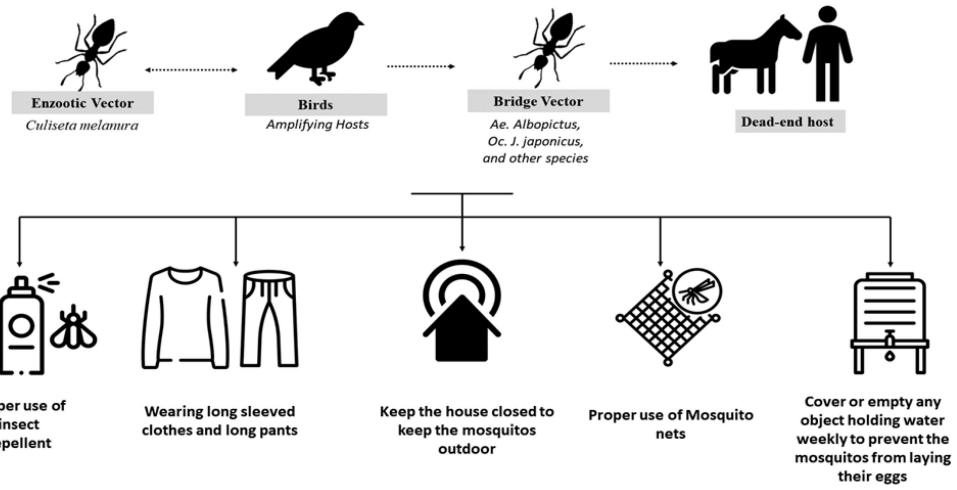

FIGURE 1
Transmission cycle of the Eastern equine encephalitis virus (EEEV) and the measures recommended by the Centers for Disease Control and Prevention (CDC) to disrupt this cycle and prevent infection.

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vectors are also linked to different feeding patterns in birds and correlated with the number of cases of infection in dead-end hosts (4).

Outbreak history

The first detection of EEEV was reported in 1831; the virus was isolated from horses in Massachusetts, USA, where 75 horses died with neurological disease sequelae. Thereafter the virus was isolated and identified as the cause of encephalitis during an outbreak in Delaware, Maryland, New Jersey, and Virginia in 1933 (12, 13). The disease was first detected in humans in 1938, in Massachusetts, USA, when 25 out of 38 infected individuals died (14). The major outbreak of the disease in a human was recorded in 1959, in which 32 encephalitis cases were detected in New Jersey, USA (3). The incidence of EEEV infection in the period from 1964 to 2002 is summarized in Figure 2. The numbers of confirmed or suspected cases of EEE are summarized in Table 1, which is adapted from the Centers for Disease Control and Prevention (CDC)'s resources for disease surveillance between 2003 and 2022 (15).

A study by Lindsey and colleagues revealed that over 14 years, from 2003 to 2016, 121 human cases of EEE were reported from 74 counties in 20 states of the USA. The majority of patients (119) had neuroinvasive disease, with only two having non-neuroinvasive disease, and almost all patients with neuroinvasive disease (110 out of 119) had encephalitis or meningoencephalitis. In total, 118 patients were hospitalized and there were 50 fatalities. The CFR was 75% in patients aged over 70 years and 31% in patients aged less than 70 years. Those aged less than 5 years or over 60 years were more likely to develop neuroinvasive disease (16).

According to the CDC, the total number of reported cases of EEE from different states in the USA in the period 2011–2020 was 110: 26 cases were reported from Massachusetts (owing to the presence of the Hockomock Swamp, which is a natural habitat for the birds and the mosquitoes, which in turn increases the likelihood of disease transmission), 18 from Michigan, nine from Florida, seven from North Carolina, seven from Georgia, six New York, five from New Jersey, and five from Connecticut; the remainder were sporadic cases from different states. This demonstrates that the disease has spread

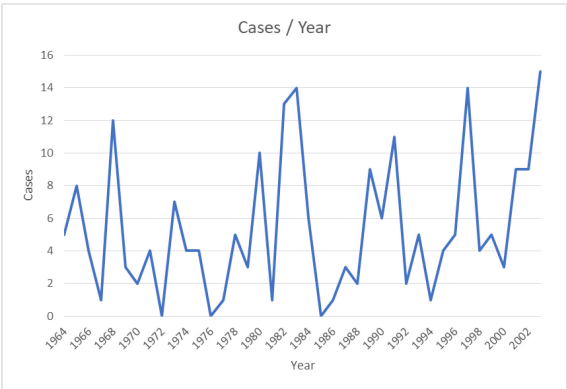

FIGURE 2
Confirmed and suspected cases of EEEV infection between 1964 and 2002 (based on data obtained from the CDC) (15).

across different states, as shown in Figure 3 (18). The overall numbers of reported cases between 2011 and 2020 were as follows: four cases in 2011, 15 cases in 2012, eight cases in 2013, eight cases in 2014, six cases in 2015, seven cases in 2016, five cases in 2017, six cases in 2018, 38 cases in 2019, and 13 cases in 2020 (19). According to the ArboNET, the total number of cases of EEE in the USA in 2021 was four in three states: Michigan, North Carolina, and Georgia (Liberty and Camden counties) (17).

According to the CDC, the total number of fatal cases of EEE in the period between 2011 and 2020 was 47, giving a mortality rate of 43% (19).

In 2022, cases reported in animals included as a dead horse in Antwerp in New York, a dead dog in Albion (as reported by Oswego County Health Department), and a dead horse in Mexico; the EEE virus was also detected in sentinel chickens. Although there were no reported human cases, cases in animals present a risk for virus transmission to horses and humans (20–23).

Clinical manifestations

The incubation period of EEEV ranges between 4 and 10 days; the carrier may be asymptomatic, febrile, or have neurological manifestation. The febrile period is associated with chills, aches, and joint pain, which can last from 1 to 2 weeks, with fewer than 5% of cases developing meningitis and encephalitis (19, 20). The neurological manifestations include encephalitis and meningitis along with other symptoms such as fever, vomiting, headache, diarrhea, seizure, behavioral change, drowsiness, and coma. One-third of encephalitis patients die, and those who survive have impairments that can be mild or severe, including seizures, paralysis, and coma (24, 25).

In 2019, four cases of the EEEV infection were detected in Connecticut, USA. All four patients experienced severe and progressive disease despite empiric treatment, and certain manifestations, such as fever, coma, weakness, confusion, and seizures, were common to all patients. Examination revealed pleocytosis in the cerebrospinal fluid (CSF), but initial immunoglobin M (IgM) testing was negative, so the patients were referred to the CDC, where EEE was diagnosed. This unexpected outbreak of EEE in this state shows the importance of public health departments being connected to the CDC and having the ability to administer diagnostic tests that will enable the detection of EEE in an evidence-based manner (26).

According to the CDC, the number of confirmed cases of EEE in 2019 was 38. Four patients (three men aged between 50 and 60 years and one girl aged 6 years) had neuroinvasive manifestations and presented to a hospital in New England. Some symptoms, such as fever, ataxia, dizziness, seizures, and mental changes, were common to all patients. Two patients diagnosed as having a severe form of EEE required ventilation. Two of the four patients died, and the other two experienced a full recovery. This shows the importance of early diagnosis of this disease, which should be suspected if there is pleocytosis in the CSF and hyperintensity of gray matter is detected on an MRI scan (27).

In a study that examined the risk factors associated with EEE, it was found that, in 15 cases of EEE in children occurring between 1970

TABLE 1 Confirmed and suspected cases of Eastern equine encephalitis virus (EEV) infection between 2003 and 2022 (adapted from the ArboNET surveillance system of the CDC) (15).

State	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Alabama	2	0	2	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Alaska	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arizona	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arkansas	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
California	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colorado	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connecticut	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	0
Delaware	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
District of Columbia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Florida	3	0	5	0	0	1	0	4	0	2	3	0	0	0	1	3	0	0	0	0
Georgia	2	0	1	1	0	0	0	0	0	1	1	0	0	1	2	1	1	0	2	0
Hawaii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idaho	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Illinois	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Indiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Iowa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kansas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kentucky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Louisiana	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Maine	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Maryland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Massachusetts	0	4	4	5	0	1	0	1	1	7	1	0	0	0	0	0	12	5	0	0
Michigan	0	0	0	0	0	0	0	3	0	0	0	1	0	2	0	1	10	4	1	0
Minnesota	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mississippi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Missouri	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Montana	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Nebraska	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Continued)

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TABLE 1 Continued

State	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Nevada	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
New Hampshire	0	1	7	0	2	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0
New Jersey	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0
New Mexico	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
New York	0	0	0	0	0	0	1	1	1	0	0	2	3	0	0	0	0	0	0	0
North Carolina	1	1	0	1	0	1	1	0	0	2	1	0	1	2	0	0	1	0	1	0
North Dakota	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ohio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oklahoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oregon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pennsylvania	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Puerto Rico	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhode Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
South Carolina	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
South Dakota	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tennessee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Texas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Utah	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermont	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Virginia	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Washington	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
West Virginia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wisconsin	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2	0	1
Wyoming	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	15	7	21	8	3	4	4	9	4	15	8	8	6	7	5	6	38	13	4	1

10.3389/fitd.2022.1077962

FIGURE 3
Incidence of EEEV infection in different states of the USA from 2011 to 2020 (adapted from the CDC) (17).

and 2010, certain manifestations, such as fever, headache, seizure, leukocytosis, and pleocytosis in the CSF, were common to all. In terms of outcome, five patients experienced severe neurological slippage, two experienced mild neurological slippage, four patients died, and four made a full recovery. It was observed that a long prodromal period of the disease is associated with less severe outcomes and that, in children, EEE is associated with a characteristic pattern of multifocal lesions that are correlated with the high incidence of complex partial lesions (28).

Another reported case of EEE occurred in 42-year-old man who was working in a wooded area, which is considered a risk factor for EEEV, and was admitted to a New Jersey hospital. It was reported in this case that the patient had received multiple mosquito bites in the week before admission. The patient was admitted with intractable headache and facial paresthesia, and required to be ventilated. After 9 days the patient started to improve, which shows the importance of taking a history from patients and the importance of administering symptomatic treatment (29).

Diagnosis

EEE should be suspected in any patient presenting with a febrile disease or neurological disease in geographical regions where EEE is prevalent and who has a history of mosquito bites, blood transfusion, and organ transplantation. However, the disease also needs to be confirmed, as other viral infections have similar manifestations. Clinical evaluation, in the form of neuroimaging and scanning, can reveal encephalitis in the form of brain lesions, destruction of neurons, and vasculitis in different brain regions, such as the cortex, brain stem, and midbrain (30). The first diagnostic method to be used should be serological testing, which includes the detection of EEEV-specific IgM. Infection can then be confirmed by the detection of neutralizing antibodies at the CDC or another official health facility, as virus isolation from clinical samples is challenging. The diagnosis of EEE is therefore based on clinical manifestations and laboratory detection using a molecular technique, such as polymerase chain reaction (PCR) analysis, which is considered more accurate and sensitive (31, 32).

In a study of all cases of EEE occurring in the period from 1988 to 1994, 36 cases were identified and the neurological manifestations associated with the disease were reported. Confusion, somnolence, focal weakness, seizures, and meningeal signs were common, and in most cases were are followed by deterioration and coma. The period between the appearance of the symptoms and neuroimaging findings of lesions in different parts of the brain, such as basal ganglia, thalami, and cerebral cortex, ranged from 1 to 14 days (33).

The diagnosis of the disease includes the serological identification of the IgM antibodies, nucleic acid identification using reverse transcription-PCR (RT-PCR) analysis of a sample of blood and CSF, and neuroimaging using MRI and CT scanning. However, in the initial stage of the disease, serology may be negative, so tests must be repeated multiple times, supported, if still negative, byPCR; therefore, clinicians should test patients early with all available techniques, as this can be helpful in case identification with the help from the local branch of the CDC (3, 31).

Treatment and prevention

Disease management is mainly supportive, including the use of antipyretics for fever, pain relievers for headache, antiemetics and fluid replacements for nausea and vomiting, and close monitoring for encephalitis as a result of the increased risk of raised intracranial pressure (25). Multiple EEEV vaccines are now in research and development, but the infrequent, localized, and widely dispersed nature of outbreaks means that there may not be significant incentives to move through with development and licensing. Although a vaccine against EEEV is available for horses, there is as yet no human equivalent (2). However, an early-generation investigational EEEV vaccine is currently available through the US Army Investigational New Drug program, which may be useful for people who are at high occupational risk (such as laboratory workers). Vaccines made from mosquito saliva that would be effective against a wide variety of mosquito-borne diseases are still in the research and development phase. An expected benefit of these vaccinations is the incorporation of salivary proteins from mosquitoes chosen for their ability to transmit numerous human arboviruses (7, 34). Prevention measures mainly involve protection from mosquito bites, such as covering the skin and the clothes with an insect repellent, such as picaridin (with adherence to the product instructions on safe use); wearing long-sleeved tops, pants, and hats, paying particular attention to areas of the body most likely to experience mosquito bites; clearing standing water that is considered a source for mosquitoes gathering; and closing all the openings and holes in the home that might enable mosquitoes to enter (35).

Various methods have been used to produce EEEV vaccines; for example, the replicon particle-based vaccine removes the genes responsible for the structural proteins. These vaccines were observed to be effective as individual and trivalent based (36). Another type is the viral vector-based vaccine, in which multiple viruses are used as a vector for the transmission of the genetic material that helps the body generate an immunity against the virus. Examples of such vaccines include the EILV/EEEV vaccine, which uses the *C-E3-E2-6K-E1* gene in an animal model (CD-1 mice); the EILV/EEEV vaccine, which uses the *C-E3-E2-6K-E1* and *C-E2-E1* genes in an animal model (CD-1 mice using the Eilat virus as a viral vector); the rISFV-EEEV vaccine, which uses the *E3-E2-6K-E1* gene in an animal

model (CD-1 mice, using the Isafahan virus as a viral vector); the SIN/NAEEEV vaccine, which uses the *C-E3-E2-6K-E1* gene in an animal model (either NIH Swiss mice or *Cynomolgus macaque*, using the Sindbis virus as a viral vector); the MVA-BN-E vaccine, which uses the *E3-E2-6K-E1* gene in an animal model (BALB/c mice); and the MVA-BN-W +E+V vaccine, which uses the *E3-E2-6K-E1* gene in an animal model (BALB/c mice, using vaccinia virus as a viral vector) (37–41).

Another vaccine strategy is the plasmid DNA vaccine, which, among other advantages, is low cost, has high stability, can be manufactured on a large scale, and involves no live parts. Among the disadvantages include the possibility that the vaccine will induce autoimmunity and the fact that large amounts and multiple doses of the are needed to provide adequate protection. Examples include the pcDNA^{TM}3.1$^{(+)}$-C-E vaccine, which uses the *C-E3-E2-6K-E1* gene in a BALB/c mice animal model (42).

Another form of vaccine uses the inactivated form of the PE-6 strain of EEEV and the FY 06-31 protocol. The vaccine is administered on day 0, day 28, and month 6; patients with an inadequate immune response, prior EEEV vaccination, and other eligible conditions also receive booster doses. It was reported that the vaccine elicited a high immune response in the primary series and when administered on an annual basis to laboratory personnel at risk, which demonstrates that the vaccine is safe and immunogenic (43).

The safety and tolerability of a trivalent vaccine have been investigated in a phase 1, randomized, open-label clinical trial. Healthy volunteers aged 18 to 50 years were given the Venezuelan equine encephalitis (WEVEE) virus like particle (VLP) vaccine at doses 6, 30, and 60 µg at day 0 and week 8. It was reported that the vaccine was safe and well tolerated, with only a few reported side effects, such as injection site pain and tenderness (44).

Conclusion and future prospects

As most reported human cases of EEE are caused by the North American type of EEEV, we should focus our research on the prevention of the prevalence of this type of disease.

EEE is a rare but deadly disease, but it is anticipated that the risk of EEEV infection may vary as a result of the projected influence of climate change on mosquito populations, leading to a greater disease burden or the spread of the illness into previously unaffected geographical areas. Hence, there is a need to increase awareness of this disease and more research should be undertaken to bridge the knowledge gap and prevent this illness.

Local health departments in every endemic country can monitor equids, birds, and mosquitoes for signs of human illness in the absence of vaccinations or specialized treatments; nevertheless, underfunding of public health activities is a constant danger to even these crude prevention methods. Several American public health specialists have recently advocated for a national defense strategy against arboviruses and other vector-borne diseases, a concept that has been endorsed by specialists from other countries. Piecemeal efforts to combat arboviruses are unlikely to be successful. Throughout the USA and the rest of the world, multiple potentially lethal viruses are always present in virologically occult enzootic foci. An additional cause for concern is the potential for climatic and weather-related factors, such as variations in temperature and precipitation, to influence the life cycles and geographic distribution of arthropod vectors and viral transmission patterns. Such vectors pose a genuine and immediate threat, and there is high probability that further arbovirus emergencies will occur. Although EEE is not yet a global health emergency, the recent uptick in cases has highlighted our lack of preparedness for unexpected infectious disease outbreaks. It would be wise to follow proactive active control measures and increase vigilance in the face of these threats. There is also a need for enhanced awareness among public health and medical personnel concerning the increase in EEE cases, and the significance of adopting appropriate prevention and control measures, particularly in regions with high prevalence. Early diagnosis of the disease, followed by timely treatment and management of EEEV-infected patients, is essential.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 07 February 2023
DOI 10.3389/fphar.2023.1090717

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 05 November 2022
ACCEPTED 19 January 2023
PUBLISHED 07 February 2023

CITATION
Islam MA, Shahi S, Marzan AA, Amin MR, Hasan MN, Hoque MN, Ghosh A, Barua A, Khan A, Dhama K, Chakraborty C, Bhattacharya P and Wei D-Q (2023), Variant-specific deleterious mutations in the SARS-CoV-2 genome reveal immune responses and potentials for prophylactic vaccine development.
Front. Pharmacol. 14:1090717.
doi: 10.3389/fphar.2023.1090717

Variant-specific deleterious mutations in the SARS-CoV-2 genome reveal immune responses and potentials for prophylactic vaccine development

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Introduction: Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has had a disastrous effect worldwide during the previous three years due to widespread infections with SARS-CoV-2 and its emerging variations. More than 674 million confirmed cases and over 6.7 million deaths have been attributed to successive waves of SARS-CoV-2 infections as of 29th January 2023. Similar to other RNA viruses, SARS-CoV-2 is more susceptible to genetic evolution and spontaneous mutations over time, resulting in the continual emergence of variants with distinct characteristics. Spontaneous mutations of SARS-CoV-2 variants increase its transmissibility, virulence, and disease severity and diminish the efficacy of therapeutics and vaccines, resulting in vaccine-breakthrough infections and re-infection, leading to high mortality and morbidity rates.

Materials and methods: In this study, we evaluated 10,531 whole genome sequences of all reported variants globally through a computational approach to assess the spread and emergence of the mutations in the SARS-CoV-2 genome. The available data sources of NextCladeCLI 2.3.0 (https://clades.nextstrain.org/) and NextStrain (https://nextstrain.org/) were searched for tracking SARS-CoV-2 mutations, analysed using the PROVEAN, Polyphen-2, and Predict SNP mutational analysis tools and validated by Machine Learning models.

Result: Compared to the Wuhan-Hu-1 reference strain NC 045512.2, genome-wide annotations showed 16,954 mutations in the SARS-CoV-2 genome. We determined

that the Omicron variant had 6,307 mutations (retrieved sequence:1947), including 67.8% unique mutations, more than any other variant evaluated in this study. The spike protein of the Omicron variant harboured 876 mutations, including 443 deleterious mutations. Among these deleterious mutations, 187 were common and 256 were unique non-synonymous mutations. In contrast, after analysing 1,884 sequences of the Delta variant, we discovered 4,468 mutations, of which 66% were unique, and not previously reported in other variants. Mutations affecting spike proteins are mostly found in RBD regions for Omicron, whereas most of the Delta variant mutations drawn to focus on amino acid regions ranging from 911 to 924 in the context of epitope prediction (B cell & T cell) and mutational stability impact analysis protruding that Omicron is more transmissible.

Discussion: The pathogenesis of the Omicron variant could be prevented if the deleterious and persistent unique immunosuppressive mutations can be targeted for vaccination or small-molecule inhibitor designing. Thus, our findings will help researchers monitor and track the continuously evolving nature of SARS-CoV-2 strains, the associated genetic variants, and their implications for developing effective control and prophylaxis strategies.

KEYWORDS

COVID-19, SARS-CoV-2, deleterious mutation, unique mutation, delta variant, omicron variant, immune response, vaccine designing

1 Introduction

The ongoing COVID-19 caused by SARS-CoV-2 has wreaked havoc on global economies, businesses, communities, and public health due to widespread infections in humans (Hoque et al., 2020; Islam et al., 2021; Jakariya et al., 2022; Rakib et al., 2022). Causingflu-like symptoms and nearly 2%–5% mortality, the COVID-19 pandemic worsened due to continuously emerging SARS-CoV-2 variants from time to time, contributing to the surge in infections and deaths in multiple waves (Dhama et al., 2022; Jacobs et al., 2023; Islam et al., 2022a; Islam et al., 2022b).

Similar to other RNA viruses, SARS-CoV-2 is prone to mutations that produce new variants, creating difficulties in developing effective antiviral drugs and vaccines against this virus (Ahmed et al., 2020; Jacobs et al., 2023; Sakib et al., 2021; Wang et al., 2020). Over 2 years, the SARS-CoV-2 virus has evolved through multiple new mutations and various genetic variants, such as Alpha (B.1.1.7) with seven, Beta (B.1.351) with nine, Gamma (P.1) with 12, Delta (B.1.6, B.1.6.2) with 17, Omicron (B.1.1.529) and Neocov variant with 32 new mutations in the spike protein gene (Ahmed et al., 2021; Chakraborty et al., 2022a; Chakraborty et al., 2022b; Chakraborty et al., 2022c; Chakraborty et al., 2022d; Chakraborty et al., 2022e). These variants have spread to various regions of the world, and among them, variants of concern (VOCs) such as Delta, Omicron, and their sub-lineages have caused significant risk to public health (Hossain et al., 2021; Islam et al., 2021, Islam et al., 2022a). However, the number of fatalities caused by various SARS-CoV-2 variants fluctuates considerably (Islam et al., 2022b; Islam et al., 2022c; Islam et al., 2022d). So far, only a few comprehensive studies have been conducted, incorporating all SARS-CoV-2 variants (Davies et al., 2021; Imai et al., 2021; Walensky et al., 2021). Each variant of SARS-CoV-2 evolves with greater pathogenicity, infecting and evading the immune system of the host, leading to vaccine breakthrough infections, re-infections *via* overpowering vaccine efficacy and antibodies-based therapies (Roy et al., 2022a; Chakraborty et al., 2022b; Roy et al., 2022b). Eight of the 23 mutations from the original Wuhan-Hu-1 strain (Accession NC_045512, version NC_045512.2) that make up B.1.1.7 are in the spike protein, in which N501Y, spike deletion 69–70 del, and P681H are the three mutations that are considered to have the most biological impact (Yang et al., 2021). In addition to D614G, B.1.351 contains a cluster of mutations (242–244 del and R246I) in the N-terminal domain, three mutations (K417N, E484K, and N501Y) in the receptor-binding domain (RBD), and one mutation (A701V) near the furin cleavage site. Among the three significant mutations that the spike protein RBD carries, E484K is situated in a loop region away from direct hACE2 (human angiotensin-converting enzyme 2) contact, while mutations in P.1: N501Y and K417T interact with human ACE2 (hACE2). (Faria et al., 2021; Hossain et al., 2021; Chandran et al., 2022). Compared with the first strain (alpha strain) of SARS-CoV-2, the Delta variant B.1.617.2 has 23 mutations. Twelve such mutations have been found in the spike protein, including T19R, L452R, T478K, D614G, P681R, and D960N (Shiehzadegan et al., 2021; Asghar et al., 2022).

To prevent SARS-COV-2 infections, a few COVID-19 vaccines utilizing distinct vaccine platforms have been developed (Hossain et al., 2021). In addition, vaccination campaigns and booster shots are underway in majority of nations to provide the populace with protective immunity. Some drugs, including recent oral antiviral drugs nirmatrelvir/ritonavir and molnupiravir, and therapies have been found effective and used for emergency purposes. However, an effective vaccine to tackle the menace of emerging variants of SARS-CoV-2, particularly such as Delta and Omicron, is still awaited (Aleem et al., 2022; Barouch, 2022). Designing effective vaccines against SARS-CoV-2 variants is a very challenging issue as it is required to develop mutation-proof, variant-specific, and universal vaccines to prevent the spread of COVID-19 (Gong et al., 2022; Park et al., 2022). In this regard, investigating SARS-COV-2 variants and unique mutations is essential for developing effective anti-COVID-19 drugs and vaccines (Rakib et al., 2021; Jakariya et al., 2021).

Therefore, in the present study, we analyzed the mutation patterns of 10,531 SARS-CoV-2 genomes of 12 variants from around the world in terms of frequency, type, the ratio of synonymous to non-

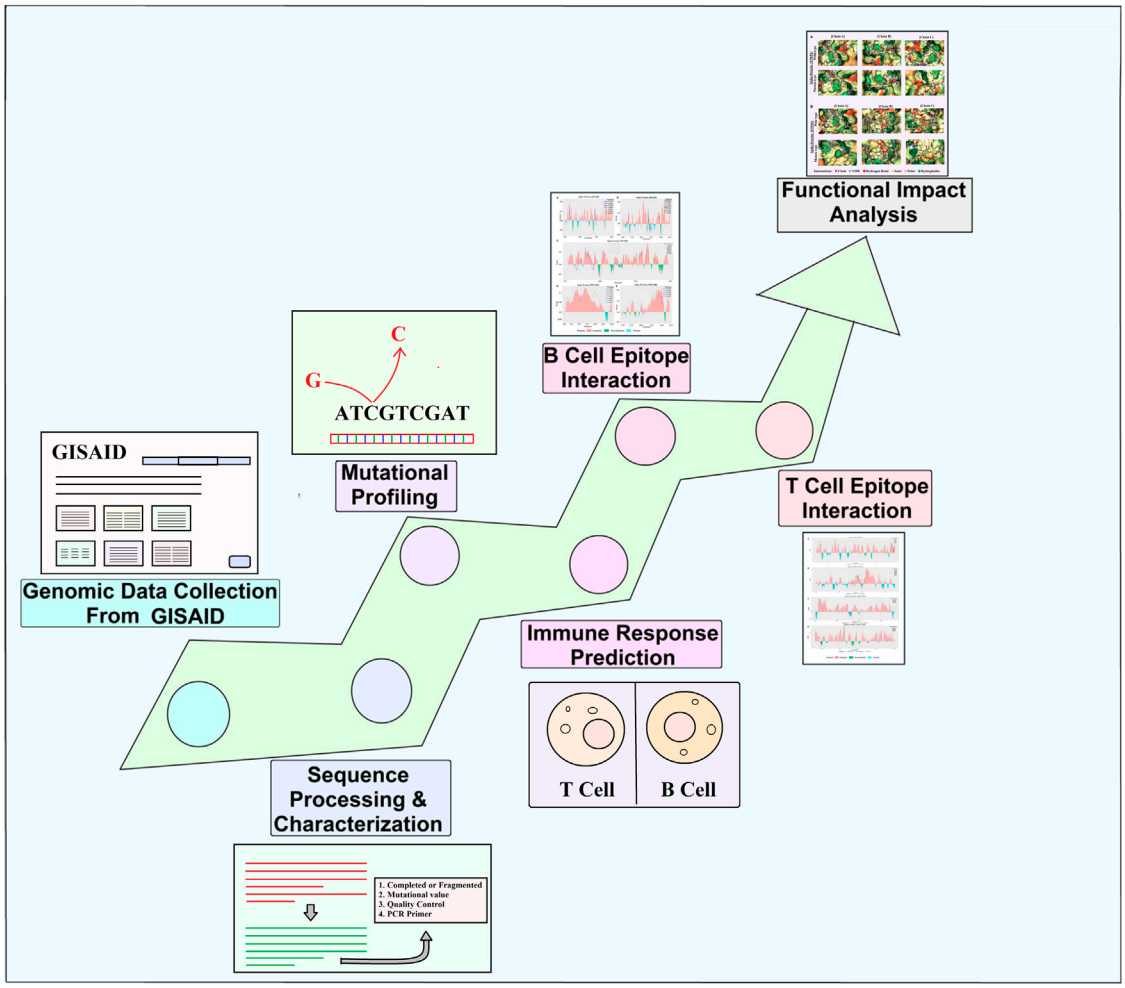

FIGURE 1
Schematic diagram representing several stages starting from genomic data collection to analysis.

synonymous mutations, and zone analysis. Based on the most significant mutations, we focused primarily on the Delta and Omicron variants. The impact of deleterious mutations on B- and T cell responses has also been investigated to identify the specific mutations responsible for the downregulation of the host immune system. The structural conformation of the most concerning mutations, such as mutations of spike proteins, was also determined. The results of this study will aid the scientific community in the development of variant-specific, more accurate, effective, and successful vaccines, as well as provide possible indications for the regions of deleterious variant viral proteins in the Omicron variant. Therefore, in the present study, we analyzed the mutation patterns of 10,531 SARS-CoV-2 genomes of 12 variants from around the world in terms of frequency, type, the ratio of synonymous to non-synonymous mutations, and zone analysis. Based on the most significant mutations, we focused primarily on the Delta and Omicron variants. The impact of deleterious mutations on B- and T cell responses has also been investigated to identify the specific mutations responsible for the downregulation of the host immune system. The structural conformation of the most concerning mutations, such as mutations of spike proteins, was also determined. The results of this study will aid the scientific community in the development of variant-specific, more accurate, effective, and successful vaccines, as well as provide possible indications for the regions of deleterious variant viral proteins in the Omicron variant.

2 Materials and methods

2.1 Genomic data collection and filtering

The first SARS-CoV-2 whole genome sequence (WGS) was deposited on 5 January 2020, in GenBank (accession number: NC_045512.2). Currently, the total number of submitted sequence numbers is 127,108,83 (accordingly, Global Initiative on Sharing All Influenza Data (GISAID), 20 August 2022) (https://www.gisaid.org/) (Khare et al., 2021).

In this study, we filtered 214,459 sequences from the whole extracted GISAID dataset based on specific criteria (all of the complete genome sequences retrieved including both death and alive p with high coverage read, patient status, and collection date from the human host). It can be

noted that the collected sequences retrieved from dead patients do not influence the whole analyzed dataset. Only complete genomes with a size greater than 29,000 bp were selected, and those with low coverage, possessing >5% of N, were filtered out. Finally, the filtered process ultimately resulted in 10,531 complete genomes of SARS-CoV-2 for this study, which ranged from January 2020 to January 2022 (Figure 2) (Supplementary Table S9). Pyfasta (https://github.com/brentp/pyfasta) was used to split the total genome into six separate files. The entire procedure used in this study is presented in Figure 1.

2.2 Mutation analysis

Sequence analysis, alignment, and clustering were performed using NextClade (https://clades.nextstrain.org/), an advanced tool for SARS-CoV-2 sequence analysis (Aksamentov et al., 2021). Mutational frequency count was executed using Python script (uploaded to GitHub). Error-free sequence data were normalized (filtered to reduce data redundancy and eliminate undesirable characteristics) using Python script and advanced Excel options. The most frequent mutations and their deleterious impact were further analyzed using PredictSNP (https://loschmidt.chemi.muni.cz/predictsnp/) (Bendl et al., 2014), PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al., 2013), SIFT (https://sift.bii.a-star.edu.sg/) (Ng, 2003), PROVEAN (http://provean.jcvi.org/index.php) (Choi & Chan, 2015), and the I-Mutant Suite (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) (Capriotti et al., 2005). Deleterious or non-synonymous mutations are critically analyzed and cross-checked using these tools. To define deleterious mutation, a threshold value of -2.5 was determined to ensure highly balanced accuracy (Choi & Chan, 2015). Therefore, mutations having a value smaller than -2.5 were identified as deleterious.

2.3 Prediction of immune responses

2.3.1 B cell epitope prediction

The antibody epitope prediction tool (http://tools.iedb.org/bcell/) from IEDB (Immune Epitope Database) was used to detect the B cell antigenic regions. In this tool, a semi-empirical method named “Kolaskar & Tongaonkar antigenicity” was used (Kolaskar & Tongaonkar, 1990) as the analyzed method for B cell antigenic prediction as the method ensures 75% accuracy. The antigenic region refers to the epitope that is most likely to bind to B cells whereas the non-antigenic region shows lower or no affinity to bind to B cells. Each mutation sequence was derived using Python script, which is available from GitHub on request (Islam et al., 2022e; Islam et al., 2023). Based on the antigen propensity score collected from the tool, linear B cell epitope area graphs of wild sequences were constructed using ggplot2 (version 3.3.5), ggh4x (version 0.1.2.1.9), and cowplot (version 1.1.1) in the R statistical environment (version 3.6.1). Graphs are presented as antigenic (>threshold value) and non-antigenic (<threshold value) regions based on a threshold value of 1 as the average score for most of the sequence is around 1. Mutations having increased antigenic scores are likely to bind B cells, therefore, enabling the host immunity to act to sort the fight. But the opposite case refers to the mutations that are becoming immune to the host B cell. Codes generated for the graphs can be accessed from GitHub upon request. Mutations are marked as line graphs with a point mark on the mutation position. The significant mutation was marked by comparing the antigenic propensity score of mutated proteins with that of wild-type proteins. If the score of the mutated proteins dropped in the antigenic region, it was marked as significant.

2.3.2 T Cell epitope prediction

Wild type sequenced for each protein was then used to perform T Cell epitope prediction with the “CD4 T Cell immunogenicity prediction tool” of IEDB (http://tools.iedb.org/CD4episcore/) (Paul et al., 2015; Dhanda et al., 2018). The threshold value of the combined score was set to 50%. The results give immunogenicity scores of several predicted immunogenic segments for a particular peptide. However, for more accuracy combined scores were taken into concern as the score not only includes immunogenicity scores but also scores from the seven allele method (an optimized method for prediction of HLA responses) (Dhanda et al., 2018). The mutations occurring in these predicted immunogenic segments were then used to perform with the same tool to observe the change in combined score due to that particular mutation. According to the result, a higher value indicates a lower tendency to provoke an immune response. Therefore, the mutations which caused an increase in the combined score beyond 50%, were marked as the most significant.

2.4 Effects of missense mutation on protein stability and protein-protein binding affinity

Mutations likely to suppress the immune response for both B- and T cells are selected for the next level of analysis to demonstrate their functional impacts. Most of the data, except spike protein, were collected with the COVID-3D database (http://biosig.unimelb.edu.au/covid3d/). For the spike protein of the delta variant (PDB ID:7jji), the “DynaMut” database (http://biosig.unimelb.edu.au/dynamut/) (C. H. Rodrigues et al., 2018) was used to find ΔΔG to identify the stabilizing effect of the mutations."Δ Vibrational Entropy Energy”, was also determined to identify the impact on structural flexibility, and deformation and fluctuation analysis. “mCSM-PPI2" (http://biosig.unimelb.edu.au/mcsm_ppi2/) (C. H. M. Rodrigues et al., 2019) was used to predict the affinity changes and differences in the interaction between the wild-type and the mutant for both delta and omicron. The effects of missense mutation on protein stability for omicron were analyzed with “mCSM Stability” (https://biosig.lab.uq.edu.au/mcsm/) (Pires et al., 2014). To perform protein and/or gene functional analysis, the 3D structure of spike protein was collected from the Protein Data Bank (https://www.rcsb.org/).

2.5 Normalization, data validation, and machine learning

In order to overcome the biases in the various factors or platforms in mutation-based studies normalization is a crucial pre-processing step. A sample-wise normalization is a typical approach in intra-study analysis. Numerous established sample-wise normalization techniques have been created and used, such as simple standardization (standardize to zero mean and unit variance). This data was subjected to standard normalization or standardization prior to validation under machine learning, which confirmed independent and unbiased findings of the mutational structure and at the same time standardized each sample to a mean of zero and a unit variance.

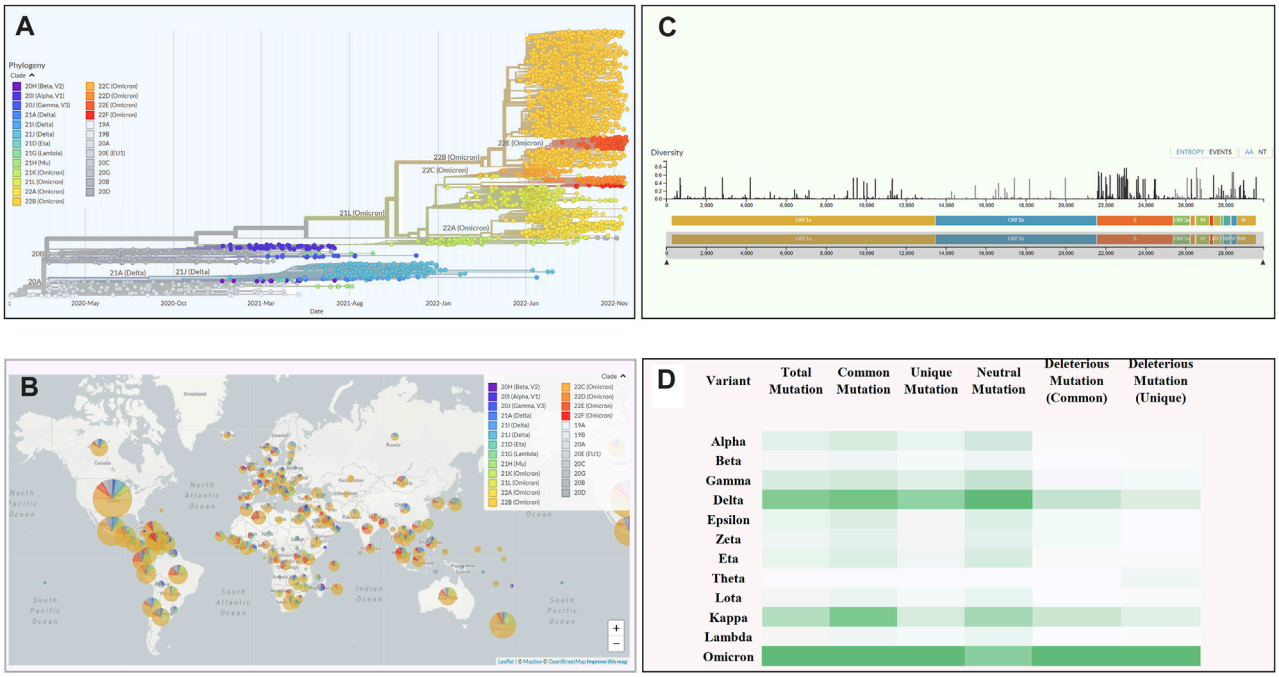

FIGURE 2
An overview of the scenario of coronavirus. **(A)** Phylogenetic tree of SARS-CoV-2 depicting variant emergence from January 2020 to January 2022. **(B)** A world map depicting the frequency of variants occurring across the world. **(C)** The total number of mutations occurring across the whole genome of SARSCoV-2. The length of the bar determines the diversity of mutations at a specific position on the genome. (D) The total number of mutations along specific types like common, unique, neutral, and deleterious across all the variants of SARS-CoV-2. (Analysed by Nextstrain: https://nextstrain.org).

The overall analysis was validated using machine learning and Bio-python. We used the following set of different classifiers, which includes probabilistic ones: Logistic Regression, Linear Discriminant Analysis, Support Vector Machines (SVM) (Cortes & Vapnik, 1995), and Neural Networks (Jantzen, 1998). We used a specific performance measure under the ROC curve, which is the proportion of correctly classified samples. The classes were balanced so that the accuracy metrics worked correctly.

We trained and turned the classifiers on the SARS-CoV-2 mutational databases (Supplementary Table S7). To avoid overfitting, five-fold cross-validation was performed. Specifically, a dataset was split into five approximately equal parts (or folds), of which four parts were used for training and the fifth part for validation. This procedure was repeated five times with different parts used for validation each time. The performance measure is an average of the values computed at each iteration. While the dataset is usually split into folds randomly, we created folds such that all mutations in the same protein fell into the same fold. This was done to avoid over fitting in the situation where we train and test a classifier on the same protein.

3 Results

3.1 Mutational analysis of SARS-CoV-2 variants

Through a comprehensive mutational analysis of 10,531 complete genomes (The number of sequences had been retrieved in a sequential way mentioned in the Genomic data collection and filtering portion of the methodology Section 2.1 and neither these sequences nor the partial sequences under different variants were compromised by any factor or biased) of SARS-CoV-2, we detected 16,954 common and unique mutations (which were continuously filtered according to the mutation score, and only a specific amount of highly deleterious mutations were focused for further mutational analysis) compared to the Wuhan-Hu-1 reference strain (Accession NC_045512, VersionNC_045512.2). Based on the mutational spectra, we found 12 variants of SARS-CoV-2, including 1,076 genomes from Alpha, 686 from Beta, 1,350 from Gamma, 1884 from Delta, 805 from Epsilon, 461 from Zeta, 1,468 from Eta, 133 from Theta, 305 from Lota, 207 from Kappa, 209 from Lambda, and 1947 from Omicron variants. The Omicron variant showed a comparatively higher mutation rate (Figure 2D).

Among these variants, the Delta variant (B.1.617.2) showed 4,468 mutations, of which 1,204 were deleterious having a value less than the threshold (−2.5). Moreover, among the detected mutations in the Delta variant, 66% were unique, and not reported in other variants. By contrast, the highest number of mutations (n = 6,307) were found in the Omicron variant in which 4,178 (67.8%) were predicted to be unique while 1,092 (26.14%) were identified as deleterious (Table 1).

In the mutational analysis, the highest number of mutations were detected in the S protein while the E protein contained the lowest (Figure 3). The mutation rate of the Omicron variant's S protein was significantly higher than that of other variants.

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TABLE 1 Comparative view of different types of mutations in 12 variants of SARS-CoV-2.

Variant	Pango lineage	Total retrieved sequences	Total mutations	Common mutations	Unique mutations	Deleterious mutations (common)	Deleterious mutations (unique)
Alpha	B.1.1.7	1076	1102	648	454	145	96
Beta	B.1.351	686	451	279	172	3	42
Gamma	P.1	1350	1427	812	615	173	133
Delta	B.1.617.2	1884	4468	1513	2952	361	844
Epsilon	B.1.427 B.1.429	805	1002	604	398	139	92
Zeta	P.2	461	726	461	265	108	62
Eta	B.1.525	1468	811	504	307	124	64
Theta	P.3	133	161	109	52	23	14
Lota	B.1.526	305	438	291	147	59	30
Kappa	B.1.617.1	207	1218	871	347	209	114
Lambda	C.37	209	367	224	143	58	40
Omicron	B.1.1.529	1947	6307	2129	4178	563	1092

Amidst 876 mutations detected in the spike protein of the Omicron variant, 364 were common, 512 were unique, 412 were neutral, and 187 were deleterious. Further, the deleterious mutations included 187 common deleterious and 256 unique non-synonymous mutations. The Delta variant, on the other hand, had the second-highest number of mutational patterns, with 53 being unique deleterious mutations, whereas Kappa was found as the third-highest variant possessing 44 unique deleterious mutations. Conversely, the other variants such as Alpha, Gamma, Eta, Theta, Lota, Zeta, etc. had more neutral mutations in the S protein region than Omicron, Delta, and Kappa variants (Table 2).

3.2 Comparative analysis of the deleterious mutations

A deleterious mutation can be referred as a mutation for which the protein compound of a gene is not produced, or does not function, or interferes with normal function. Moreover, impairment of regulatory functions can be a possible outcome of deleterious regulatory agents. Accordingly, the functional impact of mutations was analyzed using PROVEAN (Protein Variation Effect Analyzer), which predicts the effect of amino acid (aa) substitution on the overall function of a protein. The lower scores for mutant (<−2.5) in PROVEAN, were marked as the most deleterious. In this study, the lowest score of Omicron was −11.248, found in the C3408S, while Delta showed the lowest score −12.869, was found in W55C. Interestingly, these three deleterious mutations were found in the ORF8 region of the SARS-CoV-2 genome. We further compared the mutation patterns between Omicron and Delta variants, which revealed that unique-deleterious mutations of these variants were significantly stable in the protein configuration (Table 3). By comparing all mutations of the SARS-CoV-2 variants, we found that mutations in the Omicron variants were more deleterious than the other 12 variants. The non-synonymous score of the Omicron variant was higher for most structural proteins and/or genes.

Further, The Omicron variant showed a higher degree of deleterious mutations in ORF1a, ORF1b, ORF7a, ORF 8, and ORF 9b (Table 2). According to PROVEAN, −2.5 was the threshold value for deleterious mutation. Therefore, mutations with a score less than −2.5 were considered deleterious. Consequently, our findings indicate a negative correlation between the severity of a deleterious mutation with its score.

By analyzing the common mutations among the 12 SARS-CoV-2 variants, our analysis found that the Omicron variant possessed the highest number of mutations in spike protein (Figures 4A, B), whereas the Delta variant possessed the lowest number (Table 3). While the Omicron variant showed an overall higher frequency of common mutations in N, ORF1a, ORF1b, ORF7, and ORF7b of the S protein, Alpha, Gamma, and Delta variants were exposed to numerous unique mutations (Figure 3A). Among these mutations, the Omicron variant had the highest number of unique mutations. Accordingly, the deleterious/non-synonymous mutation scores varied among variants (Figures 3B, D). A predominant score zone was observed for the Delta variant. The S protein of this variant (Omicron) was more virulent because of its immense unique-deleterious mutation score (Table 3).

3.3 B−cell epitope prediction

The probability of altered proteins becoming B−cell epitopes (up and down-regulation of B cell immunological response) for specific detrimental mutations in the Delta variant was visualized using the "ggplot2" package of the R statistical environment (Figures 3, 4; Figure 5). The threshold value used to define the region was set to 1.00. The upper portion of the threshold value can be referred to as antigenic regions that are most likely to be an epitope for B cells, whereas the lower portion showed non-antigenicity for B cells.

The deleterious mutations for which the antigenic response decreased were considered significant, and an increase in the

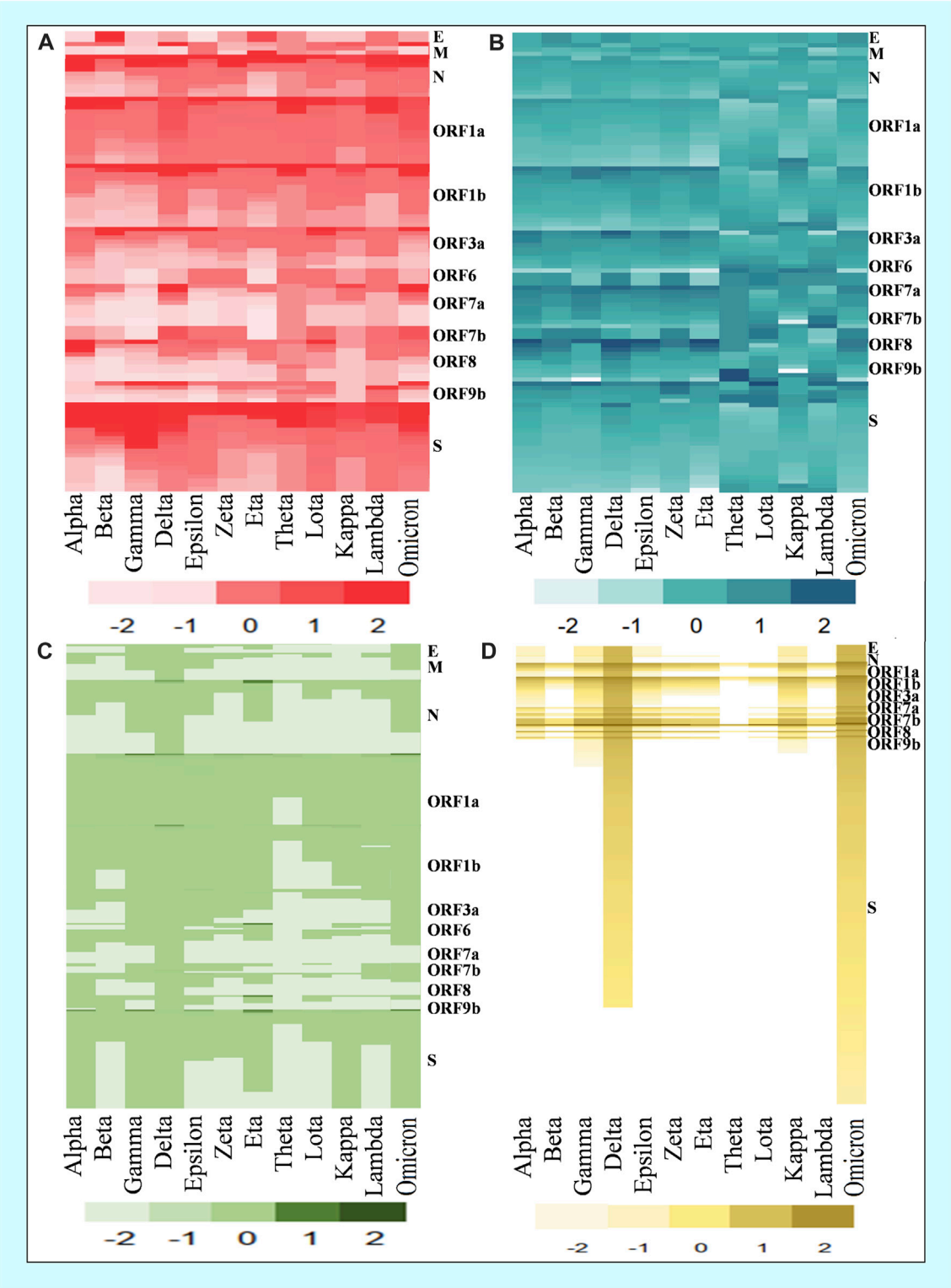

FIGURE 3
Comparison of common and unique deleterious mutation patterns in different variants. **(A)** Frequency of top common deleterious mutations of different proteins in twelve variants. **(B)** Deleterious mutation score of common mutations. **(C)** Frequency of top unique deleterious mutations of different proteins in twelve variants. **(D)** Deleterious mutation score of unique mutations.

antigenic response represented unchanged, neutral, or insignificant mutations. The mutations for which the B cell response rose significantly in the antigenic portions were extremely beneficial to the host immune system. By contrast, a decrease in the scores in the antigenic region indicates a threat to the host immune system.

TABLE 2 Comparison of mutations in the spike protein among 12variants of SARS-CoV-2.

Variant	Total mutation	Common mutation	Unique mutation	Neutral mutation	Deleterious mutation (common)	Deleterious mutation (unique)
Alpha	162	100	62	155	4	3
Beta	69	48	21	62	3	4
Gamma	215	119	96	200	8	7
Delta	678	326	352	560	65	53
Epsilon	125	92	33	118	5	2
Zeta	101	70	31	95	5	1
Eta	136	84	52	129	4	3
Theta	31	21	10	14	2	15
Lota	75	54	21	70	2	3
Kappa	421	295	126	316	61	44
Lambda	71	48	23	66	2	3
Omicron	876	364	512	412	187	256

3.3.1 Delta variant

3.3.1.1 ORF1a

Out of 666 deleterious mutations detected in ORF1a, 281 had lower antigenic score. To present these on the graph, we further predicted T Cell epitopes. Mutations that passed both analyses (n = 37 mutations) were presented in five segments featuring positions 2200–2648, 2700–3190, 3300–3500, 3700–4000, and 4000–4300 (Supplementary Figure S1). Among these mutations, L2218D and F2598N, L2948T, L3754G, F4034Q, L4234S, and V4242W (Supplementary Figure S1) appeared to have the greatest impact on lowering antigenic scores.

3.3.1.2 ORF1b

Among 262 deleterious mutations detected, 92 had a lower antigenic score for B cells. Based on the significance level in both B- and T Cell epitope prediction, the Y894M and L898D (Supplementary Figure S1) mutations were significant in both contexts.

3.3.1.3 ORF3a

Out of 47 deleterious mutations detected, 18 occurred in the B cell epitope region of the ORF3a.Among theseI35T and Y107H (Supplementary Figure S1) mutations were found to be significant in B- and T Cell epitope prediction.

3.3.1.4 ORF6

Of the 10 deleterious mutations detected in ORF6, I37T had a lower score.

3.3.1.5 ORF7a

Of the 30 deleterious mutations found in ORF7a, 13 significant mutations were found. Six of these were the most significant, including I4T, V24F, C58F, C67F, V74F, and V82A. The C67F mutation occurring in the antigenic region had a lower score than the threshold value, indicating the region was a non-antigenic portion. Among the most significant mutations, V82A decreased the likelihood of being a B cell epitope and had a higher mutation count of 1,831 (Supplementary Figure S1). Similarly, the I4T mutation remained significant in both contexts.

3.3.1.6 ORF7b

A total of eight deleterious mutations such as M1I, E3A, E3Q, L14S, F28Y, W29C, E39A, and C41F were analyzed (Supplementary Figure S1). Among these, L14S was the most significant mutation observed in the antigenic region, while C41F was found in the non-antigenic region. A positive antigenic response was detected due to six different mutations (M1I, E3A, E3Q, F28Y, W29C, and E39A) in ORF7b.

3.3.1.7 ORF8

Out of the 22 deleterious mutations detected, five were found to be significant. The C37F mutation occurred in the antigenic region with a mutation count of 38 and lowered the likelihood of binding to B cells (Supplementary Figure S1). Furthermore, the I10N, C102F, C102Y, and F120A mutations occurred in the antigenic portion and contributed to the negative antigenic response. However, none of the mutations dragged the antigenic region into a non-antigenic region.

3.3.1.8 ORF9b

Among the 22 deleterious mutations detected in ORF9b, five occurring in the antigenic regions may contribute to lowering the likelihood of binding to B cells. These five mutations (P3S, A11S, L14F, I45T, L52P, and V76F), along with others, are presented in Supplementary Figure S1. Among these, P3S and V76F attribute the antigenic region to be non-antigenic. However, L52P was significant in both B- and T cells.

3.3.1.9 Envelope protein

Three deleterious mutations were identified in the envelope protein. Among these, V58F was the most significant, as it decreased the score and lowered the immune response of B cells in

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TABLE 3 Comparison of the deleterious mutations between Omicron and Delta variants of SARS-CoV-2 in their different genes.

Gene	Omicron (O, o)		Delta (Δ, δ)		Gene	Omicron (O, o)		Delta (Δ, δ)	
	Position	Score	Position	Score		Position	Score	Position	Score
E	R61L	−1.733	R61L	-2.8	ORF1b	C1367T	-10.71	H962I	-10.31
N	Q306T	−3.878	W55C	-12.869		Y470S	-9.75	C928W	-10.31
	G164S	−2.918	G99S	-3.861		Y1944T	-9.397	C942F	-10.31
	N150T	−2.645	P162L	-3.655		C143T	-8.549	C939F	-10.26
ORF1a	C3408S	−11.248	C2989K	-11.183		G343C	-8.378	G670L	-9.667
	W3004Y	−11.153	W4096L	-11.111		R1600N	-8.654	G674I	-9.667
	G2815L	−11.044	C4370F	-10.248		D1815T	-8.43	G550L	-9.667
	P4211S	−10.398	W3481L	-10.121		V345E	-8.289	P618I	-9.667
	Y3364C	−10.051	C2445S	-9.618		L428V	-8	Y916G	-9.663
	P2870S	−9.499	C2851S	-9.319		M557H	-8.8	Y822G	-9.663
	Y2301C	−9.404	G3795L	-9.316		G1022T	-8.627	D675V	-8.7
	L2298S	−9.305	G3809L	-9.316		T267N	-7.533	N694F	-8.7
	P3015L	-9.21	G3372I	-9.316		M915S	-7.398	Y537A	-8.7
	Y4280H	-9.11	F2834P	-9.293		V344E	-6.965	Y665A	-8.7
	G3546T	−9.05	Y4013P	-9.216		P1645S	-6.729	D456I	-8.4
	S2493Y	−8.185	Y4013G	-9.183		T453A	-6.533	G328C	-8.3
	D2627C	−8.143	P3642I	-9.042		R712C	-6.366	Y737T	-8.229
	S3264D	−7.948	C2913H	-8.534		C2551S	-6.206	Y819A	-8.167
	S3195N	−7.901	G2987L	-8.531		L1088T	-5.751	G2436C	-8.138
	N1576D	−7.817	C3867I	-8.416		V1012N	-5.751	N619I	-8.043
	D1600C	−7.804	C2984A	-8.387		N2694T	-5.708	G662L	-8.033
	G2258Y	−7.746	Y3811A	-8.385		H1897S	-5.583	Y1759C	-7.875
	F3554T	−7.657	G4244F	-8.385		E720Y	-5.09	P821A	-7.731
ORF3a	W193T	−7.276	G187C	-7.248		E1623A	-5.026	Q923F	-7.731
	G188L	−6.6	G172S	-4.457		C688V	-5.003	P528S	-7.73
	T32E	-5.505	T221K	-4.276		S583T	-4.971	Y971L	-7.502
	T229T	-4.276	N257Y	-4.257		T984E	-4.958	G940T	-7.502
	S162K	-3.886	K235T	-3.276		V2501Y	-2.89	D295Y	-7.419
	K61L	-3.6	S58I	-3.276		G2510T	-2.877	D842A	-7.396
	I62T	-3.39	A103P	-3.229		L342R	-2.767	P970C	-7.278
	V256D	-2.886	I47T	-3.067	ORF9b	P39T	-8.478	L52P	-7
ORF7a	E41T	-6	Q57R	-2.629		R47E	-5	I45T	-5
	Y97E	-5.825	Q38K	-2.629		L64T	-4	V76F	-4.87
	K2Y	-5.684	M19T	-5.571		A29S	-3.304	L14F	-4
	H47I	-4.667	H3Y	-3.071		V15T	-3	N35S	-3.43
	R78Y	-4.333	C58F	-7.333	S	D1199Y	-10.528	C301R	-9.89
	T57H	-4.333	C67F	-7.333		N824S	-10.076	N919W	-9.35
	V82Y	-4.667	G42D	−7		F318R	-9.813	C301L	-7.9

(Continued on following page)

TABLE 3 (*Continued*) Comparison of the deleterious mutations between Omicron and Delta variants of SARS-CoV-2 in their different genes.

Gene	Omicron (O, o)		Delta (Δ, δ)		Gene	Omicron (O, o)		Delta (Δ, δ)	
ORF7b	F19S	−7	W29C	−13		G496S	-9.424	T478K	-7.062
ORF8	C90T	−10.389	I10N	−5.389		Q498R	-9.0665	L452R	-6.066
	C25V	−10.389	Q23H	−4.722		Y505H	-8.709	R158G	-5.071
	G96C	−6.611	F120A	−4.056		N501Y	−8.3515	T19R	-4.075
	T80Y	−4.667	P70L	−4.056		Q594H	−7.994	P681R	-3.08
	H40T	−3.556	R115C	−3.722		N764K	−7.6365	D950N	-2.084
	H40S	−3.167	G8V	−3		N856K	−7.279	E484A	-1.09
	G77T	−2.944	D119V	−2.917		L981F	−6.9215	E484K	-0.09

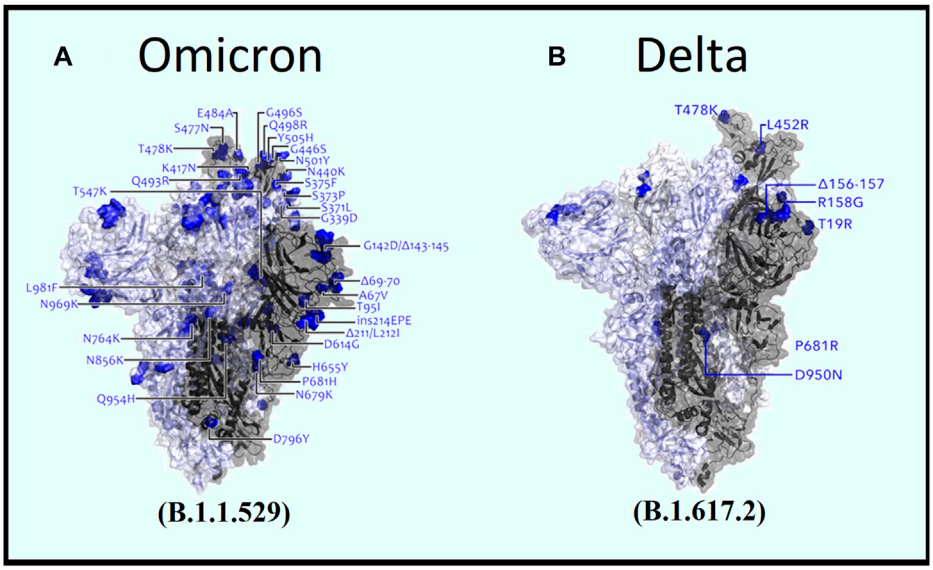

FIGURE 4
Comparative view of spike mutations within **(A)** Omicron and **(B)** Delta variants (Image source: Modified from COG-UK Mutation Explorer: http://sars2.cvr.gla.ac.uk/cog-uk).

the antigenic region (Supplementary Figure S1). The other two mutations were observed to increase the antigenic score, making the envelope more susceptible to the adaptive immune system (Supplementary Figure S1).

3.3.1.10 Membrane glycoprotein

Of the three deleterious mutations detected in membrane protein, mutations at residue position 82 were found to be significant, as they lowered the antigenic response of the antigenic portion. As observed, the replacement of isoleucine with threonine had a much greater effect than serine. In addition to this mutation, threonine had a higher mutational count of 1,864 (Supplementary Figure S1).

3.3.1.11 Nucleocapsid proteins

Deleterious mutations are presented in the context of B cell responses. Three mutations (e.g., K248M, R203M, and P168S) were highly significant out of 16 deleterious mutations found in the nucleocapsid. K248M and P168S mutations occurred in antigenic regions and contributed to lowering the immune response to non-antigenic regions, whereas R203M occurred in the non-antigenic portion with a high mutation count of 1,868 (Supplementary Figure S1).

3.3.1.12 Spike (S) protein

Significant mutations detected in Sprotein were divided into five sections (Figures 5A–E). Out of 116 mutations identified, 48 mutations were deleterious, occurring in the antigenic region and lowering the score. Thirteen mutations, including C301R (Figure 5A), Y423W, L533K, V539Y, V551Q (Figure 5B), C738N, L763W, V781D (Figure 5C), V915W, V915S, L916S, I923W, and A944R (Figure 5D) were found to drag the antigenic portion to a non-antigenic region of the spike protein. These mutations had the highest occurrence compared with other mutations. For example, V539Y, C738N, and L763W mutations were found to have occurred 16-times while A944R mutation was found 15-times, Y423W for 13-times, V915W, L916S, and I923W 12-times and V915S occurred 3-times.

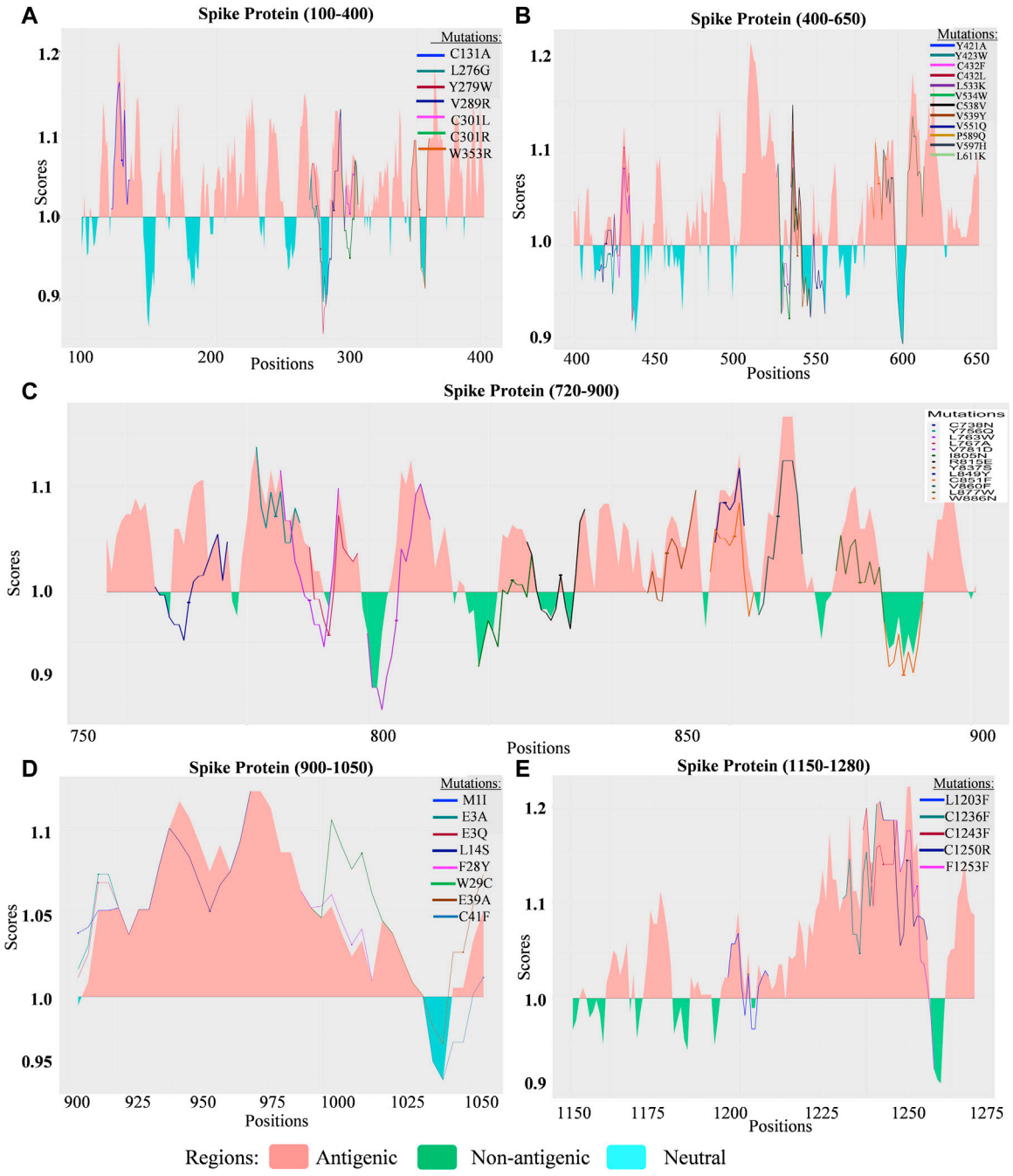

FIGURE 5
Comparison of the B cell epitope for spike (S) protein of SARS-CoV-2 Delta variant. **(A)** B cell epitope prediction score from 100 to 400 amino acids (aa) of the S protein. **(B)** B cell epitope prediction score from 400 to 650 aa of the S protein. **(C)** B cell epitope prediction score from 720 to 900 aa of S protein. **(D)** B cell epitope prediction score from 900 to 1050 aa of the S protein. **(E)** B cell epitope prediction score from 1150 to 1280 aa of the S protein.

3.3.2 Omicron variant

As mutational analysis and B cell epitope prediction on delta variant directs the most significance towards spike protein, the analysis narrowed down to explore mutations of omicron on spike protein too. The mutational analysis of the top 100 mutations found most deleterious are selected for further analysis (Supplementary Table S8). Among these A67V, G142D, N211I, L212V, G339D, S371L, S375F, G446S, S477N, T478K, Q493R, G496S, Y505H, D614G, N764K, Q954H, N969K, L981F mutations found to be occurring in antigenic regions of spike protein (Figure 6). Its is a noticeable result that along with the other mutations of delta variant, additional mutations G339D (Figure 6A), S477N (Figure 6B), Q493R (Figure 6B), and Y505H (Figure 6B), occurring in RBD region of spike protein, shows significance in the context of B cell epitope prediction.

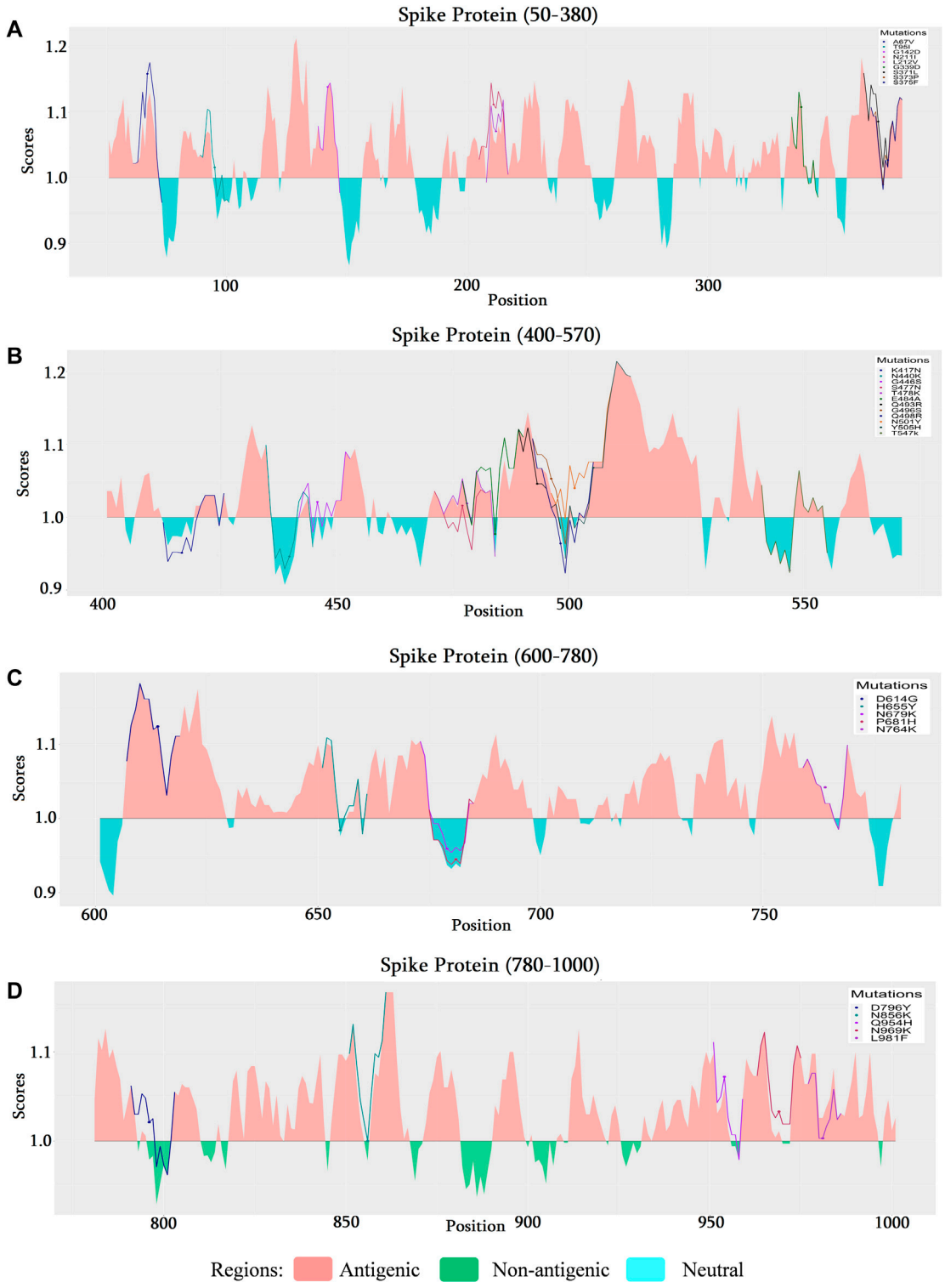

FIGURE 6
Comparison of the B cell epitope for spike (S) protein of SARS-CoV-2 omicron variant. **(A)** B cell epitope prediction score from 50 to 380 amino acids (aa) of the S protein. **(B)** B cell epitope prediction score from 400 to 570 aa of the S protein. **(C)** B cell epitope prediction score from 600–780 to 900 aa of S protein. **(D)** B cell epitope prediction score from 780 to 1000 aa of the S protein.

3.4 T Cell epitope prediction

CD4 T Cell immunogenicity prediction tool gives scores of segments from the peptides which can be identified as immunogenic based on a threshold value (50%). Most of the significant T Cell epitope mutation scores were predicted as less than 50 (Supplementary Table S1). An increment of the combined score for a mutant was taken into concern as it depicts susceptibility

towards survival into host immunogenic conditions. Envelope protein mutations V58F and R61L were observed to have a significant score (<50%) in the two epitopic segments (Supplementary Table S1). It was also predicted that the combined score increase is noticeable in the epitope region covering positions 51–65 (50% in this case). However, mutations exhibited a decreased score in the region 56–70, which indicated greater immunogenicity of T Cell epitopes. Among the 281 mutations that lowered B cell epitope potency, 50 mutations were found to significantly lower T Cell epitope potency by crossing the threshold. Therefore, the F2598N, L2948T, L3754G, F4034Q, L4234S, and V4242W mutations were found to be the most significant in both B- and T Cell epitope analyses (Supplementary Table S1).

Amidst the identified 92 mutations from B cell epitope prediction, 25 were found to lower the immune potency of T cells. while 11 mutations suppressed T Cell responses. The L898D and Y894M mutations were most significant for B- and T cells. For ORF3a, the I35T and Y107H mutations appeared to cross the threshold. ORF6 and ORF8 did not show many similarities to the expected result, suggesting that the mutations are safe for the host. For ORF7a, ORF7b, and ORF9b, the mutations I4T, L14S, and L52P showed the expected significance by crossing the threshold. In T Cell epitope prediction V911Y, V915S, V915W, L916S, A924Q, and I923W increased the combined score over the threshold values. However, V915W, V915S, L916S, and I923W were the most significant mutations in both analyses (Supplementary Table S1).

As, the analysis suggested, additional six mutations of omicron variant A67V, G142D, S371L, S373P, S375F, and L981F occur in the positions that were T Cell responsive in the wild variant. Among the six A67V, G142D, and L981F are seen (Supplementary Table S4) to induce the increase of combined score. L981F is the most important in this context as it crosses the threshold value indicating that the region is becoming less responsive towards CD4 T cells.

3.5 Effects of missense mutations on protein stability and protein-protein binding affinity

3.5.1 Delta variant

Physical characteristics of two mutations (V58F and R61L) of the envelope protein were collected from the COVID-3D database. However, both mutations were destabilizing, along with decreased molecular flexibility (Supplementary Table S1). Both I82S and I82T mutations of membrane protein, as well as P168S mutation of nucleocapsid protein, are destabilizing and contribute to increased molecular flexibility of spike protein (Supplementary Table S2). The I35T mutation had a more destabilizing effect on ORF3a than Y107H with increased molecular flexibility (Supplementary Table S2).

The I4T mutation in ORF7a, being destabilized and increased molecular flexibility, showed significance in B and T Cell epitope prediction too. The L14S mutation of ORF7b was also significant, as it was found to be a destabilizing mutation that increases molecular flexibility (Supplementary Table S2). A detailed graphic overview of the molecular interactions of these mutations is shown in Figure 7.

3.5.2 Omicron variant

Except for N440K, Q493R, and Q498R, all other 12 mutations (G339D, S371L, S373P, S375F, K417N, G446S, S477N, T478K, E484A, G496S, N501Y, Y505H) on receptor binding domain (RBD) were found as destabilizing indicating that these mutations are most likely to go through further several mutations (Supplementary Table S6). Among these, G339D (Figure 6A), S477N, and Q493R occurring in the B cell epitope region and Y505H occurring in the non-epitope region (Figure 6B) induce less likeliness towards B cell as suggested B cell epitope prediction section. These destabilizing mutations are of concern as further mutations on the same position could turn the protein less immunogenic and more specific toward the host receptors.

The increasing affinity of protein-protein binding (PPB) phenomena could be an issue to focus as this may enable the spike protein to bind host receptor with higher affinity. Therefore, in this context, G339D, N440K, S477N, Q498R, and N501Y (increase affinity in chain C) mutations could be used to explore in the future as each of these mutations contributes to increasing PPB affinity (Supplementary Table S5). It is a must to mention that both G339D (Figure 6A) and S477N (Figure 6B) also have significant respect for the B cell epitope prediction tool. A comparison of the molecular interaction of these mutations with the wild variant could ease the mind with a proper understanding of the impact of mutations on the protein-protein interaction (Figure 8).

3.6 Machine learning validation

Logistic regression, linear discriminant analysis, and artificial networks have similar kind of results aligned with each other in the context of deleterious and neural mutation perspectives (Table 4).

Whereas the result from the support vector machine classifier slightly differed from the result of rest of the models. However, all the models ensure at least 75% accuracy compared to the deleterious tool prediction and scenario. Therefore, in that context, it can be stated that machine learning models can validate the results at least 75% of cases and artificial neural network can validate the result up to 85% which is enough to maintain a balanced error-free mutational analysis (Table 5).

As it is evident from Tables 4, 5, the classifiers can reach, on average, 80.5% accuracy in the initial feature space and 74.5% sensitivity can be attained most of the time. For Logistic Regression, Linear Discriminant analysis the classification in transformed feature space leads to more accurate predictions. The significance level is undoubtedly confirmed over the test ($p < 0.0001$). Therefore, the deleterious mutations can be confirmed to a most significant percentage maintaining all the machine learning validation. The ROC curve estimated from the result section portraited a more transparent and visually vivid explanation with precise interpretation (Supplementary Figure S4).

4 Discussion

Since the first emergence of SARS-CoV-2 in late December 2019, multiple genomic variants have emerged, among which the Delta variant has been declared a 'variant of concern' (VOC) until November 2021 due to distinct characteristics (Araf et al., 2022; Mohapatra et al., 2022). The first Omicron variant (B. 1.1. 529) was identified on 09 November 2021 from a clinical sample that possessed comparatively higher mutations than other variants. The VOC was attributed to higher transmissibility with a severe disease course, decreased treatment efficacy, and many other concerning

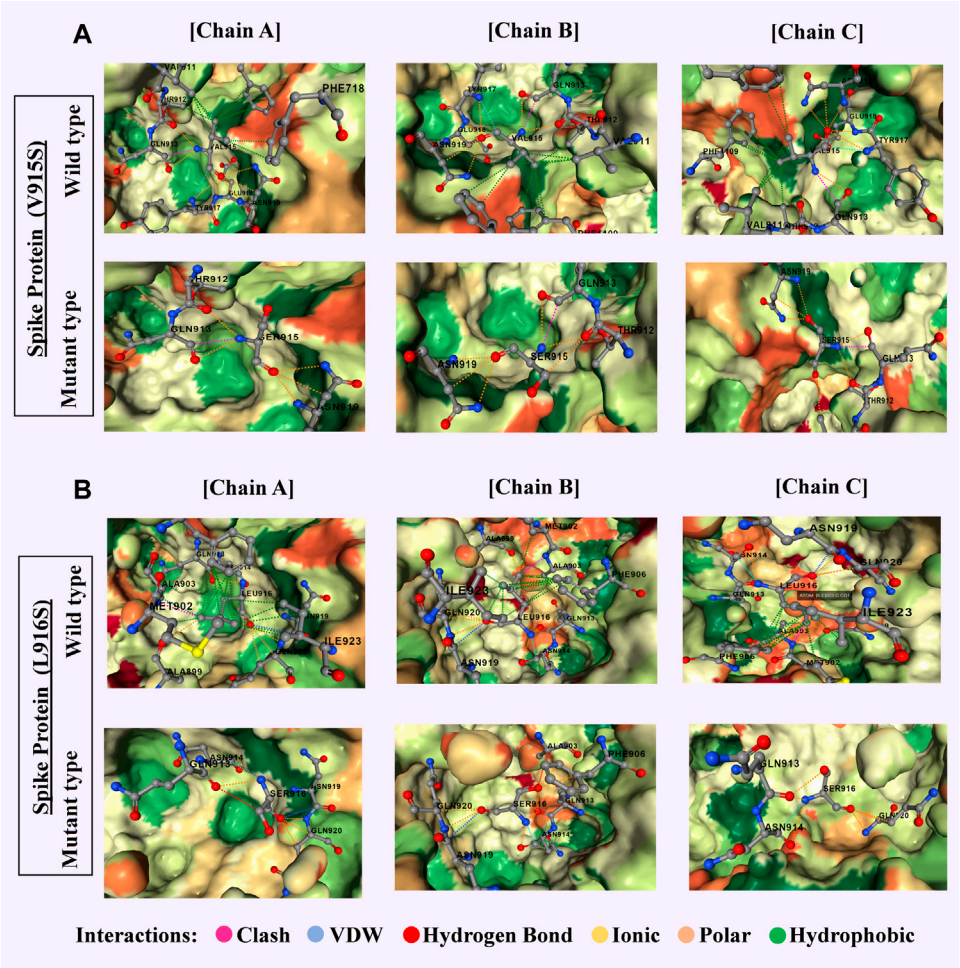

FIGURE 7
Most significant two mutations of spike proteins of delta variant with a comparative overview of molecular interaction **(A)** V915S-chain A-absence of 9 hydrophobic bonds, 2 polar bonds, and 1 Vander Waals bond in the mutein. Chain B- the absence of 9 hydrophobic bonds, 1 polar bond, and 1 Vander Waals bond in the mutein. Chain C- absence of 9 hydrophobic bonds, 1 polar bond, and 1 Vander Waals bond in the mutein. **(B)** L916S-chain A-the absence of 12 hydrophobic bonds in the mutein. Chain B- absence of 17 hydrophobic bonds, increase of 3 polar bonds and 1 hydrogen bond, decrease of clash in the mutein. Chain C- the absence of around 10 hydrophobic bonds, 1 carbonyl bond, and 1 clash in the mutein. Clashes are defined as unfavorable interactions where atoms are too close together.

features by the Centers for Illness Control and Prevention (CDC) (https://www.cdc.gov/coronavirus/2019-ncov/index.html). However, The World Health Organization designated the Omicron variantB. 1.1.529 as a VOC on 26 November 2021. A recent study reported that protection against the Omicron variant was only moderately conferred by the primary COVID-19 vaccination and prior SARS-CoV-2 infections (Chin et al., 2022). Although protection against Omicron infection was greatly enhanced by booster dosage, it gradually reduced with time (Andeweg et al., 2022). Mutation in the SARS-CoV-2 is a continuous process leading to multiple variant introductions. Though the latest SARS-CoV-2 variant's infectivity, prevalence, and severity are still unknown, investigations along with genomics analysis should be an ongoing process to get every detail to recommend efficient ways to prevent the upcoming surge (Araf et al., 2022).

New variants of SARS-CoV-2 had been observed during the pandemic. According to Majumdar and Niyogi. (2020), mutations in the ORF3a protein of SARS-CoV-2infectionis associated with a high mortality rate. Previous research has indicated that the D614G mutation of SARS-CoV-2 plays a role in the severity and mortality of COVID-19 patients, along with other factors, especially age and co-morbidity (Cong et al., 2020; Dutta et al., 2020; Grubaugh et al., 2020).

In this study, we filtered out 16,954 unique and common mutations from 10,531 sequences for 12 strains (Table 1). These unique mutations being present in a variant could be used to explain why that particular variant is much more transmissible than the previous one. For example, among 100 mutations of omicron in spike protein around 30 mutations were found to be unique (Supplementary Table S8) giving one of the clue that why this variant is more transmissible than delta. However, the presence of a destabilized common mutation in a new variant could be detrimental for future variants as these mutations tend to mutate over time. Based on these detrimental impacts, we characterized the mutations in neutral and deleterious sections. The mutations that is contributing to the detrimental impact on the host are therefore defined as

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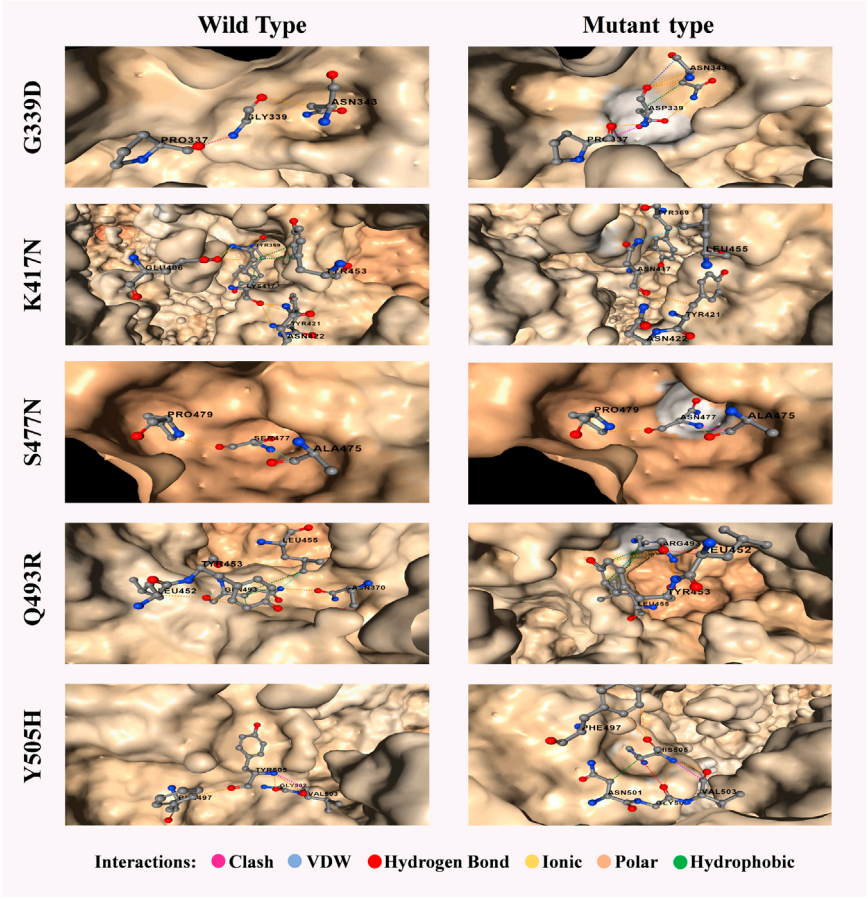

FIGURE 8
Most significant five mutations of spike proteins of the omicron variant with a comparative overview of molecular interaction. A wild variant of G339D has 1 clash and 2 polar bonds whereas the mutein has an additional 3 polar, 1 hydrophobic and 1 van der waals bonds. Accordingly, wild variant of K417N has 6 polar, 1 ionic and 5 hydrophobic bond whereas the mutein has an additional 2 polar and 1 van der waals bond but lacks ionic and hydrophobic bonds. Wild variant of S477N has 2 polar and 1 van der waals bond whereas the mutein lacks van der waals bond but has an additional 1 clash. Wild variant of Q493R has 6 polar, 1 van der waals, and 3 hydrophobic bond whereas the mutein lacks van der waals bond and 2 polar bonds but has an additional 1 hydrogen bond. Finally, Y505H has 3 polar and 1 clash which results in additional 1 polar, 1 hydrogen, 1 van der waals, and 1 hydrophobic bonds with the lackings of the clash. Clashes are defined as unfavorable interactions where atoms are too close together.

TABLE 4 Prediction of deleterious mutation depending on four classifiers: Logistic Regression, Linear Discriminant Analysis, Artificial Neural Network, and Support Vector Machine.

		True values							
		Logistic regression		Linear discriminant analysis		Artificial neural network		Support vector machine	
		*D	*N	*D	*N	*D	*N	*D	*N
Predicted Values	Deleterious	29	31	29	24	32	23	25	38
	Neutral	6	95	6	100	3	101	10	86

*D, Deleterious mutation *N, Neutral mutation.

deleterious and on the contrary, the other is defined as neutral having no significant impact on the host. All the deleterious mutations and their score predicted from some specific mutational analysis tools were further validated by four machine learning models: Logistic Regression, Linear Discriminant Analysis, Artificial Neural Network and Support Vector Machine (Tables 4, 5). At most 85% match score had been achieved by Artificial Neural Network while the lowest similarity scenario was achieved by a Support Vector Machine (Table 5). The significance level is confirmed over the test at its best ($p < 0.0001$).

TABLE 5 Comparative analysis of classification quality (accuracy) for classifiers trained without transformation (in initial feature space) and with transformation (in transformed space from the use of a neutral network) on mutational data.

	Accuracy	Kappa	Sensitivity	Specificity	Auroc	Mcnemar's test *p*-value
LR	0.8151	0.0694	0.8286	0.6984	0.5635	<0.001
LDA	0.8214	0.0742	0.8286	0.8065	0.5675	<0.001
ANN	0.8559	0.0498	0.9143	0.7855	0.5499	<0.001
SVM	0.7597	0.1134	0.4286	0.7968	0.7513	<0.001

Analysis of the unique mutation frequency of each variant indicated that the Omicron and Delta variants showed more frequent deleterious mutations. Surface glycoproteins also showed the notable feature of a unique mutation. Overall, the Omicron variant covered 512 unique mutations in its S protein, whereas Delta, Alpha, Gamma, and Kappa variants covered 352, 62, 96, and 126 unique mutations, respectively (Table 2).

The Omicron variant changes the structural pattern of the spike protein more rapidly than expected (Figure 8), which can be well explained by its rapidly transmissible nature. Various studies have focused on mutations in spike proteins (Duan et al., 2020; Huang et al., 2020; Xie et al., 2020). Therefore, mutations that occur in the surface glycoprotein may be of concern (Islam et al., 2020).

In addition, the neutral and deleterious mutation scores (Supplementary Table S7) of different variants were analyzed. Neutral or synonymous mutations were observed among all variants (Lucas et al., 2008; Shah et al., 2020) in their spike proteins (Table 2). The highest number of neutral mutations was observed in the Omicron variant, whereas in Alpha, Gamma, Epsilon, Eta, and Kappa variants, 155, 200, 118, 129, and 316 neutral mutations, respectively (Table 1). Accordingly, more deleterious mutations were observed in the spike protein, indicating the attempts of the spike protein to break the immune defense system of the host (Table 2).

In this study, we took a computational approach to analyze deleterious mutations among common and unique mutations in the variants. Accordingly, we have compared all the mutations in different variants by some specific markers (deleterious score, average deleterious score, average deleterious mutations per sequence of different variants, and mutations severely affecting the stability of the protein). After considering all of the factors it was estimated that the degree of deleterious mutations was far higher in the Omicron variant compared to other variants. While Omicron variants showed the highest mutation score pattern for both common and unique mutations, the Delta variant showed the second-highest pattern. A comparative analysis of the top 184 unique-deleterious mutations in Omicron revealed it as one of the most transmissible variants (Table 3), containing more deleterious mutations than that of Delta. The Omicron variant showed a high degree of deleterious mutations in S, ORF1a, ORF7a, ORF 8, and ORF 9b. This result indicated that the effective and non-synonymous mutations scaled up the Omicron variant to be more virulent and transmissible. The Delta variant, originating in India had already claimed numerous lives in the previous year, while the Omicron variant had been shown to infect more included as hospital cases (Sigal et al., 2022).

The spike protein of the Omicron variant showed a high frequency of deleterious mutations (Table 3). The deleterious mutation in this protein was cross-checked using four tools (Predict SNP, SIFT, PolyPhen2, and PROVEAN). With a high deleterious score, the surface glycoprotein of the Omicron variant confirmed its more virulent and transmissible nature. The heatmap of unique mutation scores of different proteins within different variants indicated that the Omicron variant had the highest possible deleterious mutation in its spike protein compared with the other variants (Figure 3). Moreover, other protein portions of the Omicron variant showed more non-synonymous mutations responsible for protein function alteration and immune escape mechanisms (Figure 8 and Supplementary Table S4).

From these observations, we can conclude that mutations of the omicron variant in the spike protein have the greatest impact. V915S and L916S are both destabilizing mutations that occur with a serine residue. Both have been shown to lower the likelihood of binding with B- (Figure 5D) and T cells (Supplementary Table S1), increase molecular flexibility, and decrease affinity (Supplementary Table S3). In ORF3a (I35T, Y107H), both mutations are of concern considering B and T cells (Supplementary Figure S1), although I35T is the most destabilized with increasing molecular flexibility (Supplementary Table S2). ORF7a (I4T), ORF7b (L14S), and ORF9b (L52P) proteins have one destabilizing mutation (Supplementary Table S2) that increases the likelihood of binding with B (Supplementary Figure S1) and T cells (Supplementary Table S1) and also increases molecular flexibility (Supplementary Table S2). ORF1a and ORF1b proteins give concerned mutation lists considering both B- (Supplementary Figure S1) and T Cell (Supplementary Table S2) epitope prediction. Mutations in membrane protein (I82T), nucleocapsid protein (K248M and P168S), ORF6 (I37T), and ORF8 (C37F, I10N, C102F, C102Y, and F120A) were significant for lowering B cell epitope potency (Supplementary Figure S1). Mutations in the envelope (V58F) were also significant for B cell epitope prediction (Supplementary Figure S1) while providing mixed results for T Cell epitope prediction (increase in score in one peptide but decrease in other peptide portion) (Supplementary Table S2) Asghar et al., 2022. These results from our study corroborated the results of many of the previous research.

In comparison, 10 mutations (W353R, Y421A, Y423W, C432F, C432L, L533K, V534W, C538V, V539Y) were found to be significant in RBD region ranging from 319 to 541 (Kumar et al., 2022) of the spike protein of delta variant in context of B cell epitope prediction (Figures 5A, B) whereas in omicron variant's spike protein additional four mutations (G339D, S477N, Q493R, and Y505H) (Figures 6A, B) were enlisted confirming the significance level of omicron spreading.

Although the T Cell epitope prediction context does not give much of a clue about the deleterious effects of mutations on the

RBD region, both variant stability, and protein-protein binding affinity gives some interesting aspect. As seen for the omicron variant most destructive mutations that affect the protein stability (Supplementary Table S6) reside between the residues 339–505 featuring mutations G339D, S371L, S373P, S375F, K417N, G446S, S477N, T478K, E484A, G496S, N501Y, Y505H, each of which are in RBD. But in the case of the delta, these effects were observed by mutations V911Y, V915S, V915W, V916S, I923W, and A924Q which is in the heptad region (HR1) and ranged from 902 to 952 (Xu et al., 2004). For protein-protein binding affinity, the most significant value is also observed between 911–924 residues in the case of the delta variant (Supplementary Table S3) and 339–505 residues for the omicron variant (Supplementary Table S5).

While several studies have shown that the Alpha variant is associated with an overall higher case fatality rate than the original lineages (Brainard et al., 2022), only a few studies indicated Delta and Omicron variants (Challen et al., 2021; Nyberg et al., 2022). In a cohort study in the Netherlands, van Gils et al. (2022) reported that vaccines were less neutralized by the Omicron variant even after booster dosing. According to Zhang et al. (2022) two-dose mRNA-1273 and BNT162b2 vaccines were able to neutralize against Alpha, Beta, Gamma, and Delta variants but after 6 months, antibodies declined for BNT162b2. Both the two vaccines, mRNA-1273 and BNT162b2 were found weakly active against Omicron for neutralizing antibodies.

Despite few antiviral drugs and vaccines available on the market to control the spread of infections, new SARS-CoV-2 variants continue to emerge, making it difficult to combat without complete immunization (M. Hossain et al., 2021; Sakib et al., 2021). The heatmap in our study (Figure 3) revealed that the Omicron variant had more common mutations in comparison with other variants, where most of the common mutations of the Omicron variant are deleterious (Table 1), causing alterations in protein function. We analyzed the association of host immune response with each deleterious mutation of the Omicron variant (Figure 6) as it is a variant of concern and revealed important outcomes. The deleterious mutations of the Omicron variant were cross-checked and the B cell responses to the different deleterious mutations of different proteins were analyzed (Figure 6). Most of the deleterious mutations were responsible for the downregulation of the immune response, especially the mutations in the ORF1a, ORF3a, ORF7a, ORF8, and ORF9b regions (Supplementary Figure S1; Supplementary Table S1). The spike protein had the greatest impact on suppressing the host adaptive immune response (Figures 5, 6; Supplementary Tables S1, S4). This indicates a comparative graphical analysis of the special immunosuppressive capability of all proteins, especially within the spike protein for the Omicron variant. The specific mutations responsible for the highest immunosuppressive nature were further cross-checked with their respective mutation frequencies suggesting they occurred in a repeated manner which makes them too virulent to challenge humanity for their existence.

5 Conclusion

The presence of unique and common deleterious mutations in delta and omicron suggests the reason for their aggressive virulence nature which has compelled the human being to face an epidemic in this modern era. Observing all the mutations of both delta and omicron variants, our study concludes that among all proteins spike protein is the most significant for both delta and omicron variants. In the delta, the significance has been drawn towards the heptad region as the most significant mutations are V911Y, V915S, V915W, L916S, I923W, and A924Q. Whereas, for omicron, the attention moves towards mutations G339D, K417N, S477N, Q493R, and Y505H which reflects the RBD region. This explains that although the delta variant is more deadly, omicron has its obvious ability to spread faster.

6 Future prospects

The prediction of the epitope of B cells and T cells does not wholly ensure the clinical conditions as it's based on a computational method. It's a guide for researchers to study specific mutations. Further study could be explored on the mutations to determine the effect on the epitopic region. The changes in molecular interactions are needed to study further to find out the chemistry of the mutein that increases the sustainability of an emergence variant.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

MI conceived the idea and designed the experiments. AM and SS performed all the experiments including data acquisition, analysis, and interpretation. MI wrote the initial draft of the manuscript. MH critically interpreted the results and edited the manuscript. All authors read the manuscript and approved the final version.

Funding

This study was supported by President Abdul Hamid Medical College, Noakhali Science and Technology University, Bangladesh, and KTH Royal Institute of Technology, Sweden.

Acknowledgments

This SARS-CoV-2 whole genome sequences data-based research article has taken all of the sequences from an open data source GISAID. We gratefully acknowledge all of the authors and their laboratories responsible for obtaining the specimens and submitting laboratories for generating the genetic sequence and metadata and sharing via the GISAID Initiative (https://gisaid.org/). We added a supplementary table indicating all of the SARS-cOV-2 sequences used in this manuscript (Supplementary Table S9). We are also thankful to all three reviewers and editors for their suggestions and comments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1090717/full#supplementary-material

SUPPLEMENTARY TABLE S1
T cell epitope prediction score for all the mutations of different proteins of SARS CoV-2 genome (Delta Variant).

SUPPLEMENTARY TABLE S2
Effects of missense mutations on different deleterious mutations of several proteins (Delta Variant).

SUPPLEMENTARY TABLE S3
Effects of missense mutations on different deleterious mutations of Spike Proteins (Delta Variant).

SUPPLEMENTARY TABLE S4
T cell epitope prediction score for all the mutations of different proteins of the SARS CoV-2 genome (Omicron Variant).

SUPPLEMENTARY TABLE S5
Effects of missense mutations on protein-protein binding affinity.

SUPPLEMENTARY TABLE S6
Effects of missense mutations on protein stability.

SUPPLEMENTARY TABLE S7
Learning (left) and Testing (right) dataset for machine learning validation.

SUPPLEMENTARY TABLE S8
All neutral and deleterious mutations for Delta and Omicron variant.

SUPPLEMENTARY TABLE S9
All of the SARS-CoV-2 Whole Genome Sequences list.

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frontiers | Frontiers in Pharmacology

TYPE Data Report
PUBLISHED 10 February 2023
DOI 10.3389/fphar.2023.1101894

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 18 November 2022
ACCEPTED 24 January 2023
PUBLISHED 10 February 2023

CITATION
Branda F, Mahal A, Maruotti A, Pierini M and Mazzoli S (2023), The challenges of open data for future epidemic preparedness: The experience of the 2022 Ebolavirus outbreak in Uganda.
Front. Pharmacol. 14:1101894.
doi: 10.3389/fphar.2023.1101894

The challenges of open data for future epidemic preparedness: The experience of the 2022 Ebolavirus outbreak in Uganda

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On 20 September 2022, the Ministry of Health in Uganda, together with the World Health Organization—Regional Office for Africa (WHO AFRO) confirmed an outbreak of EVD due to Sudan ebolavirus in Mubende District, after one fatal case was confirmed. Real-time information are needed to provide crucial information to understand transmissibility, risk of geographical spread, routes of transmission, risk factors of infection, and provide the basis for epidemiological modelling that can inform response and containment planning to reduce the burden of disease. We made an effort to build a centralized repository of the Ebola virus cases from verified sources, providing information on dates of symptom onset, locations (aggregated to the district level), and when available, the gender and status of hospitals, reporting bed capacity and isolation unit occupancy rate according to the severity status of the patient. The proposed data repository provides researchers and policymakers timely, complete, and easy-accessible data to monitor the most recent trends of the Ebola outbreak in Ugandan districts with informative graphical outputs. This favors a rapid global response to the disease, enabling governments to prioritize and adjust their decisions quickly and effectively in response to the rapidly evolving emergency, with a solid data basis.

KEYWORDS

Uganda, viral infections, Ebola virus, infection control, outbreaks, surveillance, epidemiology

1 Introduction

During the emergence of a novel pandemic, real-world data (RWD) are fundamental for informing public health policy decisions and improving clinical trials. In particular, in the early stages, there is a need to gain fundamental knowledge about the epidemiological characteristics of a new infection, from transmission potential to natural history (Branda et al., 2022a; Branda et al., 2022b). As outbreaks grow, there is a need to predict disease dynamics, estimate potential burden, and evaluate interventions (Branda et al., 2023). In the next steps, attention turns to estimating vaccine efficacy and monitoring outbreaks and evolutionary dynamics (Branda et al., 2020).

Although the African regions face recurrent epidemics and other health emergencies every year, the capacity to implement and analyze complex surveys tends to be limited as funding for data collection competes with other pressing needs. In particular, fragility, conflict and violence

(FCV) affect data collection in many ways. For example, data collection during conflicts is affected by poor roads, inadequate telecommunications infrastructure, and sometimes populations hostile to central government representatives that provide few essential public services. In other cases, risks in FCV countries are often high due to disease. In Somalia, for example, it was not possible to conduct a traditional household consumption survey, with interviews lasting several hours, because of the level of insecurity and the danger interviewers faced if they spent more than an hour with a household. During the Ebola crisis, interviewers could not travel and collect information from respondents with face-to-face interviews because of the risk of infection.

The rapid outbreak sequencing of Ebola virus in 2022 demonstrated that the resurgence of Sudan virus disease (SVD) is a major public health concern in Uganda. On 20 September 2022, Ugandan health authorities declared an outbreak of Ebola disease, caused by Sudan virus, following the confirmation of a fatal case in a young male resident of Ngabano village of Madudu sub-county in Mubende district (World Health Organization, 2022). On 11 January 2023, after 42 days with no new cases, the outbreak was declared over. A total of 164 cases (142 confirmed, 22 probable) and 77 deaths (55 among confirmed cases and 22 among probable cases) were reported from September 20 to 10 January 2023. Uganda has reported in its history four SVD outbreaks in 2000, 2011 and two in 2012, before the last one in 2022. It is therefore likely that filoviruses are present in the reservoir of wild animals in the region. Therefore, the risk of re-emergence of any filovirus through exposure to an animal host or from a persistent virus cannot be ruled out. More details on Ebola virus are given in the Appendix section.

As we have seen with COVID-19, a critical component of a coordinated response is the rapid sharing of research results and data. Although we are fortunate that the Ebola virus has been well studied and that countermeasures exist to prevent and treat the disease, it is an evolving situation and there is still much to learn in order to anticipate the epidemic. According to a publication by the Johns Hopkins Center for Health Security, the African continent is the least prepared to respond to health emergencies, treat the sick and protect health workers (Johns Hopkins Center for Health Security, 2022) and has the lowest capacity to provide critical and intensive care in the world (World Economic Forum, 2022). The weakness of the health system and the high prevalence of malnutrition, malaria, HIV/AIDS and tuberculosis pose additional challenges. Therefore, strengthening surveillance capacity (Hoogeveen and Pape, 2020) can help detect future outbreaks, preventing their further spread. Our study describes a real-time database that we created to support epidemiological understanding of the origins and transmission dynamics of the Ebola epidemic in Uganda in 2022 and highlights the importance of having open data to quickly plan effective control measures should this epidemic grow further in the future.

2 Methods

To support global response efforts, we build an epidemiological surveillance for Ebola continuously and systematically collects, compares and analyzes information on all cases of EVD infection reported by the World Health Organization - Regional Office for Africa (WHO AFRO) (World Health Organization Uganda, 2022). Updates are not always available on a daily basis because there is a lag between the date of disease onset, the date of detection, and the date of reporting, resulting in a delay in reporting. Delays in reporting have the potential to distort the incidence curve of the epidemic, and in turn, estimates of transmission potential, forecasts of the outbreak

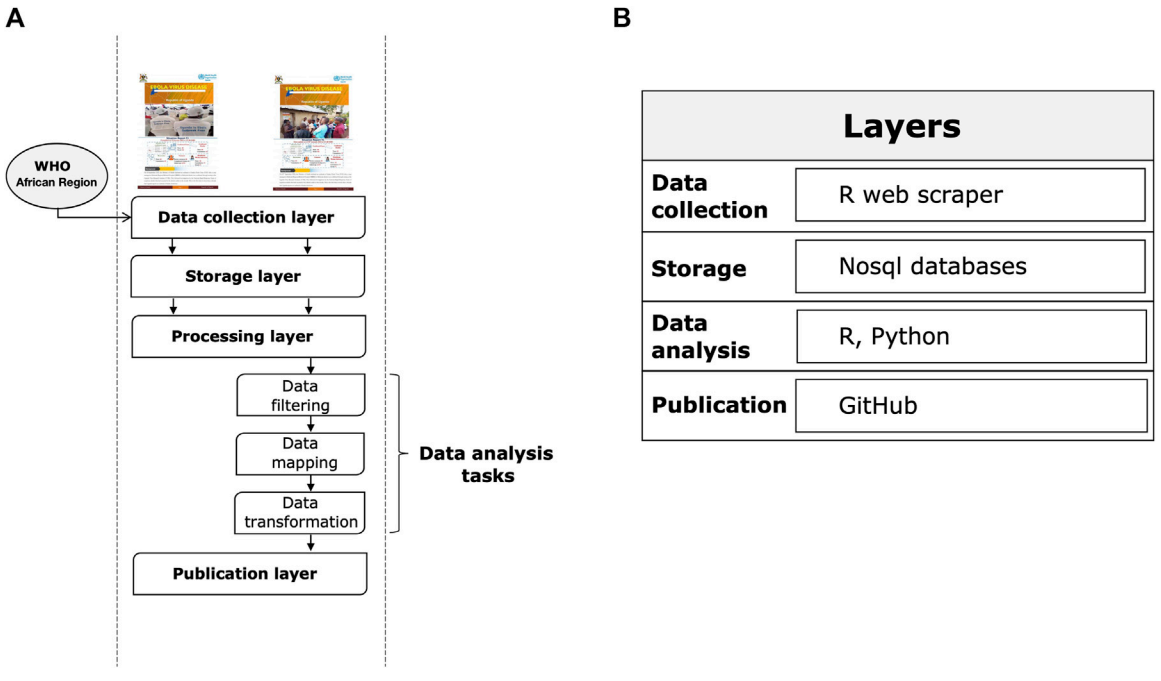

FIGURE 1
Layers of the Ebola information management system. **(A)** System execution flow. **(B)** Reference architecture.

TABLE 1 Database specifications.

Subject	Public health and health policy
Specific subject area	Infectious diseases and virology
Data accessibility	Public repository: GitHub (https://github.com/)
	Repository name: ebola
	Direct URL to data: https://github.com/fbranda/ebola
	License: CC-BY-4.0
Files and fields	1) Surveillance_data_Ebola_outbreak.csv
	• Date as of: Case reporting date
	• ConfCases: Daily number of new confirmed cases
	• CumCases: Cumulative number of confirmed cases
	• ConfDeaths: Daily number of new confirmed deaths
	• CumDeaths: Cumulative number of confirmed deaths
	• ConfRecoveries: Daily number of new confirmed recoveries
	• CumRecoveries: Cumulative number of confirmed recoveries
	• ConfHCWcases: Daily number of new confirmed cases of healthcare workers
	• CumHCWCases: Cumulative number of confirmed cases of healthcare workers
	• ConfHCWDeaths: Daily number of new confirmed deaths of healthcare workers
	• CumHCWDeaths: Cumulative number of confirmed deaths of healthcare workers
	2) Surveillance_data_Ebola_outbreak_by_district.csv
	• Date as of: Case reporting date
	• District: District name
	• ConfCases: Daily number of new confirmed cases
	• CumCases: Cumulative number of confirmed cases
	• ConfDeaths: Daily number of new confirmed deaths
	• CumDeaths: Cumulative number of confirmed deaths
	• ConfRecoveries: Daily number of new confirmed recoveries
	• CumRecoveries: Cumulative number of confirmed recoveries
	• ConfHCWcases: Daily number of new confirmed cases of healthcare workers
	• CumHCWCases: Cumulative number of confirmed cases of healthcare workers
	• ConfHCWDeaths: Daily number of new confirmed deaths of healthcare workers
	• CumHCWDeaths: Cumulative number of confirmed deaths of healthcare workers
	3) Surveillance_data_Ebola_outbreak_by_subcounty.csv
	• Date as of: Case reporting date
	• District: District name
	• SubCounty: Subcounty name
	• CumCases: Cumulative number of confirmed cases
	• CumDeaths: Cumulative number of confirmed deaths
	4) Surveillance_hospital_data_Ebola_outbreak.csv
	• Date as of: Case reporting date
	• Hospital: Hospital name

(Continued on following page)

TABLE 1 (*Continued*) Database specifications.

Subject	Public health and health policy
	•# of beds in the Isolation Unit: Cumulative number of beds occupied in the Isolation Unit (IU)
	•# of ETU beds: Cumulative number of beds occupied in the Ebola Treatment Units (ETU)
	•# of beds occupied in the Isolation Unit today: Daily number of beds occupied in the IU
	•# of beds occupied in the ETU today: Daily number of beds occupied in the ETU
	•# of suspect cases admitted to the Isolation Unit today: Daily number of suspect cases in the IU
	•# of Cases admitted to the ETU today: Daily number of cases in the ETU
	•# of walk in patients to the isolation Unit: Cumulative number of walk patients in the IU
	•# of Mild cases in the ETU today: Daily number of mild cases in the ETU
	•# of Critical cases in the ETU today: Daily number of critical cases in the ET
	•# of patients discharged from the ETU: Cumulative number of patients discharged from the ETU
	•# of patients discharged from the Isolation Unit: Number of patients discharged from the IU
	•# of suspect cases that died in the Isolation Unit: Number of suspect cases that died in the ETU
	•# of patients that died in the ETU: Number of patients that died in the ETU
	5) epicurve_by_notification_sex.csv
	• Date as of: Case reporting date
	• Sex: Sex of reported cases
	• ConfCases: Daily number of new confirmed cases
	• CumCases: Cumulative number of confirmed cases
	6) epicurve_by_onset_date.csv
	• Date as of: Case reporting date
	• Type of case: Type of case reported (confirmed/probable)
	• ConfCases: Daily number of new confirmed cases
	• CumCases: Cumulative number of confirmed cases

trajectory, and the impact of control interventions (Kelly-Hope, 2008; Reijn et al., 2011). In the context of Ebola, factors influencing reporting delays include i) difficulties in tracing and monitoring contacts for rapid case isolation, ii) deliberate attacks on healthcare workers and suspension of healthcare outreach, iii) resistance of sick individuals to seek medical care as soon as the symptoms start and iv) population displacements (Shearer, 2018).

The system consists of the steps described below (see Figure 1A): i) a *data collection layer* that collects shared data from verified sources, including reports from governments and public health organizations and statements from health officials reported in the media; ii) a *storage layer* that facilitates the storage and organization of data in an easily identifiable structure; iii) a *processing layer* that efficiently transforms, combines, and organizes data; iv) a *publication layer* that appropriately provides data and information to end users that they can use as a basis for epidemiological modeling to accelerate scientific discovery and response to the Ebola outbreak.

Figure 1B summarizes the main tools used for each step. The main types of data we collected using an automated web scraping in R: a) key dates, which include the date of laboratory confirmed cases, including infections among healthcare workers; b) demographic information about the sex of patients/cases; c) geographic information, at the highest resolution available down to the district level; d) any additional information such as the status of hospitals, i.e., the bed capacity and occupancy rate of isolation units according to the severity status of the patient. Note that point b) and d) are not always shared in public official reports. For the rapid evolution of the epidemic and a data pattern not defined *a priori* given the dynamic context, we have chosen to adopt a No-SQL approach for data storage. Data processing was conducted using several programming languages, including R and Python. Specifically, data engineering activities, such as resolving inconsistencies in text formats through conversion, string matching and manipulation, merging files, reorganizing folders, and maintaining archives and folder locations that contained the latest version of official reports, were performed using R packages. These activities were programmed to operate semi-automatically and required human supervision to monitor and perform quality checks. All processed data were analyzed daily by a dedicated team of epidemiologists, data scientists, and statistical experts through Python scripts. Data analysis focused primarily on trends, geo-spatial distribution, and epidemiological characterization of cases by disease severity and sex. Other types of analysis performed included risk profiling of Ugandan districts by outbreak intensity.

Finally, Ebola data were published through a GitHub repository (https://github.com/fbranda/ebola).

3 Data description

Table 1 provides a short description of the database. In addition, the README file of the GitHub repository reports code snippets that can be used by a user to import such data into a variety of software programs.

4 Usage notes

These data can be used to investigate the origins and transmission dynamics of the 2022 Uganda Ebola outbreak. This includes the estimation of key epidemiological parameters such as the incubation period and serial interval using mathematical models. Such models could be adapted to monitor the Ebola epidemic in other African regions, or for future outbreaks. In Supplementary material, we show a preliminary view of the collected epidemiological data and how they can be useful for direct visual assessment of the geographic distribution of risk areas as well as insights on the evolution of the outbreak over time. The data are openly available, and we will continue to curate the database as new information is made available.

While every effort has been made to standardize the data collected, some limitations must be recognized. The first is that although the data have been checked periodically wherever possible, conversion errors may occur when extracting data from the parent pdfs in machine-readable format. We have provided the sources consulted (i.e., the *Bulletins* folder in the GitHub repository) so that users can do further verification. There are then possible changes in reporting during the outbreak. For example, we found that demographic information or the status of hospitals reported initially were subsequently no longer made public. Although we have made every effort to report data as accurately as possible, given the dynamic nature of the outbreak, we caution that the database cannot be guaranteed to be error-free, and we apologize in advance if there are missing entries that were not detected using our standardized protocol. We invite database users to contact us directly if potential errors or omissions have been found. You can do so by emailing the corresponding authors or, preferably, by submitting a request *via* the Github repository.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://github.com/fbranda/ebola.

Author contributions

FB: Conceptualization; Data Curation; Resources; Visualization; Writing–original draft; Writing–review and editing. AM: Writing–review and editing. SM: Investigation; Supervision; Validation; Writing–original draft; Writing–review and editing. AM: Investigation; Validation; Writing–review and editing. MP: Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1101894/full#supplementary-material

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Appendix: Ebola virus disease

Ebola virus (EBOV) is a *Filovirus* involved in hemorrhagic, rare, high fatality rates and lack of effective treatment or vaccines, outbreaks in Sub-Saharan Africa. It recognizes probably in fruit bats Pteropodidae the reservoir animals, with spillovers in humans and primate apes (Taylor et al., 2010). Although there is evidence of wild mammals infected, the biology of host-filovirus interactions is not jet well understood (Emanuel et al., 2018), and it appears difficult to identify potential reservoir species with an expected long-term co-evolutionary history. The existence of filovirus-like elements, recorded as paleo viral, among mammalian genera, whose divergence dates have been estimated, suggests that filoviruses are at least tens of millions of years old (Emanuel et al., 2018), showing the possible co-existance of these viruses with humans and mammals from the beginning of their presence on Earth. Emerging hemorrhagic diseases has made the search for reservoir species a priority (Emanuel et al., 2018), seen the very high deaths rates: in some cases, the mortality in primates was so severe as to raise potentiality for extinction (Walsh et al., 2003).

Filovirus outbreaks are a known risk in Africa, with the first human case in 1976 (CDC. History, 2022) near the River Ebola in an area now known as the Democratic Republic of the Congo. Several outbreaks have been observed in recent years in other African countries. Here, we focus on the multiple outbreaks in Uganda where species of EBOV were observed over the last 20 years: i) *Sudan ebolavirus* (2000-2001, 2011, 2012, 2012/2013, 2022); ii) *Bundibugyo ebolavirus* (2007-2008); and iii) *Zaire ebolavirus* (2018-2020) which was imported from the Democratic Republic of the Congo (CDC. History, 2022).

This last 2022 EVD outbreak in Uganda is sustained by *Sudan ebolavirus;* no safe nor protective vaccine exists for this viral species. ERVEBO Vaccine, FDA approved, is protective only against *Zaire ebolavirus* species (CDC. History, 2022). Blood, secretions, organs, or other bodily fluids of dead or living infected people or animals contact are the dominant mode of transmission, but there is increasing evidence that different routes of transmission, including blood-borne, vertical, sexual, and aerosol transmission, can be impacting (MacIntyre and Chughtai, 2016). In recent years, a new paradigm of outbreaks has been suggested. It has been discovered that Ebola virus can be latent and persistent in infected persons and animals, with recovery of viral particles in human semen (EBOV RNA semen positive rate of 75.4% at 6 months from infection) (Thorson et al., 2021) and breast milk from women without previous infection (Sissoko et al., 2017). EBOV can reactivate in previous outbreaks survivors, also after long periods of time (Garry, 2022). This has been the starting event in recent Ebola virus Zaire species outbreaks in 2021 in Guinea [(Keita et al., 2021)]. Suspected are small unrecognized chains of human-to-human transmission are believed to sustain the constant viral presence in the population in Guinea. This outbreak was not due to a new spillover from an animal reservoir but to the resurgence of latent Ebola virus particles, latent and persistent, in survivors: a reactivation. This new phenomenon epidemiologically implies detailed investigation of the index cases: in fact, latentization can be present in asymptomatic, pauci-symptomatic EBOV infections during previous outbreaks. Important is the survivor's surveillance for monitoring eventual reactivations and relapses and viral strains genotyping and phylogenetic reconstruction. In the case of New Guinea Outbreak the index case was a nurse. The greatest risk of acquiring the infection is in healthcare workers due to direct contact with patients and/or local communities in affected areas. In addition, staff members of humanitarian, religious and other organizations, who have a large presence in the country, may be exposed to the virus, but the likelihood of infection for this group is considered low if infection prevention and control measures are followed.

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TYPE Review
PUBLISHED 09 March 2023
DOI 10.3389/fphar.2023.1125305

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 16 December 2022
ACCEPTED 09 February 2023
PUBLISHED 09 March 2023

CITATION
Rana R, Kant R, Kumra T, Gupta S, Rana DS and Ganguly NK (2023), An update on SARS-CoV-2 immunization and future directions.
Front. Pharmacol. 14:1125305.
doi: 10.3389/fphar.2023.1125305

An update on SARS-CoV-2 immunization and future directions

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Millions of people have died as a result of SARS-CoV-2, which was first discovered in China and has since spread globally. Patients with SARS-CoV-2 infection may show a range of symptoms, including fever, coughing, and shortness of breath, or they may show no symptoms at all. To treat COVID-19 symptoms and avoid serious infections, many medications and vaccinations have been employed. However, to entirely eradicate COVID-19 from the world, next-generation vaccine research is required because of the devastating consequences it is having for humanity and every nation's economy. Scientists are working hard to eradicate this dangerous virus across the world. SARS-CoV-2 has also undergone significant mutation, leading to distinct viral types such as the alpha, beta, gamma, delta, and omicron variants. This has sparked discussion about the effectiveness of current vaccines for the newly formed variants. A proper comparison of these vaccinations is required to compare their efficacy as the number of people immunized against SARS-CoV-2 globally increases. Population-level statistics evaluating the capacity of these vaccines to reduce infection are therefore being developed. In this paper, we analyze the many vaccines on the market in terms of their production process, price, dosage needed, and efficacy. This article also discusses the challenges of achieving herd immunity, the likelihood of reinfection, and the importance of convalescent plasma therapy in reducing infection.

KEYWORDS

COVID-19, SARS-CoV-2, vaccine efficacy, herd immunity, reinfection, convalescent plasma therapy, Pfizer, Moderna

Introduction

Millions of people have died as a result of SARS-CoV-2, which was first discovered in China and has since spread globally (Wu et al., 2020; Zhou et al., 2020; WHO, 2022). Of the four different genera of the Coronaviridae family, i.e., alpha, beta, gamma, and delta-coronavirus, SARS-CoV-2 belongs to the beta genus. The characteristic features of the

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 19; ACE 2, angiotensin-converting enzyme 2; WHO, World Health Organization; CDC, Centers for Disease Control and Prevention; PCR, polymerase chain reaction; RBD, receptor-binding domain; If. U, infectious units; CPT, convalescent plasma therapy; MERS-CoV-2, Middle East respiratory syndrome coronavirus; MHC, major histocompatibility complex.

Coronaviridae family are a positive-sensed RNA virus enclosed by an envelope (Almeida and Tyrrell, 1967; Kapikian et al., 1969; Peiris et al., 2004; Van Der Hoek et al., 2004; Woo et al., 2005; Zaki et al., 2012). Since its discovery, the genome of the virus has undergone numerous modifications resulting in numerous mutant strains, including alpha, beta, and delta variants. According to the genetic sequence of the virus, which was first published in January 2020, SARS-CoV-2 has distinct characteristics, such as a strong affinity for the angiotensin-converting enzyme 2 (ACE2) receptor and a polybasic cleavage site at the S1/S2 spike junction that determines infectivity and host range (Nao et al., 2017; Andersen et al., 2020). SARS-CoV-2-infected patients can be asymptomatic or symptomatic and may show a number of symptoms, such as fever, cough, and shortness of breath. Occasionally, infected patients can also show symptoms including vomiting, diarrhea, and abdominal pain (Wang et al., 2020). Individuals who acquire pneumonia after COVID-19 infection show mottling and ground-glass opacity in chest X-rays (Zhu et al., 2020). Along with the primary target of the lungs, other organs of the body, such as the kidneys and liver, are also affected by COVID-19 infection (Renu et al., 2020). SARS-CoV-2 transmission occurs with high efficacy and infectivity, mainly through the respiratory route and primarily through droplet transmission (Han et al., 2020; Leung et al., 2020). At present, coronavirus is a dominating concern throughout the world. The severe effects of COVID-19 on humanity and the economy of every country require next-generation vaccine development to completely end this virus. Every non-profit organization and country in the world is attempting to fund vaccine companies to provide a vaccine development fund. Through valiant efforts by the scientific community, the first COVID-19 vaccine entered human clinical trials in 2020 (Thanh Le et al., 2020). However, the major issues in vaccine development are the absence of an animal model, the time-consuming process, and an unknown mechanism of pathogenesis (Mukherjee, 2020). Additionally, the continuous development of new genetic variants of SARS-CoV-2 is also an issue in generating an effective vaccine (Aljabali et al., 2020; Amawi et al., 2020; Shereen et al., 2020; Velavan & Meyer, 2020). Utilizing a small number of human trials, an ideal vaccine dosage and administration schedule should be established. Existing drugs for other viruses can also be examined for use as drugs for the COVID-19 virus (Dhama et al., 2020). Apart from vaccine development, there is also a need to check the time period for which antibodies are present in an individual after recovery, because the level of antibodies may relate to the probability of reinfection with the COVID-19 virus, and a great deal of research is ongoing to determine the probability of reinfection with the virus. Reinfection occurs when a person develops an infection once, recovers, and then becomes infected again, either with the same infectious agent or with a different variant (Yahav et al., 2021). The CDC states that recovered individuals must have at least one negative PCR test result for SARS-CoV-2. Reinfection of a patient can be immensely important because if reinfections are common, natural immunization will not be sufficient to confer herd immunity. Herd immunity is the indirect protection of susceptible individuals from the infection due to the presence of a large proportion of immunized individuals. Scientists are also working to determine the role of convalescent plasma therapy to treat or reduce the severity of COVID-19 infection. Convalescent plasma has already been tested for efficacy against other respiratory viruses. This therapy is hypothesized to initiate a temporary immune response against infectious virus particles and serve as a safeguard before the peak-level production of antibodies by the immune system of the infected individual (Luke et al., 2006; Mair-Jenkins et al., 2015; Arabi et al., 2016). In this article, we compare the different available vaccines in terms of their methods of production, cost, and effectiveness. Additionally, we discuss the potential for reinfection with the COVID-19 virus and how convalescent plasma therapy is used to treat infected people.

SARS-CoV-2

The SARS-CoV-2 virus, which was first discovered in China, has already spread to every country in the world. The genome of SARS-CoV-2 contains a positive-sensed RNA virus enclosed by an envelope. The genomic sequence of SARS-CoV-2 was first made public in January 2020, and comparisons with other coronaviruses show that it differs from these in terms of its strong affinity for the ACE2 receptor and the presence of a polybasic cleavage site at the S1/S2 spike junction that controls infectivity and host range. After its discovery, various mutations occurred in the virus genome, which resulted in the development of various mutant strains, such as the alpha, beta, and delta variants. The receptor-binding domain (RBD) of the spike (S) protein mediates viral entry by binding with the human cell surface protein angiotensin-converting enzyme 2 (ACE2). Figure 1 shows the structure of SARS-CoV-2. According to sequencing results, the SARS-CoV-2 genome undergoes two single-nucleotide alterations per month. In the alpha variant, 17 mutations can be seen in the genome, which includes mutations of the receptor-binding domain, such as E484K, S494 P, and N501Y, and mutations in the s-glycoprotein comprising 69del, 70del, D614G, 144del, and A570D. These mutations in the receptor-binding domain and s-glycoprotein region make the virus 70% more transmissible. Deletions in the spike protein correlate with the immune response of the infected person. In addition, increased virulence and infectivity have been linked to the N501Y mutation in mouse models (Brief, 2020; Hill et al., 2022). RBD mutations in the beta [K417N/E484K/N501Y] (Planas et al., 2021; Zhou et al., 2021), gamma [K417T/E484K/N501Y] (Burki, 2021; Lancet, 2021; Xie et al., 2021), delta [T478K, and L452R] (Goher et al., 2022), and omicron variants [L452R/F486V/F486V/L452R/L452R, F486V/R493Q] (Mohapatra et al., 2022) have also been explored by scientists. Mutations in the beta variant have been shown to have a stronger affinity (4.62 times higher) for binding hACE2 (Ramanathan et al., 2021). RBD mutations in the epsilon, beta, and theta variants resulted in an increase in infectivity in vitro and also made the virus 20% more transmissible (Ali et al., 2021a; Deng et al., 2021; McCallum et al., 2021; Zhao et al., 2021). In January 2022, a virologist discovered a new variant of SARS-CoV-2 in Cyprus and named it “deltacron.” Deltacron is a super-variant with the combined genome of the delta and omicron variants (Kreier, 2022). Genome analysis of deltacron showed that the RBD is derived from the omicron variant, and this variant may lead to enhanced disease transmission and immune evasion (Colson et al., 2022; Hosch et al., 2022).

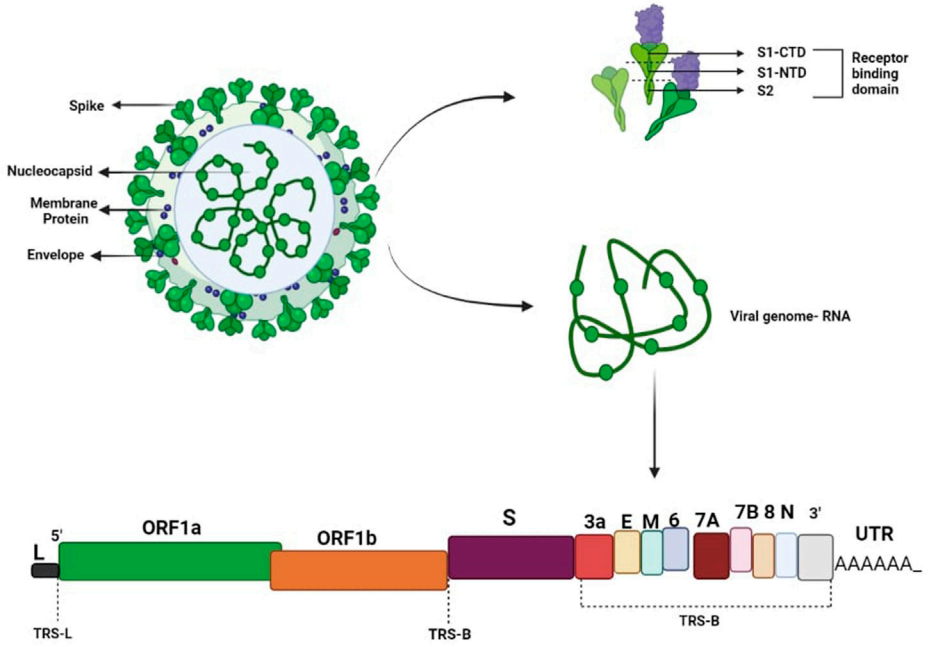

FIGURE 1
SARS-CoV-2 virus. A functioning polybasic cleavage site at the S1–S2 junction of the spike protein and the receptor-binding domain (RBD) in the S1 subunit are two important genetic traits of SARS-CoV-2.

Vaccines for COVID-19

The severe effects of COVID-19 on humanity and on every country's economy raise the need for next-generation vaccine development to eradicate this virus. Through valiant efforts by the scientific community and despite various obstacles, the first COVID-19 vaccine entered human clinical trials in 2020 (Aljabali et al., 2020; Amawi et al., 2020; Mukherjee, 2020; Shereen et al., 2020; Thanh Le et al., 2020; Velavan & Meyer, 2020). After its development, the optimal dosage and schedule was another important aspect that needed to be determined to enhance vaccine efficacy against the infection. Under normal conditions, the vaccine development process requires significant research and testing before the vaccine can be introduced into later-phase clinical trials, but due to the unprecedented circumstances, permission was granted for the emergency use of coronavirus vaccines on the basis of data on their efficacy and safety from early clinical trials (BIO, 2021). However, it is still questionable whether the existing SARS CoV2 vaccinations are safe and effective (Kuppili et al., 2021; Uddin et al., 2021). Because these vaccines were approved on the basis of an emergency situation, proper monitoring of the efficacy, safety, and side effects (if any) of these vaccines is required. Additionally, vaccinations should be evaluated for their efficacy against the several SARS-CoV-2 mutations that have recently emerged. Certain vaccines produce an appropriate immune response after a single dose, whereas others require a booster shot a month or more later. Therefore, a suitable schedule with respect to the gap between the two doses and a booster dose of each vaccine should also be developed to improve their efficacy and results (Torales et al., 2020; Rana et al., 2022). To date, a number of COVID-19 vaccines have been developed and are being globally administered in vaccination programs. Scientists have used different platforms to develop coronavirus vaccines, such as mRNA and proteins. The different platforms for vaccine production and their modes of action are shown in Figure 2. An issue that should also be considered during vaccination programs is that vaccine development requires high levels of expertise, extensive infrastructure, and a great deal of money; therefore, low-income countries are not able to produce vaccines. However, to eradicate this virus from Earth, vaccination of the majority of the population is necessary to avoid any future variants, so developed countries must support vaccination programs in these low-income countries (Saied et al., 2022). Based on the platforms used for the production of the coronavirus vaccine, we categorized the vaccines developed into four categories.

A) Nucleic acid-based vaccines (**BNT162b2 and Spikevax**)
B) COVID-19 viral vector/adenovirus vaccines (**JCOVDEN, Vaxzevria, and Sputnik V**)
C) Protein-based vaccines **(Nuvaxovid)**
D) Whole inactivated virus (**BBIBP-CorV, CoronaVac, and Covaxin**)

A) Nucleic acid-based vaccines

Nucleic acid vaccines are genetic vaccines consisting solely of DNA or RNA, which are taken up and translated into proteins by host cells and elicit immune responses. Since naked nucleic acids do not have a viral coat, they are not typically affected by pre-existing immunity, which can reduce the clinical effectiveness of recombinant virus vaccines. Nucleic acid vaccines provide several significant advantages over other forms of vaccination in terms of

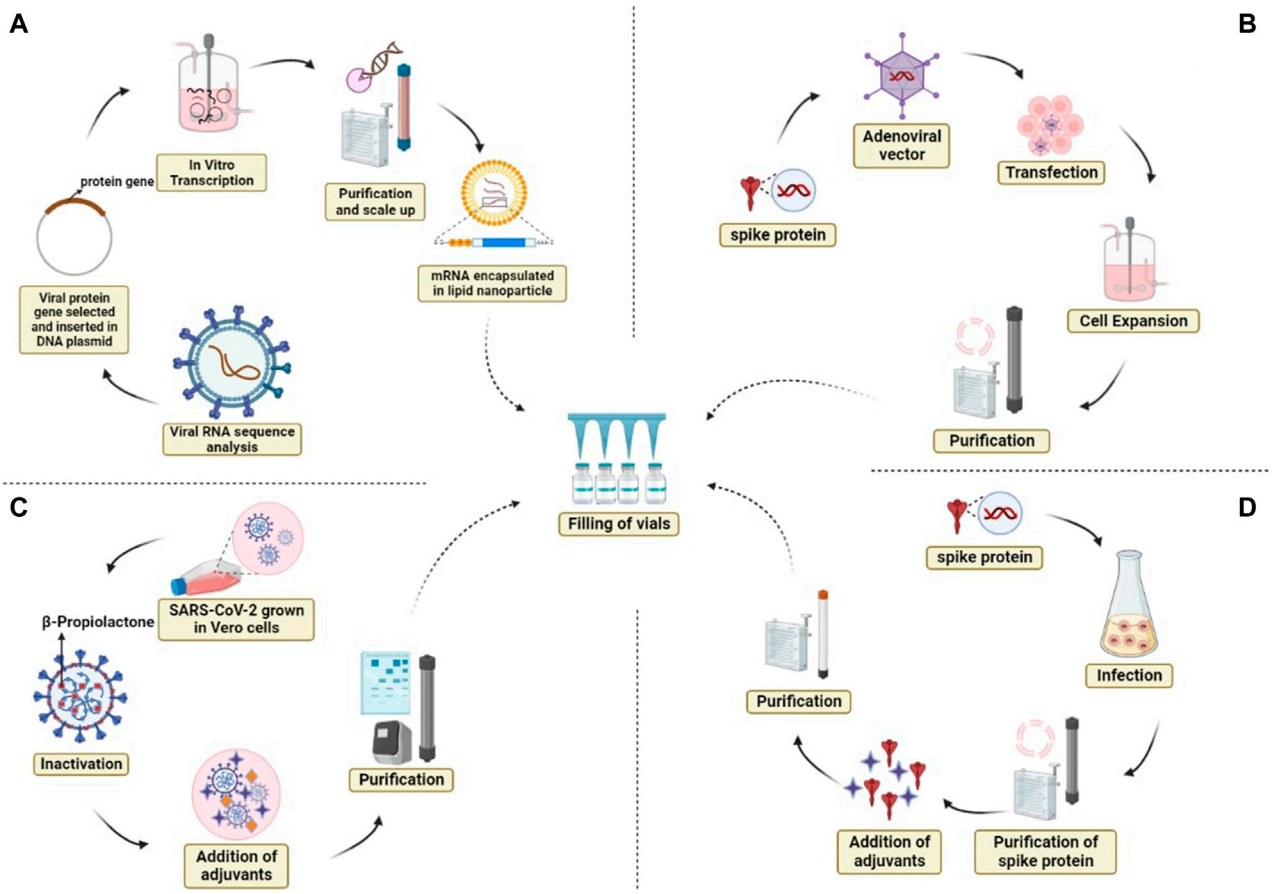

FIGURE 2
Different platforms for vaccine production and their modes of action. **(A)** mRNA vaccine, **(B)** adenoviral vector vaccine, **(C)** whole inactivated virus, and **(D)** protein subunit vaccine.

increased safety and lower production costs (Liu, 2011; Sardesai and Weiner, 2011). Despite the safety concerns regarding DNA/mRNA vaccinations, very little incorporation of viral genes into host genes occurs with the use of plasmid vectors (Sheets et al., 2006). In this article, we compare two major nucleid acid vaccines (BNT162b2 and Spikevax) that have been approved for emergency use.

a) BNT162b2/Comirnaty

The biotechnology companies Pfizer (American) and BioNTech (German) developed an mRNA-based vaccine and named it the Comirnaty/BNT162 vaccine. The SARS-CoV-2 full-length spike protein is encoded by the nucleoside-modified RNA vaccine BNT162b2, which is packaged as a lipid nanoparticle. Preclinical data provided by Pfizer indicated that immunization of a non-primate model (*Rhesus macaques*) with BNT162b2 administered intramuscularly induced the production of neutralizing antibodies and also of T_H and T_C cells, which protect *R. macaques* from SARS-CoV-2 infection (Khehra et al., 2021). After initial approval by the UK, the vaccine was subsequently also approved by the FDA. Three doses of the BNT162b2 vaccine in children (6 months to 4 years) have been found to produce similar immunity to that produced by two doses in adults (Mayo Clinic, 2021). In children, the vaccine shows different efficacy rates in different age groups (Pfizer, 2020; Pfizer, 2021). The vaccine efficacy for different SARS-CoV-2 variants is as follows: 94% for the alpha variant (Lopez Bernal et al., 2021), 75% for the beta variant (Abu-Raddad et al., 2021), 88% for the delta variant (Lopez Bernal et al., 2021), and 60% for the omicron variant (Tartof et al., 2022). To minimize cases of hospitalization and enhance the efficacy rate, many countries have also immunized their population with a booster dose of BNT162b2 (Edouard et al., 2020).

b) Spikevax/mRNA-1273

The World Health Organization approved Spikevax vaccine, which is manufactured by Moderna, for emergency use against SARS-CoV-2 on 30 April 2021. The spike protein of Coronavirus is encoded by mRNA found in the lipid nanoparticles that make up the Spikevax vaccine. The non-replicating, transiently expressed, transported mRNA is mostly found in dendritic cells and subcapsular sinus macrophages. After recognition by immune cells, this mRNA activates an immune response, which results in the production of B and

T cells and thus the protection of an immunized individual from SARS-CoV-2 [Spikevax, 2021; Baden et al., 2021). The efficacy of the Spikevax vaccine has been found to be 51% in children aged 6–23 months, 37% for children aged 2–5 years (Mayoclinic, 2021), and 93.3% for those aged 12–17 years (Verbeke et al., 2021). Immunization with a booster dose has been shown to enhance levels of neutralizing antibodies against the delta variant by 17% (Spikevax, 2021). The efficacy of the vaccine in terms of symptomatic cases and asymptomatic cases has been found to be 94.1% and 63%, respectively (El Sahly et al., 2021; Verbeke et al., 2021). A published study found that the efficacy of two dosages of Spikevax was reduced by 10 times for the delta variant and by more than 100 times in the omicron variant (Macdonald et al., 2022).

B) COVID-19 viral vector/adenovirus vaccines

Viral vector vaccines or Adenovirus vaccines use harmless adenovirus as a vector; this is modified to deliver SARS-CoV-2 genetic material. Immune cells produce antibodies against the protein encoded by this genetic material. In this article, we compare three major viral vector or adenovirus vaccines (JCOVDEN, Vaxzevria, and Sputnik V) that have been approved for emergency use.

a) JCOVDEN/Ad26. COV2-S

JCOVDEN is produced by Janssen Inc., and was approved for emergency use by the World Health Organization on 5 March 2021. A recombinant, non-replicating, human adenovirus type 26 vector that codes for the SARS-CoV-2 spike protein is present in the monovalent vaccine JCOVDEN. JCOVDEN is able to stimulate both neutralizing and S-specific antibodies (Jcovden, 2021). It has not yet been determined whether JCOVDEN is safe and effective for use in children and adolescents (under the age of 18). JCOVDEN is preferably not used over other available vaccines, because this vaccine has been found to cause various side effects, which include hypersensitivity, anaphylaxis, anxiety-related reactions, concurrent illness, and coagulation disorders such as thrombosis with thrombocytopenia syndrome (Jcovden, 2021). The efficacy of this vaccine against symptomatic COVID-19 14 days after vaccination has been found to be 70.1% for the alpha variant and 38% for the beta variant, but its efficiency has been found to be lower for the delta variant. However, the efficacy of the vaccine against severe COVID-19 14 days after vaccination has been found to be 51.1% for the alpha variant and 70.2% for the beta variant. A booster dose of JCOVDEN should be given after 2 months only in people above 18 years of age. The efficacy of a single dose of JCOVDEN has been found to be reduced by 10 times for the delta variant and by more than 100 times for the omicron variant (Macdonald et al., 2022).

b) Vaxzevria/ChAdOx1-S/Covishield

The British–Swedish multinational pharmaceutical and biotechnology business AstraZeneca produces Vaxzevria, which is commonly known as ChAdOx1-S. In addition, the Serum Institute of India produces Covishield. Vaxzevria contains a replication-deficient chimpanzee adenovirus that encodes for the coronavirus spike glycoprotein. One dose of the vaccine contains 2.5×10^8 infectious units (If. U) created by recombinant DNA technology and genetically altered HEK 293 cells (Vaxzevria, 2021). The dosage gap between the primary and secondary doses should be 4–12 weeks, as recommended by officials (Vaxzevria, 2021). To date, this vaccine has not been approved for the pediatric population, so its efficacy rate in this population is not known. The side effects of Vaxzevria reported so far include hypersensitivity, anaphylaxis, anxiety-related reactions, concurrent illness, and coagulation disorders, such as thrombosis with thrombocytopenia syndrome (Vaxzevria, 2021). The efficacy of Vaxzevria vaccine has been found to be 74.0% for symptomatic cases (Vaxzevria, 2021) and 54% for asymptomatic cases (Pramod et al., 2022). A booster dose should be administered at least 3 months after the secondary dose. The efficacy of the vaccine after the primary dose is slightly lower for the delta variant of the virus (71%) compared to the alpha strain (76%) (Lopez Bernal et al., 2021; Stowe et al., 2021).

c) Sputnik V

The Russian institute Gamaleya National Research Institute of Epidemiology and Microbiology developed Sputnik V based on two different human adenovirus vectors, for adenovirus 26 and adenovirus 5 (Jones and Roy, 2021). Both components of this vaccine (Adenovirus 26 and Adenovirus 5) contain the spike protein gene of SARS-CoV-2. Both components of the vaccine are administered in the form of two doses separated by 3 weeks (Cdsco, 2021). The vaccine induces humoral and cellular immunity against infection caused by SARS-CoV-2. The mechanism of the drug's action is based on the ability of Ad26- and Ad5-based recombinant viral particles carrying the SARS-CoV-2 S protein gene to efficiently transduce the cells of the vaccinated body; in this case, genetic sequences that code the antigen are delivered to the cells so that the transduced cells start to produce the antigen. After the first dose, the rAd26-based vector enters the body cells, which leads to the expression of SARS-CoV-2 S protein and triggers the development of SARS-CoV-2 immunity. The rAd5-based vector targets body cells following the second dose of the vaccine and strengthens protective immunity toward SARS-CoV-2. Data on the efficacy of Sputnik V are not available for the pediatric population because this vaccine is not approved for the vaccination of children. Chills, fever, headaches, soreness at the injection site, and other adverse reactions are possible with this vaccine. The efficacy of the vaccine has been found to be 73.1% after the primary dose and 91.6% after the secondary dose (Logunov et al., 2021). The efficacy of the vaccine against the alpha and delta variants after the primary dose has been reported as 85.7% (Vokó et al., 2022) and 78.6% (González et al., 2021), respectively. There is an 8.1-fold decrease in neutralizing antibody titers for the omicron version, according to a study published in *Vaccines* (Lapa et al., 2022). According to statistical data made public by the Ministry of Health of the UAE, the vaccine was found to have 97.8% efficacy in averting symptoms of COVID-19 and 100% efficacy in preventing severe illness in 81,000 people who had received two doses (Precision Vaccinations, 2022). In a study involving 40,387 adults aged 60 to 79 who were vaccinated and 146,194 individuals who were not, the Buenos Aires Health Ministry in Argentina found that a

single dose of Sputnik Light reduced symptomatic infections by 78.6%, hospitalizations by 87.6%, and deaths by 84.7% (Bianca Nogrady, 2021).

C) Protein-based vaccines

SARS-CoV-2 protein is used in protein-based vaccines; whenever this protein is detected by a person's immune system, an immune response is produced. A whole protein, protein fragment, or peptide can be used to make protein-based vaccines. The only significant protein-based vaccine that has been authorized for emergency use is Nuvaxovid.

a) Nuvaxovid

Nuvaxovid is a protein-based vaccine for coronavirus manufactured by Novavax, Inc. Nuvaxovid is composed of the full-length recombinant spike protein of SARS-CoV-2, which is adjuvanted with Matrix-M (Fractions A C of *Quillaja saponaria Molina* extract) (Nuvaxovid, 2021). Matrix-M adjuvantation helps in the enhancement of the innate immune response and activation of B and T cells in response to the s-protein. In India, Novavax Inc. has collaborated with the Serum Institute of India to market the vaccine as Covovax. The European Medicines Agency has granted permission for the emergency use of Nuvaxovid in Europe. The efficacy of Nuvaxovid has been found to be higher in children (12–17 years old) compared to adults. Overall, the efficacy of the vaccine has been found to be 89.7% (Heath et al., 2021).

D) Whole inactivated virus-based vaccines

Whole inactivated virus-based vaccines use a killed or inactivated COVID-19 virus strain; when this killed virus is recognized by immune cells, an immune response is produced. In this article, we compare three major whole inactivated virus-based vaccines (BBIBP-CorV, CoronaVac, and Covaxin) that have been approved for emergency use.

a) BBIBP-CorV

The BBIBP-CorV vaccine is manufactured by Sinopharm, a company located in Beijing, China. The BBIBP-CorV vaccine of the whole inactivated virus type. This type of vaccine contains a virus whose genetic material has been damaged by radiation, heat, or chemicals, but that still possesses the ability to induce an immunological reaction. BBIBP-CorV is produced using an aggressive WIV04 strain of SARS-CoV-2 inside Vero cells. The virus is inactivated by beta-propiolactone while the integrity of other viral particles is maintained. Aluminum hydroxide is used as an adjuvant and combined with the resultant inactivated virus to increase the immune response against viral particles. After initial approval by China, the World Health Organization approved this vaccine for emergency use throughout the world (Xia et al., 2020). A 21-day gap between the two doses has been demonstrated to induce the production of a high level of neutralizing and SARS-CoV-2-specific IgG antibodies. The efficacy of this vaccine against symptomatic COVID-19 infection was shown to be 79% in an international phase III trial (Xia et al., 2020). According to reports, a booster dose of the BBIBP-CorV vaccine causes a 1.5–5-fold increase in neutralizing antibodies against omicron compared to a two-dose regime (Ewunkem et al., 2022; Wang et al., 2022).)

b) CoronaVac

Sinovac, which is a China-based company, developed CoronaVac using a whole inactivated virus method. The active ingredient in CoronaVac is an inactivated CZ02 strain (SARS-CoV-2 Virus strain), and aluminum hydroxide is used as an adjuvant to enhance the immune response (WHO, 2021a). A phase III trial of the vaccine conducted in Brazil showed 50.7% efficacy against symptomatic infection, while efficacy of 100% was observed in the prevention of severe cases and hospitalization (Leung et al., 2022). In a population of 3- to 5-year-old children, efficacy against symptomatic cases, hospitalization, and severe illness was found to be 38.2%, 64.6%, and 69.0%, respectively (Florentino et al., 2022). The efficacy of the vaccine in 6- to 11-year-olds is 41.5% and 63.5% against symptomatic and severe cases, respectively (Cheng et al., 2022). In China and Hong-Kong, the vaccine has been approved for use in the pediatric population despite insufficient data. In adults, two doses should be administered at an interval of 28 days for improved efficacy.

c) Covaxin

Bharat Biotech produces Covaxin in partnership with the National Institute of Virology (ICMR). The vaccine is manufactured using the beta-propiolactone inactivated strain (Asp614Gly) of SARS-CoV-2. To enhance the immune response, Alhydroxiquim-II (financed by the National Institute of Health) is used as an adjuvant. In India, the vaccine was granted approval for emergency use in November 2021 and for full use in January 2022 by the Drugs Controller General of India (DGCI). In a phase III trial of Covaxin, efficacy was reported to be 77.8% against symptomatic cases and 63.6% against asymptomatic cases (Bharat Biotech, 2021; Yadav et al., 2022). The levels of neutralizing antibodies against omicron produced by two doses of the vaccine are frequently insufficient, demonstrating its weak ability to trigger immune responses against omicron. In a phase II/III trial of Covaxin in children (2–18 years old), the vaccine was found to be safe and to induce a sufficient immune response with no extreme side effects (Sapkal et al., 2022). Figure 3 shows a comprehensive overview of the coronavirus, including its risk factors and the drugs used for treatment.

As discussed above, various types of vaccines provide different efficacy rates, and their efficacy also varies for different variants of SARS-CoV-2. Therefore, a comparison is needed to determine the best available vaccine for each variant. Table 1 shows a comparison of the different types of widely used vaccines worldwide.

Convalescent plasma therapy

Convalescent plasma therapy (CPT) was discovered in the past as a therapy for other respiratory viral diseases, such as MERS-CoV-2 (Luke et al., 2006; Mair-Jenkins et al., 2015); it was found that this

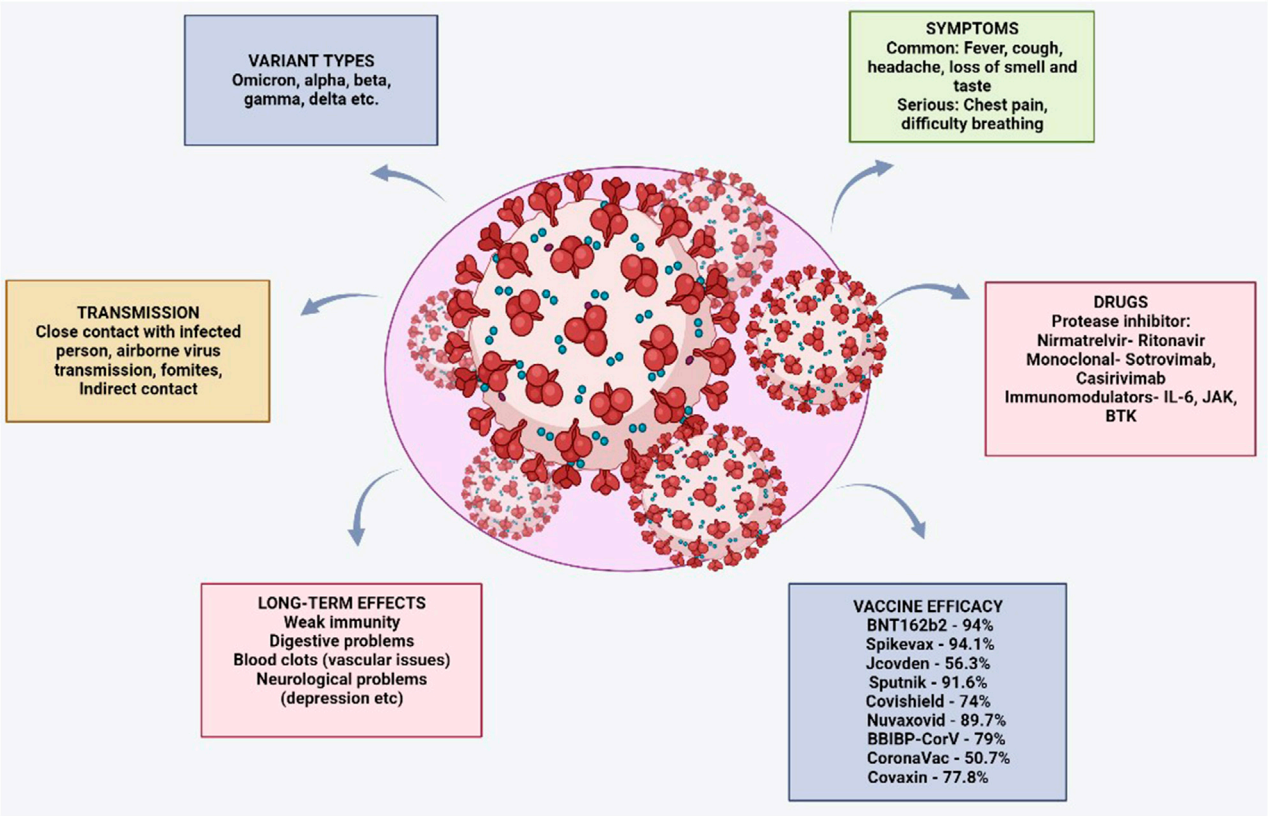

FIGURE 3
Comprehensive overview of the coronavirus, including relevant risk factors and drugs used for its treatment.

therapy results in the generation of a temporary immune response and reduction in viral particles in the infected individual, which further helps in the avoidance of cytokine storm (Luke et al., 2010; Arciuolo et al., 2017). The major advantage of CPT is that it is readily available as soon as an individual who has recovered from the infection becomes available, but it does require further development. Despite certain drawbacks, such as the need for dedicated collection, testing, dose standardization, and blood group testing, this therapy serves as a first line of defense (Luke et al., 2010). Despite being poorly defined and associated with some controversy, CPT is among the therapeutic strategies that are under investigation for efficacy against SARS-CoV-2 (Duan et al., 2020; Focosi et al., 2020; Perotti et al., 2020; Focosi and Farrugia, 2021). The FDA granted permission for the emergency use of COVID-19 convalescent plasma on 23 August 2020 for the treatment of COVID-19 patients who were taking immunosuppressive medications. Various studies have attempted to explore the role of CPT in individuals infected with SARS-CoV-2. Some of these have found that CPT plays an important role in enhancing the survival rate of patients (Agarwal et al., 2020; Klassen et al., 2021), whereas others have found that CPT does not play any significant role in survival rate, especially in severe cases (Janiaud et al., 2021). These findings created confusion for the public, scientists, and governments regarding the efficacy and safety of CPT, prompting Daniele Focosi et al. to conduct a review of several randomized clinical trials (RCTs); they found that these RCTs produced different results for several reasons, such as CPT dose and timing (Focosi et al., 2022). Reports suggest that the benefits of CPT are increased when it is administered with high neutralizing antibody titers within 3 days of the onset of symptoms (Libster et al., 2021; González et al., 2022; Paneth et al., 2022). The main issue in CPT is the administration of high-quality CPT by transfusion. Although CPT titer ≥1:320 is recommended for use in immunocompromised people, its effective/optimum dose and the timing of administration should be assessed in further clinical trials (Focosi and Franchini, 2021; Franchini et al., 2021).

Chance of reinfection

Beyond the efficacy of vaccines, the most important question is to determine the chances of reinfection with the same or a different SARS-CoV-2 variant after a patient has recovered. Patients who have recovered from COVID-19 appear to have memory B and T cells, in accordance with findings indicating that infection with SARS-CoV-2 generates both a neutralizing antibody response and a cellular response with virus-specific T cells (Wajnberg et al., 2020a; Juno et al., 2020; Le Bert et al., 2020). Approximately a week after developing symptoms, more than 90% of those with SARS-Cov2 develop antibodies that persist for at least 3 months

TABLE 1 Comparison of the different types of widely used vaccines worldwide.

S. No	Vaccine name and type	Company and manufacturing date	Efficacy	Cost	Recommended dose	Countries approved	References
1	BNT162b2/ Comirnaty (mRNA vaccine)	Pfizer and BioNTech (31 December 2020)	Alpha, 94%; beta, 75%; delta, 88%; omicron, 60%	EU and United States of America: $19.50	Two doses (21-day gap)	The United States of America, Austria, Brazil, *etc.*	Kuppili et al. (2021); Lancet (2021); Lapa et al. (2022)
2	Spikevax/ mRNA-1273 (mRNA vaccine)	Moderna (30 April 2021)	94.1% (symptomatic cases); 63% (asymptomatic cases)	EU: $25.5 United States of America: $15 Argentina: $21.5 Botswana: $28.8	Two doses (28-day gap)	European Union, Japan, Austria, the US, the United Kingdom, *etc.*	Leung et al. (2022); Libster et al. (2021)
3	JCOVDEN/ Ad26.COV2-S (adenovirus vaccine)	J&J/Janssen (5 March 2021)	56.3% (symptomatic cases); 34.2% (asymptomatic cases)	EU: $8.5 United States of America: $10 African Union: $10	One dose	India, European Union, Colombia, the United States of America, Brazil, *etc.*	Liu (2011)
4	Vaxzevria/ ChAdOx1-S/ Covishield (adenovirus vaccine)	AstraZeneca and the Serum Institute of India (16 February 2021)	74% (symptomatic cases); 54% (asymptomatic cases)	EU: $2.15	Two doses (4–12-week gap)	Europe, Africa, America, India, Australia, the United Kingdom, and the United States of America	Lopez Bernal et al. (2021); Luke et al. (2010)
5	Sputnik V (adenovirus vaccine)	Gamaleya National Research Institute of Epidemiology and Microbiology (12 April 2021)	91.6% (symptomatic cases)	<$10	Two doses (21-day gap)	Argentina, Serbia, the United States of America, India, UAE, *etc.*	Maulud et al. (2022)
6	Nuvaxovid (protein vaccine)	Novavax (20 December 2021)	89.7% (symptomatic cases)	Denmark: $20.9	Two doses (21-day gap)	India, Turkey, Malaysia, Denmark, *etc.*	Nguyen et al. (2022)
7	BBIBP-CorV (whole inactivated virus)	Sinopharm/Beijing Institute of Biological Products (April 2021)	79% (symptomatic cases)	Argentina, Mongolia: $15 Senegal: $18.6 China: $30 Hungary: $36	Two doses (28-day gap)	Asia, Africa, South America, Argentina, China, *etc.*	Nordström et al. (2022)
8	CoronaVac (whole inactivated virus)	Sinovac Biotech (1 June 2021)	50.7% (symptomatic cases); 100% (hospitalization)	China: $29.75 Ukraine: $18 Philippines: $14.5 Brazil: $10.3 Cambodia: $10	Two doses (28-day gap)	China, Indonesia, Singapore, *etc.*	Perotti et al. (2020)
9	Covaxin (whole inactivated virus)	Bharat Biotech in collaboration with the National Institute of Virology (ICMR) (3 November 2021)	77.8% (symptomatic cases); 63.6% (asymptomatic cases)	$3–$4	Two doses (28-day gap)	India, Brazil, Iran, Mexico, *etc.*	Planas et al. (2021); Polack et al. (2020)

(Wajnberg et al., 2020b; Gudbjartsson et al., 2020). However, antibody titers may eventually decrease in cases of mild illness (Ibarrondo et al., 2020). It is critical to gain a better understanding of whether COVID-19 survivors are immune to reinfection or not. Reinfection occurs when a person contracts an infection once, recovers, and then contracts it again—either from the same infectious agent or a different variation (Yahav et al., 2021). According to the CDC, recovered patients should have at least one negative PCR test result for SARS-Cov-2. In 2021, the World Health Organization stated that the presence of antibodies in patients after recovery from COVID-19 does not guarantee protection from reinfection (WHO, 2021b). The reinfection rate in different countries has been reported to range from less than 0.5% to more than 5% [117,118]. Different studies have confirmed cases of reinfection with mild-to-moderate symptoms in the second infection, depending on the time interval between the primary and secondary infections and the level of detectable IgG against SARS-CoV-2 (Ali et al., 2021b; Breathnach et al., 2021; Caralis, 2021; Hall et al., 2021; To et al., 2021; Nguyen et al., 2022; Sotoodeh Ghorbani et al., 2022). A study conducted in Sweden found that the risk of SARS-CoV-2 reinfection and COVID-19 hospitalization in individuals who have survived and recovered from a previous infection remained low for up to 20 months (Nordström et al., 2022).

Herd immunity

The major drawback of the possibility of reinfection is the reduced chance of herd immunity of the population. Herd immunity means that a sufficient percentage of immune people are present in a population to achieve the indirect protection of vulnerable people from infection. Herd immunity is important for the protection of individuals who cannot be vaccinated, including the very young and the immunocompromised. Reinfection can increase the chances of contact between infected individuals and susceptible hosts. According to various studies, the reproductive number of SARS-CoV-2 is estimated to fall within the range of 2.2 to 5.7 (Li et al., 2020; Sanche et al., 2020), whereas herd immunity is achieved when the reproductive number is less than 1. Evidence suggests that the spread of SARS-CoV-2 will not cease until at least 50% of the population has developed immunity. Given that SARS-CoV-2 has a very high case fatality rate, infection of 50% of the population would lead to a significant number of deaths (Flaxman et al., 2020; Salje et al., 2020). Therefore, vaccines may be a promising way of reaching herd immunity. However, vaccine hesitancy due to fear of side effects, religious beliefs, and misinformation about vaccines is a major hurdle that inhibits the attainment of herd immunity in the population (Wong, 2021).

Future directions

The SARS-CoV-2 pandemic has brought into focus unexpected and significant issues for humanity. Numerous containment techniques, including the utilization of genetic and community monitoring, and an increase in immunization and the provision of booster doses to the susceptible population, have been developed to reduce the harmful effects associated with the many forms of SARS-CoV-2 (Dhawan et al., 2022). Although a number of proteomic techniques are available for detection of the virus, more techniques with higher specificity should be sought (Rana et al., 2020). The pandemic has forced us to explore different existing viruses by integrating artificial intelligence and machine learning, as these viruses could potentially pose a threat to humans in the future. We cannot ignore the possibility that a completely new virus could emerge and induce another global pandemic in the future. According to studies, those who are not immunized are more prone to experience serious illness, leading to hospitalization. Based on prior experiences with other viruses, we understand that further information regarding the transmissibility of the virus, the effectiveness of vaccination, and the severity of illness caused by coronavirus will only be available with time and careful monitoring. Meanwhile, studies should focus on the development of a vaccine that is equally effective for all variants of the virus.

For the research community

The research community should focus on developing a vaccine that is equally effective against all variants. Furthermore, effective methods of detecting virus variants as soon as possible should be developed so that proper measures can be taken to prevent the spread of the disease (Maulud et al., 2022; Rana et al., 2022). Additionally, previous studies have shown that antibody levels are reduced 3–4 months after vaccination (Polack et al., 2020; Khoury et al., 2021; Naaber et al., 2021). Along with vaccines, other compounds should be tested for their role in the treatment of COVID-19. For example, studies have shown that use of 2% hydrogen as a line of treatment can enhance patient immunity and may result in an exceptional decrease in toxicity and oxidation processes, as hydrogen displays various antioxidant, anti-inflammatory, and anti-apoptosis properties (Yang et al., 2020). In support of this, various experiments have been performed to verify the effects of hydrogen in an animal model; these have shown that after inhalation of 2% hydrogen by cerebral ischemia-reperfused rats, their condition is improved, with a high positive recovery rate (Channappanavar and Perlman, 2017). This mechanism works by selective elimination of hydroxyl radicals and peroxynitrite anions by H_2. This technique also helps to lower cytokine levels (TNF-α, IL-2, IL-7, and IL-10) in COVID-19 patients. Through the use of hydrogen inhalation as a treatment, lung injury can also be prevented (Waqas et al., 2020). Additionally, COVID-19 patients often experience organ failure, which can lead to death. Organ failure occurs due to an unstable internal environment in the body and increased levels of malondialdehyde in the lungs, along with other toxic substances. Clinical therapy with hydrogen can help with the activation of the antioxidant enzyme superoxide dismutase, which helps in eliminating toxic substances and damaged DNA from the body; it also stabilizes the internal environment, which enhances defense mechanisms in patients (Rana et al., 2020). Crystallographic analysis of class I MHC/peptide complexes has shown that the majority of charged peptide core residues are exposed in MHC complexes and are recognized by T-cell receptors (Rouzbahani et al., 2022). By boosting host immunogenicity, an appropriate multi-epitope vaccine can support the immune response and thus reduce the chance of reinfection (Rouzbahani et al., 2022; Sajid et al., 2022). It is crucial to identify T-cell epitopes quickly and accurately, but doing so can considerably reduce the amount of experimental labor required in carrying out culturing to determine the *in vitro* expression required to develop vaccines based on these epitopes. Additionally, repurposing of available drugs could also be an effective method for elimination of this infection.

For governments, NGOs, and the public

Governments and non-governmental organizations can also play an important role in generating awareness and in the elimination of ethical and religious myths regarding the vaccine. They can also provide funding to the research community so that money is not an limiting factor in the development of vaccines. Additionally, governments of all countries should focus on the vaccination of people all over the world, as vaccination of the maximum possible proportion of the global population is necessary to avoid the next variants of SARS-Cov-2 (Islam et al., 2022; Mattiuzzi and Lippi, 2022) and to achieve herd immunity. Governments should heed the WHO slogan that 'none of us is safe until all of us are safe.' Furthermore, a proper channel should be established for faster approval of effective vaccines. The public can also play a major role in the prevention of other variants of this virus.

Everyone should get vaccinated, and anyone who has any doubts regarding the production and efficacy of the vaccine should resolve these doubts by referring to the relevant sources. The public should also follow the guidelines provided by the government at times, as well as complying with social distancing, wearing a mask, following good hygienic practices, and avoiding social gatherings during pandemics.

Conclusion

Since its discovery, coronavirus has spread across the globe and emerged as a significant public health threat. At this point in the pandemic, with debate underway regarding vaccine efficacy and drug repurposing, a comparative study of vaccine efficacy is much needed to combat this disease. Screening, infection prevention, quarantining of ill people, and preventive self-isolation of contacts are crucial steps to reduce the number of new cases. The discovery of new variants each day serves as a sobering reminder that the world is still dealing with a pandemic and that another SARS-CoV-2 outbreak could happen at any time. The available vaccines should be compared on the basis of their efficacy against the different variants of the virus, and authorities should develop appropriate and strict guidelines to ensure the survival of humankind. Beyond vaccines, the scientific community should also focus on the use of convalescent plasma therapy for therapeutic purposes, as this could be very promising for low-income countries where vaccine production for a large population is not possible. The achievement of herd immunity requires vaccination of the wider population for the protection of immunocompromised and susceptible individuals. Therefore, the vaccination process should be accelerated and disruption should be avoided. Scientists should also examine the production of antibodies with the use of a combination of vaccines; their efficacy should be tested; and the chances of reinfection should also be studied. The public must maintain a high level of caution when hosting social events and make sure that everyone who qualifies has received all necessary vaccinations, including the third and/or booster dose. Finally, it is the duty of all of us, and not only governments or the scientific community, to combat this disease.

Author contributions

RR assisted with the conceptualization and design of the study. Planning of the study was supported by RR, RK, and SG. The manuscript was written by RR, RK, and TK. The final draft of the text, language corrections, and critical revision of the work were all completed by RR, DSR, and NKG. The submitted version of the article was reviewed and approved by all authors.

Acknowledgments

The authors are grateful to Sir Ganga Ram Hospital, Delhi, India, for providing the necessary support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 19 April 2023
DOI 10.3389/fphar.2023.1140494

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 09 January 2023
ACCEPTED 29 March 2023
PUBLISHED 19 April 2023

CITATION
Akash S, Hossain A, Mukerjee N, Sarker MMR, Khan MF, Hossain MJ, Rashid MA, Kumer A, Ghosh A, León-Figueroa DA, Barboza JJ, Padhi BK and Sah R (2023), Modified coptisine derivatives as an inhibitor against pathogenic *Rhizomucor miehei*, *Mycolicibacterium smegmatis* (Black Fungus), Monkeypox, and Marburg virus by molecular docking and molecular dynamics simulation-based drug design approach.
Front. Pharmacol. 14:1140494.
doi: 10.3389/fphar.2023.1140494

Modified coptisine derivatives as an inhibitor against pathogenic *Rhizomucor miehei*, *Mycolicibacterium smegmatis* (Black Fungus), Monkeypox, and Marburg virus by molecular docking and molecular dynamics simulation-based drug design approach

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During the second phase of SARS-CoV-2, an unknown fungal infection, identified as black fungus, was transmitted to numerous people among the hospitalized COVID-19 patients and increased the death rate. The black fungus is associated with the Mycolicibacterium smegmatis, Mucor lusitanicus, and Rhizomucor miehei microorganisms. At the same time, other pathogenic diseases, such as the Monkeypox virus and Marburg virus, impacted global health. Policymakers are concerned about these pathogens due to their severe pathogenic capabilities and rapid spread. However, no standard therapies are available to manage and treat those conditions. Since the coptisine has significant antimicrobial, antiviral, and antifungal properties; therefore, the current investigation has been designed by modifying coptisine to identify an effective drug molecule against Black fungus, Monkeypox,

and Marburg virus. After designing the derivatives of coptisine, they have been optimized to get a stable molecular structure. These ligands were then subjected to molecular docking study against two vital proteins obtained from black fungal pathogens: Rhizomucor miehei (PDB ID: 4WTP) and Mycolicibacterium smegmatis (PDB ID 7D6X), and proteins found in Monkeypox virus (PDB ID: 4QWO) and Marburg virus (PDB ID 4OR8). Following molecular docking, other computational investigations, such as ADMET, QSAR, drug-likeness, quantum calculation and molecular dynamics, were also performed to determine their potentiality as antifungal and antiviral inhibitors. The docking score reported that they have strong affinities against Black fungus, Monkeypox virus, and Marburg virus. Then, the molecular dynamic simulation was conducted to determine their stability and durability in the physiological system with water at 100 ns, which documented that the mentioned drugs were stable over the simulated time. Thus, our *in silico* investigation provides a preliminary report that coptisine derivatives are safe and potentially effective against Black fungus, Monkeypox virus, and Marburg virus. Hence, coptisine derivatives may be a prospective candidate for developing drugs against Black fungus, Monkeypox and Marburg viruses.

KEYWORDS

Black Fungus, Monkeypox, Marburg virus, molecular docking, admet, QSAR, molecular dynamic simulation, DFT

Introduction

The SARS-CoV-2 infection, which is responsible for the ongoing disease outbreak and has caused a rise in the percentage of COVID instances with the existing new wave, is a significant concern in terms of global health, particularly for immunocompromised individuals and elderly people. Ebola, Zika, Influenza, SARS-CoV, MERS-CoV-2, Monkeypox and Marburg are just some of the viral infections that have infected millions of humans, animals, and birds alike as either a seasonal epidemic, a pandemic, or a global health emergency (Mohapatra et al., 2021). In the post COVID-19 era or second phase of SARS-CoV-2, a deadly fungal pathogen identified as black fungus (particularly the species of *Mucormycosis*) infected a large number of SARS-CoV-2 affected patients in Indian and Bangladesh subcontinents.

When mucormycosis infects patients, they form black abnormalities in color; this is one of the reasons mucormycosis is sometimes referred to as "black fungus." Mucormycosis is mainly a group of molds containing filaments that belong to the mucoromycetes. This particular kind of pathogenic fungus grows almost exclusively on rotting vegetables, bread, dirt, and dust. Humans interact with these pathogens by breathing spores, consuming food that has been compromised, or inoculating exposed skin or sores. Scientists have discovered that black fungus may damage the primary organs of the human body, such as the liver, kidney, *etc.* Based on findings from the last two decades, mucormycosis has emerged as a scary fungal illness with elevated death rates among all other fungi infections (Dubey et al., 2021).

Amphotericin B, echinocandins, flucytosine, and azoles are the most commonly prescribed medications for infections caused by the fungus, and these categories of antifungal medications offer different mechanisms of action. Among the most effective forms of antifungal drugs, azole antifungals are considered first-line treatments for fungal infections because of their high activity level across a broad range of fungal strains and their systemic availability. The great concern now is the global resistance to antifungal drugs such as fluconazole-resistant *Candida albicans* (Marchaim et al., 2012), amphotericin B, and fluconazole-resistant *Candida auris* (Sarma et al., 2013). In addition, numerous antifungal medications have been documented to have adverse effects on host tissue. While azoles are often used to treat Aspergillosis, hepatotoxicity and visual disturbances have been reported as adverse effects. Besides, no established medication is available in the market for the treatment of black fungus.

Simultaneously, two more pathogenic viral infections have recently affected numerous people, which have occurred due to the Monkeypox and Marburg viruses. The spread of these two viruses might trigger another global health emergency and may turn into a global health crisis. Monkeypox is indigenous to West and Central Africa and is infected by a virus classified in the same clade as smallpox and cowpox (MacNeil et al., 2009). Historically, Monkeypox infections have been documented mostly in Central Africa, with the first occurrence confirmed in the Democratic Republic of Congo (DRC) in 1970. Human infections with the Monkeypox virus were infrequent outside Africa until April 2022. But recently, it has been happening all across the globe, and it is not clear how infections spread, what variables put people at risk, how the disease manifests in the body, or what the consequences of infection are. According to a few studies, the Monkeypox virus is transmitted from person to person through direct animal contact (Quarleri et al., 2022). The clinical features of MPXV are comparable to those caused by the smallpox virus. MPXV is categorized as a member of the genus Orthopoxvirus and has a double-stranded DNA genome. Comparatively, the MPXV genome has around 190 genes, while the orthopoxvirus genome contains approximately 200 genes (Wang et al., 2022). The symptoms of Monkeypox include a high temperature (38.5° to 40.5°C), fatigue, a rash, and a headache. Lymph node enlargement and the presence of hard, deep, well-circumscribed, umbilicated lesions are very suggestive (Duque et al., 2022).

Secondly, the Marburg virus, often known as MARV, is a zoonotic pathogen that may spread from animals to people and produce epidemics of severe infection. It is considered a member of a distinct genus and is classed as the filovirus family, which also includes the Ebola virus (EBOV). It is an encapsulated, negative-sense RNA virus, which may produce epidemics of a severe, sometimes deadly disease in people

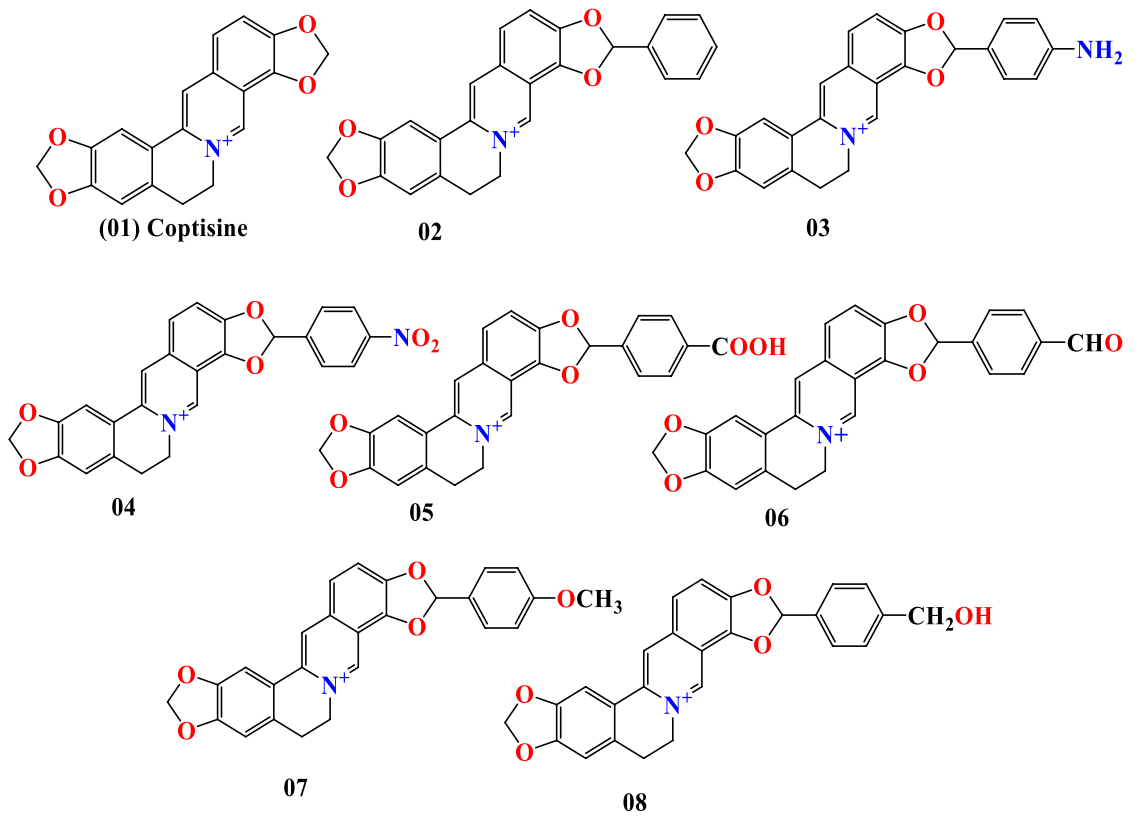


FIGURE 1
Chemical structure of coptisine and its derivatives.

(Zhao et al., 2022). Since its discovery in 1967, the Marburg virus (MARV) has been a significant cause of worry, with two crucial outbreaks occurring between 1998 and 2004 (Abir et al., 2022). In the Ashanti area of Ghana, there have been two confirmed fatal instances of the Marburg virus disease (MVD). The appropriate health authorities notified these patients of possible viral hemorrhagic fever (VHF) on 28 June 2022, and on 1 July 2022, they tested positive for the Marburg virus (Sah et al., 2022). Although these pathogenic infections are continuously happening, an effective treatment to manage them is lacking. Consequently, this urgently necessitates the development of new antifungal and antiviral drugs with more potent antifungal and antiviral effectiveness with fewer adverse effects.

Coptisine is a natural alkaloid that may be detected in Chinese goldthread. This alkaloid is used in traditional Chinese herbal medicine to treat digestion problems caused by pathogenic bacteria (Gobato et al., 2015). It has a wide variety of medicinal benefits, such as antidiabetic, antimicrobial, antiviral, anti-cancer (Wang et al., 2017). So, for the convenience of this research investigation, coptisine has been modified in addition to different functional groups to determine the probable antifungal, and antiviral efficacy and effectiveness. The synthesis of efficient medication in current medical research is a sophisticated process that calls for a substantial amount of resources, time, money, and human labor. As a direct consequence of this, the use of *in silico* methodologies has developed into a major tenet in the process of the discovery of novel bioactive compounds (Mohapatra et al., 2020). Their significance in drug development is that they are most effective in uncovering and exploring different potential drugs while minimizing costs and time.

Computational method and working procedure

Modified structure design of coptisine

Coptisine is a conventional quaternary alkaloid generated from the benzylisoquinolines through phenolic oxidation. Its chemical formula is $C_{19}H_{14}NO_4$, and it is synthesized from benzylisoquinolines by phenolic oxidation and bonding with the isoquinoline N-methyl group (Macáková et al., 2019). Structure-based drug discovery comprises creating and improving chemical structures to locate a potentially beneficial curative candidate for laboratory trials (Andricopulo et al., 2009). Following the development of the first possible lead chemical, optimization work has been carried out to ensure an efficient therapeutic candidate. It depends on a grasp of the three-dimensional structure of the substance and how its shape, properties, and charge cause it to engage with the target organism. It is also intended to treat the leads to the production of pharmacologically efficacies molecules. So, in this investigation, the primary molecule was coptisine, which was modified (01–08) in addition to different functional groups. The modifications are depicted in Figure 1.

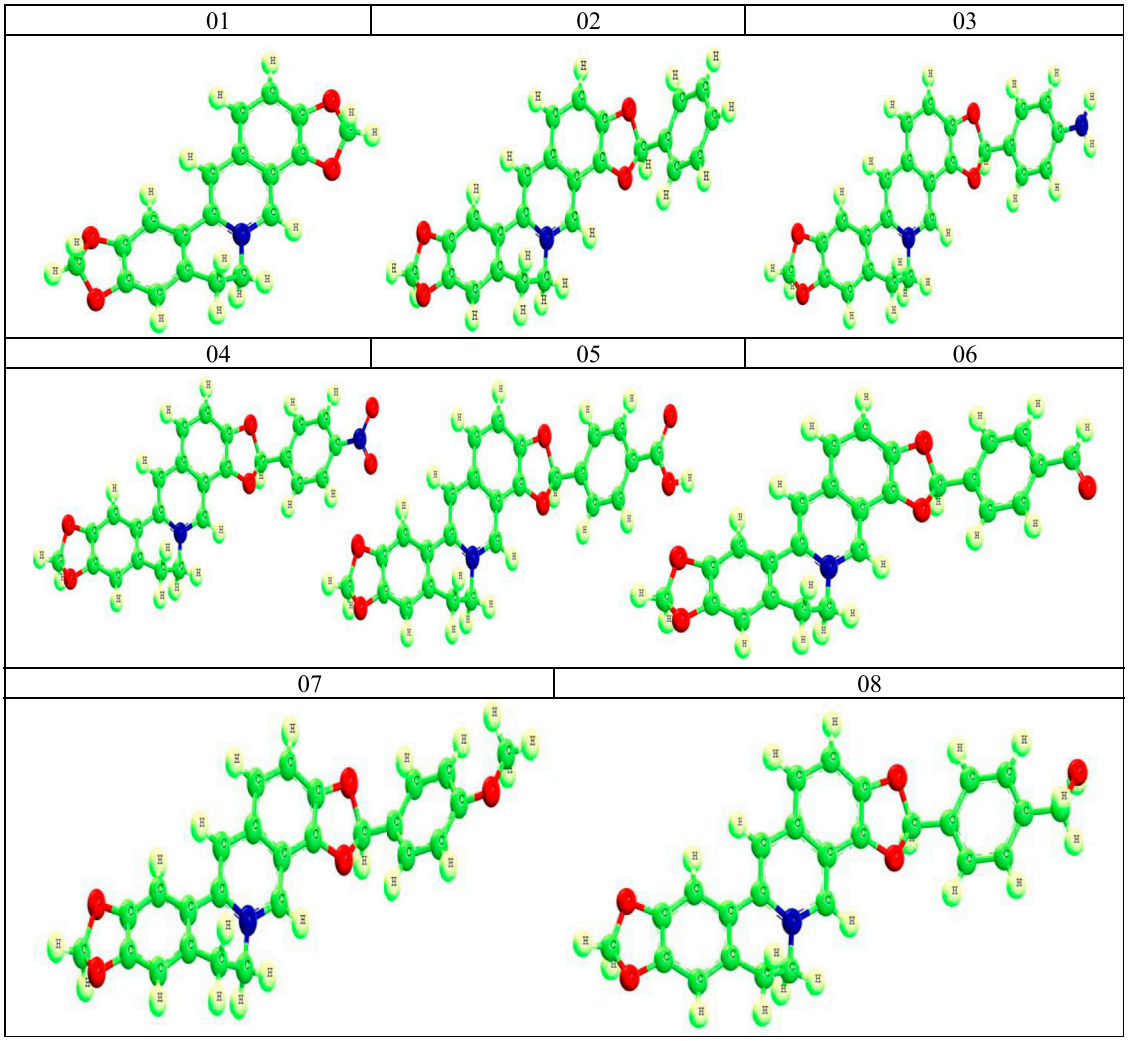


FIGURE 2
Optimized structures of Coptisine derivatives.

Since aromatic rings in compounds have a higher degree of freedom, they are able to engage more strongly with particular protein target. Because of presence in aromatic parts, they play a role in protein stability, and they are also thought to play a role in the enhancement of affinity and specificity in drug-like compounds, making them an important component in the development of therapeutics (Lanzarotti et al., 2020; Azam et al., 2021).

Optimization and ligand preparation

For geometry optimization, the Material Studio 08 version has been applied using B3LYP and the functional unit DFT procedure of DMoL3 code. This technique has been involved to achieve accurate results. Due to the existence of the electronegative atom, oxygen, the B3LYP functional and basis set DND was appropriately arranged. Following the completion of geometric optimization, the optimized lead compounds were saved as pdb files for further computational research, such as molecular docking, molecular dynamic simulation, and ADMET analysis (Kumer et al., 2022a). The Frontier molecular orbital features, HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital), were calculated using quantum mechanics approaches, known as density functional theory (DFT) in material studio.

The optimized structures are shown in Figure 2.

Determination of the data of Lipinski rule

The Lipinski rule of five is helpful for classifying substances into drug-like and non-drug-like groupings. Structural properties such as drug-likeness criteria have been used to more swiftly determine a compound's drug-like qualities. (Hydrogen bond acceptor, Hydrogen bond donor, TPSA, Bioavailability Index) The key emphasis of the Lipinski five Rule has been generated using Swiss ADME "(http://www.swissadme.ch/index.php)" (Azzam, 2023).

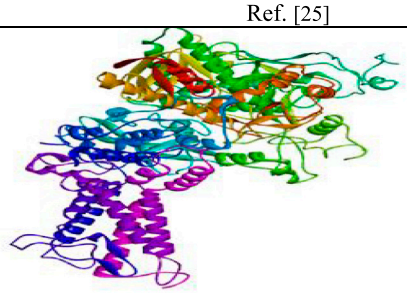
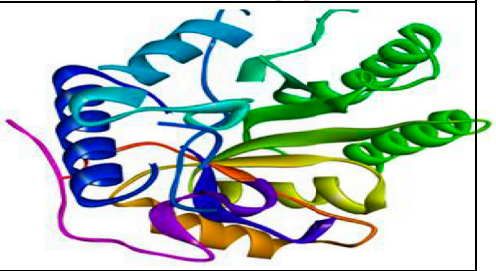
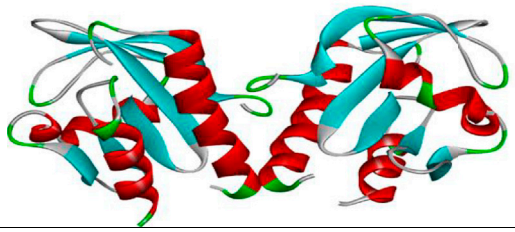
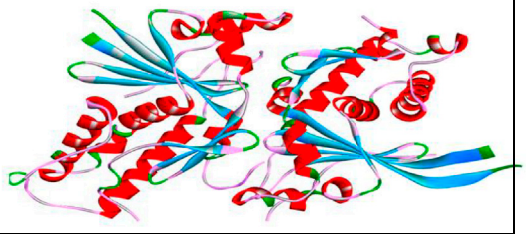
Mycobacterium smegmatis (PDB 7D6X)	*Rhizomucor miehei* (PDB ID 4WTP)
Resolution: 2.88 Å	Resolution: 1.30 Å
Method: Electron Microscopy	Method: X-RAY DIFFRACTION
Ref. [25]	Ref. [26]
	
Monkeypox Virus (PDB ID 4QWO)	Organism: Marburg virus (PDB 4OR8)
Organism: Monkeypox virus Zaire-96-I-16	Marburg virus - Musoke, Kenya, 1980
Method: X-ray diffraction	Method: X-ray diffraction
Resolution: 1.52 Å	Resolution: 2.65 Å
Ref. [27]	Ref. [28]
	

FIGURE 3
Three-dimensional protein structure of black fungal, Monkeypox, and Marburg virus target proteins used in this study.

In silico ADMET prediction

The determination of DMPK (drug metabolism and pharmacokinetics) investigation, also known as ADMET (absorption, distribution, metabolism, elimination, and toxicity) experiments, is a crucial component of the drug development process (Ajoy Kumer et al., 2022). Because many drugs cannot reach the final steps due to unfavorable effects and withdrawal from the market. The most reputable and dependable resource for forecasting the AMDET properties is the online database known as pkCSM, which can be found at ("http://biosig.unimelb.edu.au/pkCSM/") (Pires et al., 2015). ADMET features were finished using this repository and listed in the result and discussion section.

Preparation of target protein

The three-dimensional pathogenic fungal proteins *Mycobacterium smegmatis* (PDB 7D6X), *Rhizomucor miehei* (PDB ID 4WTP), and Monkeypox Virus (PDB ID 4QWO) & Marburg virus (PDB 4OR8) were collected from the PDB databank following link https://www.rcsb.org/. Pymol software version PyMolV2.3 scrutinized the protein retrieved from the PDB database (https://pymol.org/2/) (Rosignoli and Paiardini, 2022). All water molecules and unexpected ligands or heteroatoms were eliminated from the protein and preserved as PDB files to obtain the pristine protein. Finally, their energy minimization, and optimization is done with the help of swisspdbviwer (Akash, 2022). The protein information is displayed with a three-dimensional configuration in Figure 3.

Method for molecular docking

For molecular docking investigation, previously prepared optimized molecules and cleaned protein were uploaded to the PyRx virtual screening application, and run the application in the mode of AutoDock vina (Alam et al., 2016; Pawar and Rohane, 2021). During the docking experiment, each protein was wrapped by different grid box size such as, *Mycolicibacterium smegmatis* (PDB ID 7D6X) center X = 72.897, Y = 74.534, Z = 55.2881-dimension X = 158.417, Y = 155.152, Z = 119.437 and *R. miehei* (PDB ID 4WTP) center X = 48.057, Y = 41.746, Z = 51.503, dimension X = 14.321, Y = 27.735 and Z = 63.678, Monkeypox Virus (PDB ID 4QWO) center X = 12.4697, Y = 15.9818, Z = 16.0634, dimension X = 35.14496, Y = 37.6455, Z = 6.9662, and Marburg virus (PDB 4OR8) center X = 3.0194, Y = −0.7823, Z = 40.2835, dimension X = 38.36585, Y = 58.43047, and Z = 66.85451 When molecular docking was completely done, the docked molecule was loaded to the Pymol software for making them as complex file, and finally discovery studio version 2021 for viewing and analyzing the outcome of protein-ligand binding, Hydrogen bonding, 2d picture of active sites, solvent surface area, ionizability, and aromaticity.

Intermolecular hydrogen bonds between protein and ligand exhibit that 04, 07, and Fluconazole build hydrogen bonds with

the residue of the catalytic domain. Fluconazole showed maximum hydrogen bond contact rather than 04 and 07. Eventually, all analyses from the M.D. simulations suggest that 04 and 07 are more stable than Fluconazole and performed a few conformational changes of the protein.

QSAR and pIC_{50}calculation

To establish a statistically valid method for the prediction of the bioactivities of different chemical constituents, quantitative structure-activity relationships (QSARs) have been employed (Roy, 2007). QSAR is a computer modeling technique that reveals correlations between the structural features of bioactive molecules and the biological activities of such substances. The Chemdesk online webtool "http://www.scbdd.com/chemdes/" and the following multiple linear regression (MLR) equation was implemented to calculate the pIC_{50} values. Before calculation the pIC_{50}, the MLR equation was developed in excel sheet, and inputted the following data which was obtained from Chemdesk.

pIC_{50} (Activity) = −2.768483965 + 0.133928895 × (Chiv5) + 1.59986423 × (bcutm1) + (−0.02309681) × (MRVSA9) + (−0.002946101) × (MRVSA6) + (0.00671218) × (PEOEVSA5) + (−0.15963415) × (GATSv4) + (0.207949857) × J) + (0.082568569) × (Diametert) (Siddikey et al., 2022).

Molecular dynamic simulation

The dynamical behavior of nucleic acids, mutant proteins, protein-ligand complexes, and protein-protein interaction is available *via* molecular dynamics (MD) simulation. For finding the stable ligand binding pocket, it is a very handy tool (Ozalp et al., 2018). YASARA dynamics version 21.6.17 was used to run the MD simulation. The hit's best pose from the virtual screening was chosen, and YASARA Structure's scene mode was then set up using the default option. The scene mode was put through MD simulations using the YASARA Structure macro's default parameters for MD run (Prasasty and Istyastono, 2020). Force fields, which use periodic functions for bond rotations, springs for bond lengths and angles, and Coulomb's equation for ionic interactions to calculate the forces exerted on each atom started from the initial setting. The AMBER14 force field is widely applied for describing macromolecular systems (Ozalp et al., 2018; Siraj et al., 2021). The complex was positioned in the middle of a periodic standard cubic box that also included other atoms and the model. To equalize the system's charges, Na+ and Cl-ions were inserted to the Transferable Intermolecular Potential3 (TIP3P) water model (Zhang et al., 2020). For energy minimization, every system conducts with the steepest gradient approach (5000cycles). A periodic boundary condition was engaged to play simulations, where the cell size was 10 Å broad than protein size in all events. MD simulations and electrostatic interactions were performed by using particle-mesh Ewald (PME) methods and prescribe some physiological conditions at, 0.9% NaCl, pH 7.4 (Krieger et al., 2006). The setup also used 298 K and one atmosphere, respectively, for temperature and pressure parameters. Finally, 100 ns of MD simulations were executed, and YASARA MACRO's default script was used to manage further analysis (Shakil, 2021).

Results and discussion

Lipinski rule and pharmacokinetics for oral drug

In the initial stages of drug development, the concepts of Pharmacokinetics and Lipinski's rule give significant assistance that may enhance the possibilities of a biochemical entrance and therapeutic clearance. The predictions of Pharmacokinetics and the drug-likeness properties of medicinal compounds by Swiss ADME (Azzam, 2023). To design a novel medicine for black fungal species infection, Pharmacokinetics and drug-likeness have been investigated comparably to Lipinski's rule and drug activity utilizing the online source following link https://www.sib.swiss/ furnished by the Swiss Institute of Bioinformatics. This rule was established based on the five rules including the molecular weight being less than 500 g/mol, the calculated octanol/water partition

TABLE 1 Summary of ligands calculated results for Lipinski rule, pharmacokinetics and drug likeness activities.

Ligand No	Molecular weight	Number of rotatable bonds	Hydrogen bond acceptor	Hydrogen bond donor	Topological polar surface area (Å^2)	Consensus Log$P_{o/w}$	Lipinski rule		Bioavailability score
							Result	Violation	
01	320.32	00	04	00	40.80	2.40	Yes	00	0.55
02	396.41	01	04	00	40.80	3.57	Yes	00	0.55
03	411.43	01	04	01	66.43	3.07	Yes	00	0.55
04	441.46	02	04	02	86.62	3.00	Yes	00	0.55
05	440.41	02	06	01	78.10	3.18	Yes	00	0.55
06	424.42	02	05	00	57.87	3.31	Yes	00	0.55
07	426.24	02	05	00	50.03	3.61	Yes	00	0.55
08	426.44	02	05	01	61.03	3.15	Yes	00	0.55

TABLE 2 Chemical reactivity descriptor analysis.

S/N	I = – HOMO	A = -LUMO	Energy gap E (gap) = I-A (eV)	Chemical potential $(\mu) = \frac{I+A}{2}$	Hardness $(\eta) = \frac{I-A}{2}$	Softness $(\sigma) = \frac{1}{\eta}$
01	−9.886	−1.022	8.864	5.454	4.432	0.2256
02	−9.883	−1.021	8.862	5.452	4.431	0.2257
03	−9.878	−1.019	8.859	5.4485	4.429	0.2258
04	−10.008	−1.177	8.831	5.5925	4.415	0.2265
05	−9.993	−1.139	8.854	5.566	4.427	0.2259
06	−9.995	−1.141	8.852	5.568	4.427	0.2259
07	−9.974	−1.129	8.845	5.5515	4.422	0.2261
08	−9.979	−1.134	8.845	5.5565	4.423	0.2261

TABLE 3 Binding affinities of docked ligand calculated against Black Fungus.

Drug molecules No	*Rhizomucor miehei* (PDB ID 4WTP)	*Mycolicibacterium smegmatis* (PDB ID 7D6X)
	Binding Affinity (kcal/mol)	Binding Affinity (kcal/mol)
01	−9.4	−10.4
02	−11.0	−11.7
03	−10.5	−10.7
04	−10.4	−12.8
05	−10.7	−11.1
06	−10.4	−10.8
07	−10.5	−12.2
08	−10.1	−11.0
Fluconazole	−7.0	−8.0

coefficient being less than 5 (LogP 5), The number of hydrogen bond donors being less than 5, and the number of hydrogen bond acceptors (particularly N and O atoms) is not more than 10. If the drug-like biomolecules had adhered to this concept, then they should have reflected to use as oral medication. Besides, the topological polar surface area (TPSA) is another useful molecular biomarker in drug discovery and development. It calculates the surface area of a polar or hydrogen-bonding molecule, which might impact solubility, permeability, and other pharmacokinetic features, which anticipate the capability to cross the cell membranes since molecules with high TPSA values may have restricted membrane permeability. Literatures studies reported that the compounds having TPSA values larger than 140 $Å^2$ are unlikely to permeate cell membranes, while compounds with TPSA values less than 90 $Å^2$ may be able to cross the blood-brain barrier (BBB). Our reported molecules have shown that the TPSA values are less than 90 $Å^2$. So, they might cross the BBB, based on this finding (Prasanna and Doerksen, 2009). It is evident from Table 1 that the Lipinski criterion is adhered to by all of the compounds and identified as potential medications. The amount drugs are assimilated into the systemic circulation after it has been administered are called bioavailability. The typical range of bioavailability depend on which routes, the drug is taken by the patient. Our reported molecules have shown the bioavailability score is 0.55 for all compounds, which means 55% of drugs might be present in systemic circulation after administration (Chen et al., 2022).

Chemical descriptor (HOMO-LUMO) calculation

HOMO stands for the highest occupied molecular orbital, while LUMO stands for the lowest unoccupied molecular orbital. The molecular orbitals (HOMO-LUMO) and chemical reactivity descriptors that are conceived by the computer program, and they are mathematical representations of the different properties which present in the chemical structures (Alam et al., 2018). These estimations of chemical properties are obtained by utilizing the B3LYP functional to material studio, and it is mostly related to HOMO-LUMO energy gap. The optimal HOMO-LUMO energy

TABLE 4 Binding affinities of docked ligand calculated against Monkeypox and Marburg virus.

Drug molecules No	Monkeypox virus (PDB ID 4QWO)	Marburg virus (PDB 4OR8)
	Binding Affinity (kcal/mol)	Binding Affinity (kcal/mol)
01	−8.3	−8.5
02	−8.5	−8.7
03	−9.3	−8.3
04	−9.0	−8.6
05	−10.8	−8.5
06	−9.5	−8.3
07	−9.8	−8.2
08	−9.2	−8.3
Standard (Acyclovir)	−6.3	−5.8

gap for organic compounds is between −6.00 and −9.00 eV and is acknowledged as perfectly fitting for organic molecules (Nath et al., 2021). The energy gaps of molecular orbitals are explored to evaluate the electrical conduction capabilities of atoms and molecules. Good physical and chemical stability can be maintained through the use of energy gaps. The high chemical stability must be depended on the size of the HOMO-LUMO gap (Kumer et al., 2019). Compounds with a small energy gap have a greater atomic system but lower chemical stability due to HOMO-LUMO being close to each other. According to this research, the E-gap spans from 8.831 eV to 8.864 eV. This shows a large E-gap, which indicates that the molecules in consideration have better chemical stability and a lower atomic system. The chemical potential, hardness, and softness are all valuable parameters for determining the therapeutic potential of biologically active molecules. Usually, the softness of drugs like molecules should be lower than the hardness of the drug. The absorption rate is proportional to the lower softness, and the hardness must be at around 4.000 kcal/mol for optimal biological flexibility (Akter and Bhuiyan, 2022). Here, softness ranges from 0.2256 to 0.2265, and hardness ranges from 4.415 to 4.4427. So, in our current investigation, all the coptisine derivatives have greater hardness and softness scores than the standard; these mentioned drugs may take longer to break down in the physiological system. The chemical potentiality varies from a minimum of 5.4485 eV to a high of 5.5925 eV, with all these values clustering close to the standard value of 8.831 eV (See Table 2). Finally, it is concluded that all derivatives of coptisine are potential and better stable according to this finding.

Molecular docking analysis against black fungus

Docking is considered one of the most promising techniques in structure-based drug design (Fischer et al., 2021). This technique calculates the preferred orientation of a compound and when it is attached to protein with ligands to develop a stable combination (El-Demerdash et al., 2021; Ouassaf et al., 2021). Docking may also show the signatory of small molecule ligand on appropriate target region during the formation of the drug-protein complex. H-bonding and hydrophobic bonding are the essential factors in docking values because they perform a substantial role in structurally based medication development (Cichero et al., 2021). The substance is categorized as a standard medication when the docking score exceeds −6. 000 kcal/mol (Rahman et al., 2022a; Kumer et al., 2022b). In this investigation, the coptisine derivatives show outstanding binding affinities against both species of black fungus. Specifically, the binding affinities range from −9.4 kcal/mol to −11.0 kcal/mol against *R. miehei* (PDB ID 4WTP). Once a significantly improved binding affinity has been achieved compared to *R. miehei,* another species of black fungus was picked up (*Mycolicibacterium smegmatis*) and performed molecular docking experiment. Then, it is observed that the binding affinity is about −10.4 kcal/mol to −12.8 kcal/mol. In each condition, Fluconazole is used as a standard drug, and in comparison, newly developed coptisine derivatives have achieved better binding energy mentioned in Table 3**.** So, these reported molecules could be used as potential inhibitors for treating black fungal species infection.

Binding affinities analysis against pathogenic Monkeypox and Marburg virus

Historically and literature findings reported that coptisine has a broad spectrum of pharmacological effects such as antidiabetic, antimicrobial, antiviral, anti-cancer (Wang et al., 2017). So, based on the literature studies and literature, Monkeypox, and Marburg virus are also included in this research to analyze what types of activities presented the synthetic derivatives of coptisine against the Monkeypox and Marburg virus. So, the molecular docking was conducted, and it was reported that the binding affinities against Monkeypox virus (PDB ID 4QWO), −8.3 kcal/mol, −8.5 kcal/mol, −9.3 kcal/mol, −10.8 kcal/mol, −9.5 kcal/mol, −9.8 kcal/mol, and −9.2 kcal/mol in ligands (02–08). Besides that, the binding affinities ranges against Marburg virus (PDB 4OR8) is −8.3 kcal/mol to −8.7 kcal/mol for ligands (01–08). As there is no medication against Monkeypox

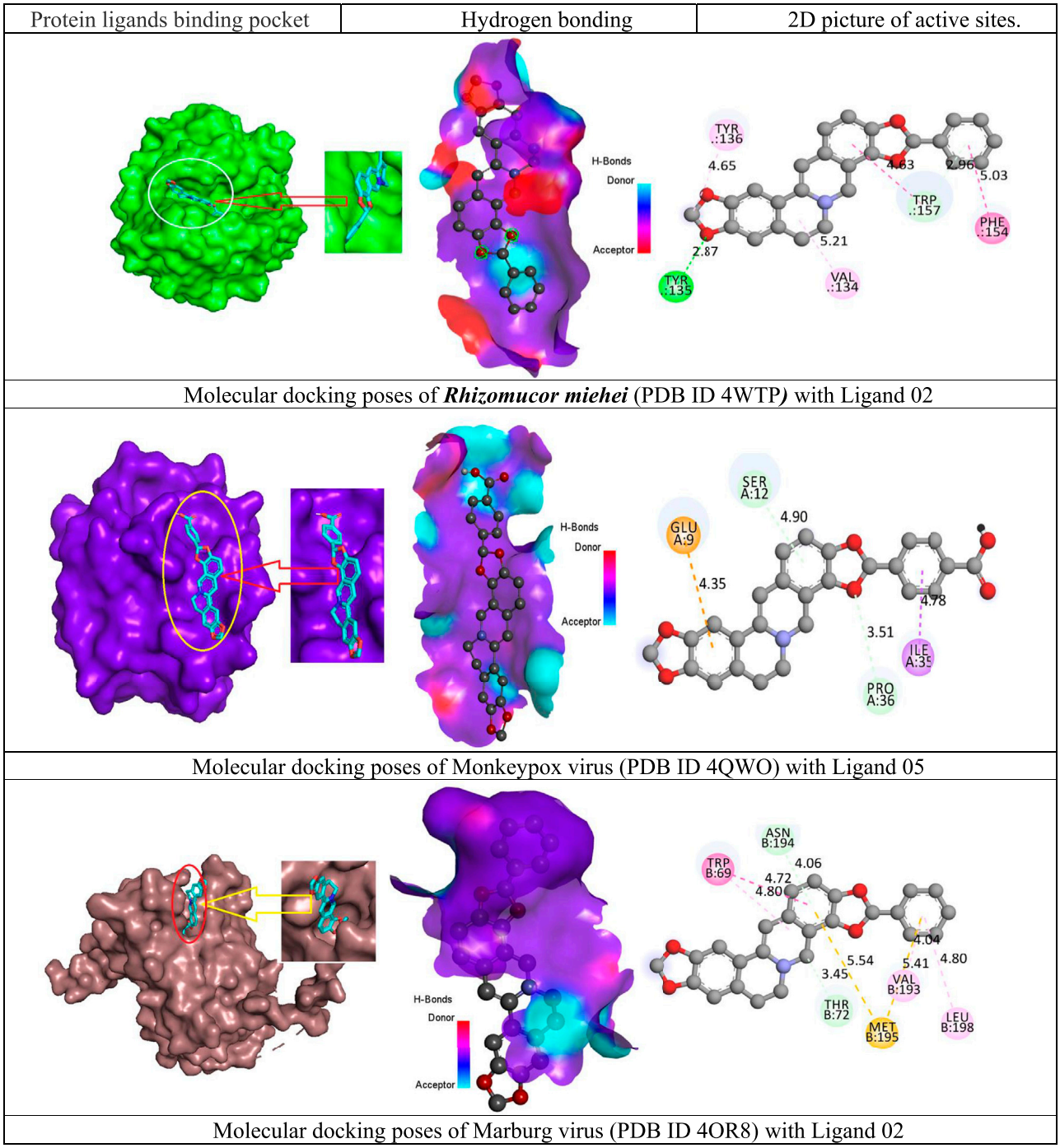


FIGURE 4
Docking interactions between the proposed compound and targeted pathogen.

and Marburg virus, we have considered an established antiviral and performed with these two pathogens to compare with our studies drugs. Overall findings against Monkeypox and Marburg virus are determined as outstanding affinities compared to standard (acyclovir) showing in Table 4. So, they might be suggested as potential drugs for inhibiting Monkeypox and Marburg virus disease.

Protein-ligand interaction and molecular docking pose

The ligand-protein interaction is essential when developing a novel medicine since it reveals how well a drug will react to the protein of a black fungus or any targeted binding receptor. The interaction of the new drug candidate with the *R. miehei* (PDB ID

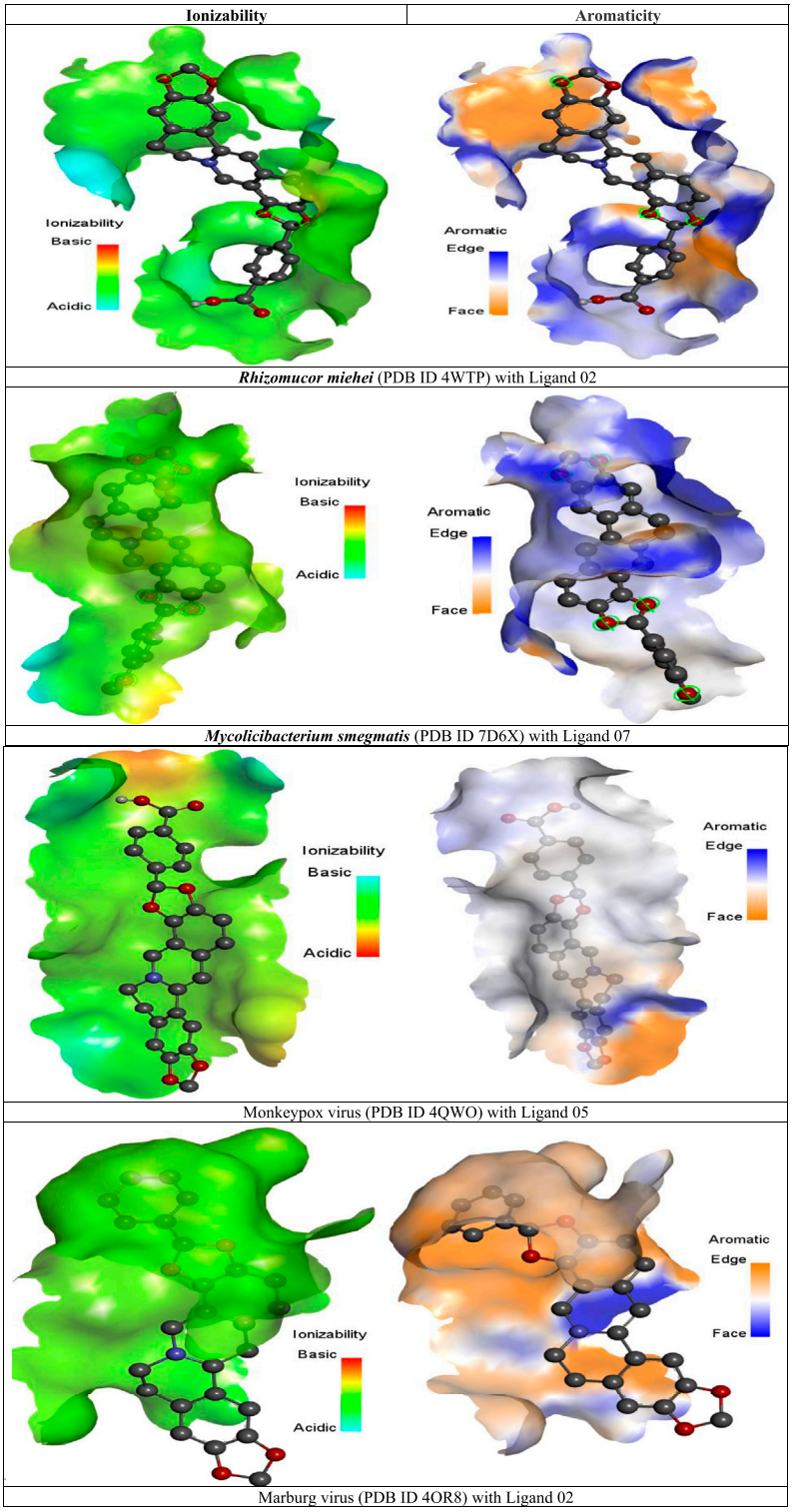


FIGURE 5
Graphical illustration of ionizability, and aromaticity analysis.

4WTP), *Mycolicibacterium smegmatis* (PDB ID 7D6X) of black fungus, and Monkeypox and Marburg virus have been explored with 2d active residue and hydrogen bonding system. Figure 4 demonstrates that there are different sorts of bonds, especially H-bond and hydrophobic bonds are denoted in most cases. However, the Van der Waal bond is not prevalent for all medications. The active is prediction TYR:135, VAL: 134, PHE: 154, TRP:157, TYR:136, in ligand 02 against *R. miehei,* LEU A:432,

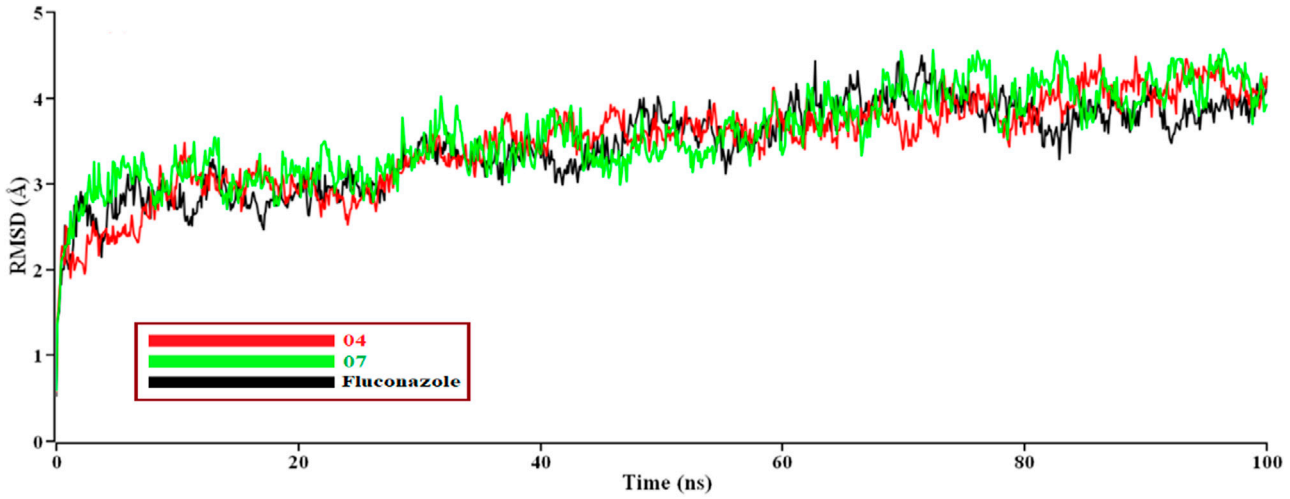


FIGURE 6
The time series of the RMSD of Cα atoms for protein *Mycobacterium smegmatis* (PDB ID 7D6X) and ligand. Here, red and green lines denote the 04 and 07 complexes, and the black lines denote the subjected drug Fluconazole, respectively.

GLU A:413, ARG A:423, HIS A:46, ALA A:50 against *Mycolicibacterium smegmatis* in ligand 07, similarly, SER A:112, GLU A:109, PRO A:36 against Monkeypox virus in ligand 05, and TRP B:69. ASN B: 194, THR B:72, MET B: 195, VAL B:193, LEU B: 198 against Marburg virus in ligand 02 mentioned in Figure 4.

Ionizability and aromaticity analysis

Ionizability

Ionization is a term used in science and chemistry to describe any mechanism by which electrostatic interactions particles or atoms may be switched to electrically charged particles or atoms (ions) while receiving or shedding electrons (Aleksandrov et al., 2010). The digestive system's epithelial cells are responsible for keeping the stomach healthy. To get into the bloodstream, a medication must penetrate through it or penetrate endothelial tissues. The cell membranes of certain medicines may operate as a barrier. Semi-permeable membranes are made up of phospholipid bilayers. Extremely tiny, uncharged substances may penetrate pristine lipid bilayers. Because ionic compounds are electrified, the assimilation of a component will be affected whether it is discharged or not. While molecules with positive charges have a higher solubility, those with negative charges have higher permeability. Exchange proteins and channels let certain chemicals pass from the lumen into the bloodstream more efficiently (Mannens et al., 2002).

In the illustration of ionizability, the red color represents acidic, the sky blue is regarded as basic, and the green color indicates neutral or slightly acidic or basic (Figure 5). But our investigation suggested that almost all the molecules have a greater possibility of neutralizing, which regards, they could rapidly penetrate semi-permeable membranes and reach the bloodstream or systemic circulation.

Aromaticity

In order to produce drugs reasonably, one must be able to anticipate and optimize the non-covalent interactions between organic ligands and protein (Brylinski, 2018). The aromatic arrangement has indeed been established for a long time as one of the primary factors of ligand-protein integrations that are responsible for maintaining–chemical bonding (π-π) (Kar et al., 2013). Based on the illustration of aromaticity, it can be seen that the edge and face of the engagement among pharmaceuticals acting as ligand and the protein of the black fungus, together with the pocket, demonstrate how the ligand has coupled with the peptide and where it has generated a bonding.

Molecular dynamic simulation

For understanding the nature of structural stability and flexibility, some selected compounds (04, 07, and fluconazole) have performed MD simulation (Based on maximum docking score with proteins); here, Fluconazole has been used as a standard reference medication, and we performed M.D. simulations for 100 ns. RMSDs of the Cα atoms for protein and ligand were calculated and plotted time-dependent (Figure 6) (Junaid et al., 2019).

Evaluate protein behavior during M.D. simulation; as seen in the plot, 07 complexes showed high fluctuation in RMSD at 95 ns–97 ns, indicating complex are not perfectly stable as complex 04 and Fluconazole. This point indicates that at 95 nano second to 97 nano second, the RMSD of complex (07) showed high fluctuation, which means lower stability compare to complex 04 and Fluconazole, or deviation from the complex 04 and Fluconazole. So, at 95 nano second to 97 nano second, the complex 07 has shown lower stability. Other protein-ligand complex results were closer to the 07 value also obtained in the graph for better results on how 04, 07, and Fluconazole influence the binding mode with *Mycobacterium*

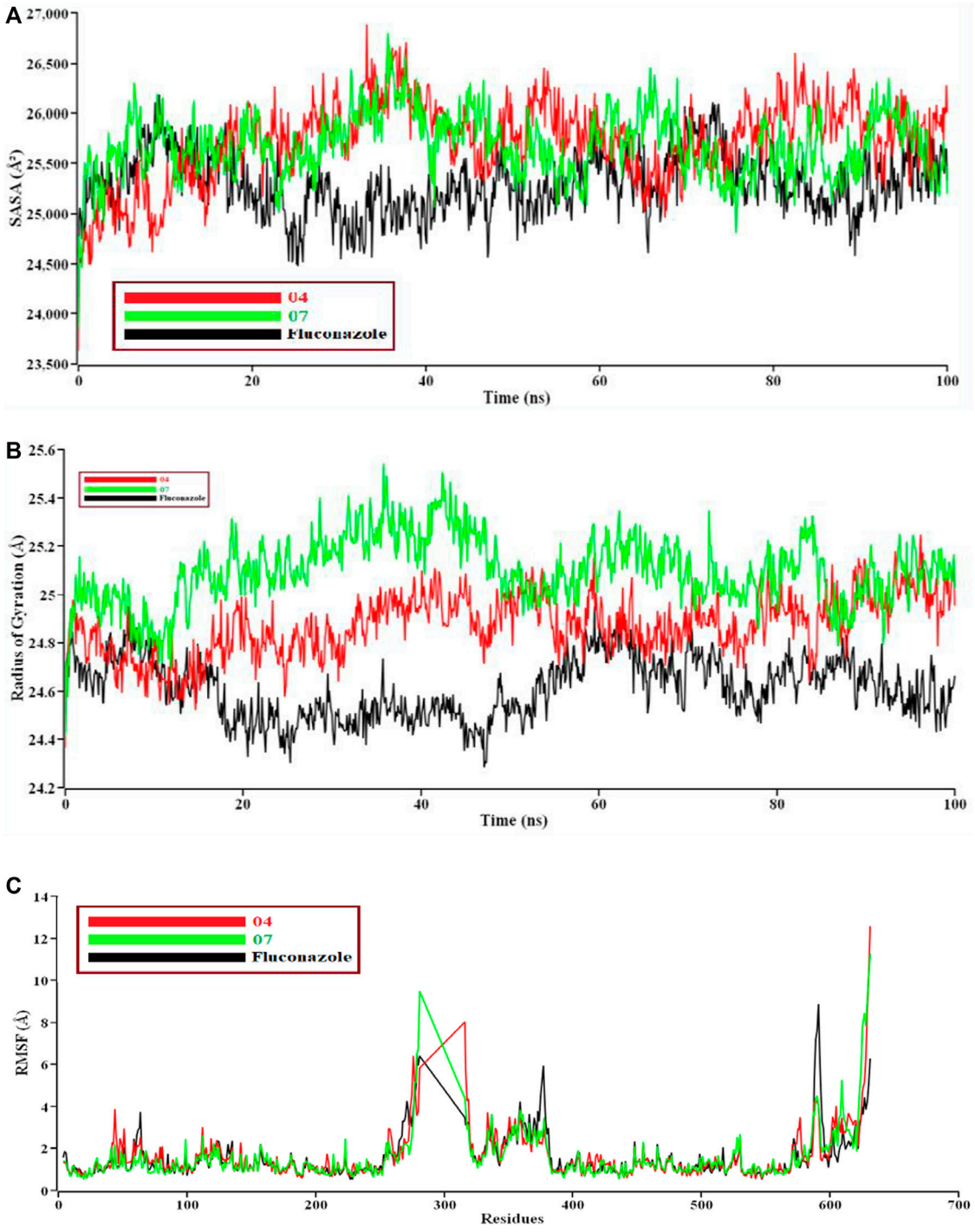


FIGURE 7
The structural behavior changes of protein employing a) solvent accessible surface area (SASA), 8b) radius of gyration, and 8c) root means square fluctuations (RMSF) analysis. Here, the red line indicates 04, and the green and black lines indicate 07 and the Fluconazole complex, respectively.

smegmatis (PDB ID 7D6X). The structural change of the three complexes was tested through the root mean square fluctuation (RMSF), the radius of gyration, and the solvent-accessible surface area of the protein-ligand complex (Figure 6).

If solvent enters into the binding site, the pocket can be destroyed. We need protein-ligand tight interaction. (Figure 7A). represents 04 compounds showed high SASA value after 33 ns of simulation, it may not reduce the protein expansion. In contrast, (Figure 7B), demonstrates the radius of gyration value; 07 compounds showed high value than 04 and Fluconazole, denoting loose packing of protein structure. RMSF value (Figure 7C) reflects the flexibility of the whole residue in the protein. High fluctuations were performed in some positions, resulting in better results, ranging from 631–635 residues,

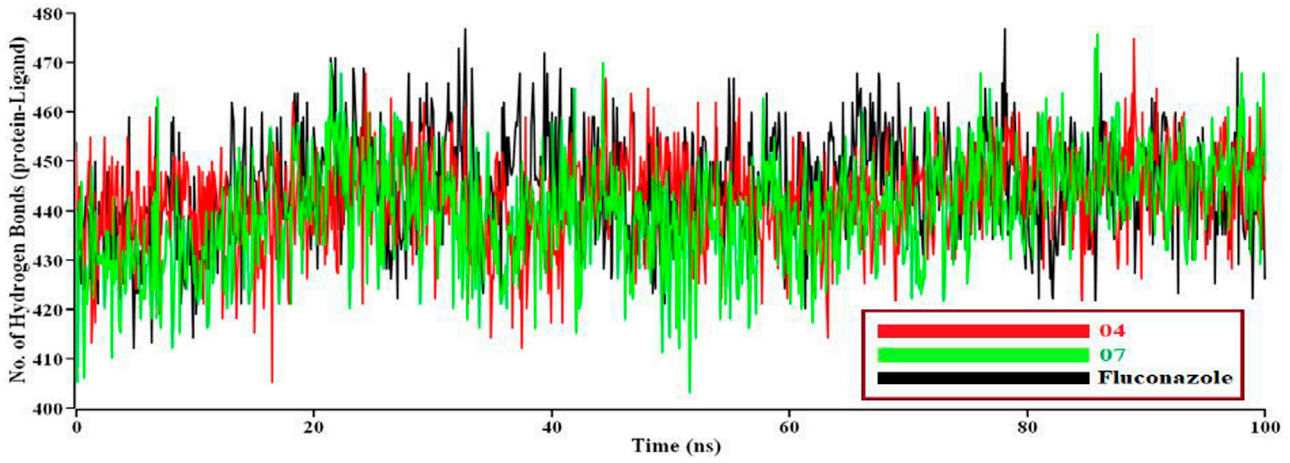


FIGURE 8
A total number of hydrogen bonds formed between protein-ligand complexes during M.D. simulations. Here, the red line indicates 04 complexes, and the green and black lines indicate 07 and the Fluconazole complex, respectively.

TABLE 5 Data of QSAR and pIC_{50}.

Ligand	Chiv5	bcutm1	MRVSA9	MRVSA6	PEOEVSA5	GATSv4	J	Diametert	pIC_{50}
1	2.852	4.121	10.772	42.092	00	0.839	1.24	11	4.87
2	3.476	4.127	10.772	77.987	30.332	1.103	1.055	15	5.31
3	3.556	4.128	16.46	71.921	00	1.086	1.039	16	5.08
4	3.572	4.128	16.46	82.035	00	1.033	1.009	17	5.14
5	3.593	4.128	16.772	87.485	12.33	1.037	1.009	17	5.20
6	3.576	4.128	17.059	77.485	24.65	1.063	1.022	17	5.30
7	3.572	4.128	10.772	71.921	00	0.996	1.022	17	5.31
8	3.614	4.128	10.772	77.485	24.265	1.063	1.022	17	5.45

including the 04 and 07 complexes. Finally, the hydrogen bond interaction formed within protein and ligand showed in Figure 8.

QSAR an pIC_{50} analysis of coptisine derivatives

The trustworthy *in silico* approach known as quantitative structure-activity relationship (QSAR) was established to simulate the bioactivity utilizing the chemical structure. Before the exact chemical synthesis begins, it is possible to make predictions about the bioactivity of the prospective bioactive compounds. The projection is built based on the structural characteristics involved in bioactivity. Through the use of QSAR models, the structural and molecular features may be expressed (Ahmad Pasha et al., 2010).

The mathematical QSAR model working of multiple linear regression which had been built by analyzing the computational IC50 values similar to pIC50 [-log (IC50)]. From the ChEMBL open-source website (Gaulton et al., 2012). ChEMBL was developed by more than a million bioactive molecules and was founded from the eight most approved biological characteristics, including hiv5, bcutm1, MRVSA9, MRVSA6, PEOEVSA5, GATSv4, J, and diameter, among others. Moreover, the IC_{50} values are closely correlated to its structural chain, and this value changes with the modification in its side chain. The score of IC_{50} increases as the molecular weights of the medicine increases, but it must remain under 10.00 in order to be considered an efficient medication. Mentioned the Table 5, it has been reported that the pIC_{50} value is reported as 4.87–5.45, which falls in acceptable ranges and could be said to be potential drug (Rahman et al., 2022b). So, the pIC50 value of drugs (01–08) should be an efficient drug since the value is not more than 10.0.

In silico ADMET data prediction

Drug metabolism and pharmacokinetics (DMPK) studies are a key component of the drug development process. Investigations like this popularly stand for ADMET (Absorption, Distribution, Metabolism, and Toxicity) because they explore how medications are metabolized and removed from the physiological system. These experiments contribute to

TABLE 6 Summary of calculation of ADMET results for selected 08 compounds.

S/N	Absorption			Distribution	Metabolism		Excretion			Toxicity		
	Water solubility Log S	Caco-2 Permeability x 10^{-6}	Human Intestinal Absorption (%)	VDss (human)	CYP450 1A2 Inhibitor	CYP450 2C9 Substrate	Total Clearance (mL/min/kg)	Renal OCT2 substrate	Max. tolerated the dose log mg/kg/day	Skin Sensitization	Hepatotoxicity
01	−2.948	1.195	99.223	0.755	Yes	No	1.287	Yes	−0.21	No	No
02	−3.22	1.019	100	−0.96	Yes	No	1.217	No	0.57	No	No
03	−3.979	0.989	96.972	0.20	Yes	No	1.378	No	0.307	No	No
04	−4.187	0.977	98.11	0.012	No	No	1.034	No	0.181	No	No
05	−3.562	0.974	97.21	−0.331	Yes	No	0.874	No	0.449	No	No
06	−4.453	0.654	99.971	0.189	Yes	No	1.257	No	0.311	No	No
07	−3.831	0.692	99.90	0.018	Yes	No	1.291	No	0.401	No	No
08	−4.043	0.643	97.701	0.181	Yes	No	1.389	No	0.325	No	No

the process of assessing the efficacy of a potential new drug. For instance, the absorption, or the amount of drug and how quickly it is absorbed into the body, distribution refers to how the drug is distributed within the body and how fast and broadly it has been supplied. The term "metabolism" refers to the pace at which a drug is broken down and its mechanism of action; the metabolites form it generates, the elimination, has been defined as outlining how and how quickly the medicine departs the body. Finally, the toxicity-has been described. Whether or not it is beneficial or harmful to human use (Selick et al., 2002; Chalkha et al., 2022).

The ADMET profile of the medicine, as determined by a search of an online database called pkCSM and displayed in Table 6 for the purpose of computational prediction. The result reported that the aqueous solubility ranges −2.948 to −4.453, which means they are moderate to highly soluble (Tallei et al., 2022); the Caco-2 Permeability ranges were found to be 0.654–1.095, while all of the drug candidates have a quick absorption rate in the human digestive tract, which is indicated by a range of values between (96.972%–100%). Besides, around 08 out of 07 drug candidates may inhibit by CYP450 1A2 Inhibitor, and none of them can be Substrate by CYP450 2C9. The Total Clearance 0.874 mL/min/kg–1.389 mL/min/kg and Max. tolerated dose 0.449 log mg/kg/day while the minimum tolerated dose −0.21 log mg/kg/day. Finally, they all are free from skin sensitization and hepatotoxicity. The overall ADMET, and Pharmacokinetics properties is satisfied to be potential medication. These drug candidates are suggested to explored further laboratory experiment such as synthesis, and *in vitro* or *in vivo* experiment.

Conclusion

Currently, there is a limited number of drugs available against pathogenic black fungus (*R. miehei* and *Mycolicibacterium smegmatis*), Monkeypox, and Marburg virus. So, in this innovative and advanced *in silico* investigation, various *in silico* approaches are applied to find potential inhibitors against two species of pathogenic black fungus (Rhizomucor miehei and Mycolicibacterium smegmatis), Monkeypox, and Marburg virus by modification of coptisine with different molecular modeling approaches. Firstly, design the coptisine derivatives by structural modification. Then, the molecular docking, drug-likeness, ADMET, Molecular dynamic simulation, and QSAR, etc., are gradually conducted. The highest docking score reported for a range such as this is 9.4 kcal/mol to 11.0 kcal/mol, and it is for *R. miehei* (PDB ID 4WTP). Furthermore, it has an over the potential of −10.4 kcal/mol to 12.8 kcal/mol for *Mycobacterium smegmatis* (PDB ID 7D6X). Similarly, the maximum affinities against Monkeypox virus (PDB ID 4QWO), −8.3 kcal/mol, −8.5 kcal/mol, −9.3 kcal/mol, −10.8 kcal/mol, −9.5 kcal/mol, −9.8 kcal/mol, and −9.2 kcal/mol in ligands (02–08). Besides that, the binding affinities ranges against Marburg virus (PDB 4OR8) is −8.3 kcal/mol to −8.7 kcal/mol for ligands (01–08).Correspondingly, compared to FDA-approved standard Fluconazole and standard (Acyclovir). It is acceptable to conclude that the evaluated bioactive coptisine derivatives have significantly superior binding affinity and dynamic molecular accounting to indicate their stability. Besides, the quantum calculation HOMO-LUMO gap is also acceptable ranges. After completing the comprehensive study, it was observed that all of the medication candidates exhibited the following characteristics: better solubility in water, absence of any toxic effect; high

gastrointestinal (G.I) absorption rate; fulfillment of the Lipinski rule; and drug-like aspects. Therefore, these mentioned drug candidates have been determined to be the effective medication for inhibition of the deadly black fungus pathogen, Monkeypox, and Marburg virus presumably, if these pharmaceuticals are put in clinical trial or laboratory investigation, they will be substantially fewer negative impacts than those of established medications according to our computational data.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

Conceptualization: SA, AH, and NM writing, and original draft preparation: SA, AH, MS, MK, AK, AG and MR; Writing: SA, .RS, MK, AK, AG AH, and NM editing: DL-F, JB and BP supervision: RS; All authors have reviewed and approved the final version of the manuscript prior to submission.

Acknowledgments

All authors would like to acknowledge their respective departments for the conduct of the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor AR declared a past co-authorship with the author RS.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 05 May 2023
DOI 10.3389/fphar.2023.1149909

OPEN ACCESS

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RECEIVED 23 January 2023
ACCEPTED 25 April 2023
PUBLISHED 05 May 2023

CITATION
Shamim MA, Satapathy P, Padhi BK, Veeramachaneni SD, Akhtar N, Pradhan A, Agrawal A, Dwivedi P, Mohanty A, Pradhan KB, Kabir R, Rabaan AA, Alotaibi J, Al Ismail ZA, Alsoliabi ZA, Al Fraij A, Sah R and Rodriguez-Morales AJ (2023), Pharmacological treatment and vaccines in monkeypox virus: a narrative review and bibliometric analysis.
Front. Pharmacol. 14:1149909.
doi: 10.3389/fphar.2023.1149909

Pharmacological treatment and vaccines in monkeypox virus: a narrative review and bibliometric analysis

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Mpox (earlier known as monkeypox) virus infection is a recognized public health emergency. There has been little research on the treatment options. This article reviews the specific drugs used to treat mpox virus infection and the vaccines used here. Instead of focusing on the mechanistic basis, this review narrates the practical, real-life experiences of individual patients of mpox virus disease being administered these medicines. We conducted a bibliometric analysis on the treatment of the mpox virus using data from several databases like PubMed, Scopus, and Embase. The research on this topic has grown tremendously recently but it is highly concentrated in a few countries. Cidofovir is the most studied drug. This is because it is indicated and also used off-label for several conditions. The drugs used for mpox virus infection include tecovirimat, cidofovir, brincidofovir, vaccinia immune globulin, and trifluridine. Tecovirimat is used most frequently. It is a promising option in progressive mpox disease in terms of both efficacy and safety. Brincidofovir has been associated with treatment discontinuation due to elevated hepatic enzymes. Cidofovir is also not the preferred drug, often used because of the unavailability of tecovirimat. Trifluridine is used topically as an add-on agent along with tecovirimat for ocular manifestations of mpox virus disease. No study reports individual patient data for vaccinia immune globulin. Though no

vaccine is currently approved for mpox virus infection, ACAM 2000 and JYNNEOS are the vaccines being mainly considered. ACAM 2000 is capable of replicating and may cause severe adverse reactions. It is used when JYNNEOS is contraindicated. Several drugs and vaccines are under development and have been discussed alongside pragmatic aspects of mpox virus treatment and prevention. Further studies can provide more insight into the safety and efficacy of Tecovirimat in actively progressing mpox virus disease.

KEYWORDS

mpox infection, antiviral, drug, management, public health emergency, tecovirimat, cidofovir, bibliometry

1 Introduction

While the world is finding ways to deal with SARS-CoV-2 and COVID-19, a novel threat of mpox has emerged in the human population. The World Health Organization (WHO) has declared this a health emergency, and the confirmed cases have risen to 84,916 and 81 deaths as of 20 January 2023. One hundred ten countries throughout the globe have reported cases (World Health Organization, 2022a). The mpox virus is a member of the orthopoxvirus family. It generally invades rodents and animals but has now escaped into the human population. There are two distinct genetic clades of MPXV: African clad (Congo basin) and West Africa clad (Kaler et al., 2022). As per reports by WHO, new cases have been identified from various regions of the world, irrespective of their historical distribution (Reynolds et al., 2017). However, the symptoms are diverse and less severe than in smallpox (Rizk et al., 2022; Satapathy et al., 2022; Gandhi P et al., 2023).

Treatment of the mpox virus is a new challenge for the entire healthcare system (Sherwat et al., 2022). There are very few studies on it, and there is an urgent need to address it. There have been a few review articles covering the treatment options being employed. However, given that mpox virus disease is a rapidly evolving field, we regularly find new original research articles cropping up. We found several original research articles reporting on treatment options and other aspects of mpox virus disease that were not discussed in previous review articles (DeLaurentis et al., 2022; McCarthy, 2022; Torres, 2022; Rabaan et al., 2023; Shamim et al., 2023). This paper gives a brief analysis of literature from the past till the current time about the pharmacological treatment of the disease and the drugs being administered along with the vaccines being used. This review gives a detailed insight into the clinical orientation of the antivirals being administered: tecovirimat (TPOXX), cidofovir, brincidofovir, trifluridine, and Vaccinia Immune Globulin which would be essential for improving the treatment protocol and for increasing the treatment efficacy of the disease (Siegrist and Sassine, 2022). We follow it up with a discussion on the new and upcoming options for both the treatment and prevention of mpox virus disease.

2 Bibliometric analysis

The bibliometric analysis involves using mathematical methods to study books and communication media (Smith, 2008). It is beneficial in assessing the trend of research on a specific topic. This can help identify gaps in the currently available literature and thus propose ideas for future research. The bibliometric analysis covered three electronic databases for all articles from the inception of each database till 22 December 2022. The keywords "monkeypox" and "mpox" were used in combination with "treatment"/"management"/"drug" in the title, abstract, and keywords in Scopus, PubMed, and Embase, yielding 722, 370, and 289 results, respectively (Table 1). The distribution of studies depending on the year of publication and type of studies is shown in (Table 2). Only the last 5 years have been mentioned for clarity. A greatly increased number of studies can be noticed in 2022 (compared to previous years) owing to the public health emergency. This pattern highlights the relevance of research in this vastly underexplored area of public health concern.

If we further look at the countries where research is ongoing in the field of mpox virus disease, we can notice a worrying trend (Table 3). Though the United States expectedly has the highest number of studies, there are very few studies from other countries. As an international issue concerning most countries across the globe, we need efforts from all countries to help combat this situation. Globally, efforts should be taken to promote equitable research efforts.

There is a clear trend in the funding patterns in research pertaining to the mpox virus (Table 4). The National Institute of Allergy and Infectious Diseases funds the highest number of studies on this public health emergency. Most (80%) of the top ten funding agencies are from the United States. The other sponsors (one each) are from China and United Kingdom.

Using the results of the aforementioned search strategy, a bibliometric map of the relevant keywords was constructed using VOSviewer. We performed a co-occurrence analysis of keywords across several databases using whole counting. This helped us visualize the critical areas discussed in the sparsely available literature on the treatment of mpox. Figure 1 illustrates this.

There is a strong co-occurrence of "humans" and "animals" keywords. Many of the studies on pharmacological management of mpox virus infection have been based on animal models, and human studies have only recently started cropping up. Cidofovir is mentioned a lot. This is because it is used in many other conditions apart from mpox virus infection, more than any other antiviral mentioned here. It is mainly used against cytomegalovirus. It is used in patients with acquired immunodeficiency syndrome (AIDS) and those undergoing organ transplantation. It also demonstrates *in-vitro* activity against several DNA viruses like herpesvirus, poxvirus, polyomavirus, and papillomavirus (de

TABLE 1 The adjusted search terms as per searched electronic databases.

Database	No	Search query	Results
PubMed			
	#1	{[monkeypox (Title/Abstract)] OR [mpox (Title/Abstract)]} OR [mpxv (Title/Abstract)]	2,262
	#2	{[treatment (Title/Abstract)] OR [management (Title/Abstract)]} OR [drug(Title/Abstract)]	6,827,326
	#3	#1 AND #2	370
Scopus			
	#1	[TITLE-ABS-KEY (monkeypox) OR TITLE-ABS-KEY (mpox)]	2,523
	#2	{[TITLE-ABS-KEY (treatment) OR TITLE-ABS-KEY (management) OR TITLE-ABS-KEY (drug)]}	19,496,403
	#3	#1 AND #2	722
Embase			
	#1	[(monkeypox:ti,ab) OR (mpxv:ti,ab)] OR (mpox:ti,ab)	1,734
	#2	[(treatment:ti,ab) OR (management:ti,ab)] OR (drug:ti,ab)	7,832,676
	#3	#1 AND #2	289

TABLE 2 Results of the bibliometric analysis of the search for "Monkeypox" in title, abstract, and keywords in PubMed, Scopus, and Embase (on 18 December 2022).

Type (Scopus)	Frequency	Year	Scopus	PubMed	Embase
Article	395	2022	309	243	147
Review	201	2021	21	7	5
Letter	38	2020	19	11	6
Note	38	2019	15	5	6
Miscellaneous	50	2018	12	3	6

[a]Refers to the total number of a specific type of publication from 2018 to 2022.

TABLE 3 Results of the bibliometric analysis with regard to country of publication as per Scopus.

Country	Frequency
United States	347
United Kingdom	61
India	51
China	36
Germany	36
Belgium	24
France	24
Italy	24
Canada	22
Russian Federation	20

Clercq, 2003). It is used off-label in respiratory papillomatosis (Ballestas et al., 2021), acyclovir-resistant herpes simplex virus (Enescu et al., 2021), skin lesions associated with herpesvirus (Ferrer et al., 2021), BK polyomavirus (Napolitano et al., 2021). We have thus built upon the existing bibliometric analyses pertaining to mpox virus disease (Cheng et al., 2022; Zeeshan et al., 2022). We have focused on the treatment aspect for our bibliometric analysis.

3 Treatments and vaccines

3.1 Tecovirimat (alternative name: ST-246, brand name: TPOXX®)

Tecovirimat is the most commonly employed antiviral in patients with mpox infection. Initially identified in a high throughput screening, the United States FDA granted it approval in 2018, and it is indicated in smallpox disease (Grosenbach et al., 2011). Tecovirimat was approved under the FDA animal rule after testing on monkeys and rabbits, and an investigational new drug protocol has been granted to the United States Centers for Disease Control to study it in non-variola orthopoxvirus infections, including mpox virus (Food and Drug Administration, 2018; Centers for Disease Control and Prevention, 2022b). Due to

TABLE 4 Results of the bibliometric analysis with regard to funding sponsor of publications as per Scopus.

Rank	Funding agency	Country	Publication count
1	National Institute of Allergy and Infectious Diseases	United States	106
2	National Institutes of Health	United States	51
3	National Center for Research Resources	United States	14
4	National Natural Science Foundation of China	China	12
5	Centers for Disease Control and Prevention	United States	11
6	National Cancer Institute	United States	11
7	Defense Threat Reduction Agency	United States	10
8	United States Department of Health and Human Services	United States	8
9	Biomedical Advanced Research and Development Authority	United States	7
10	Medical Research Council	United Kingdom	7

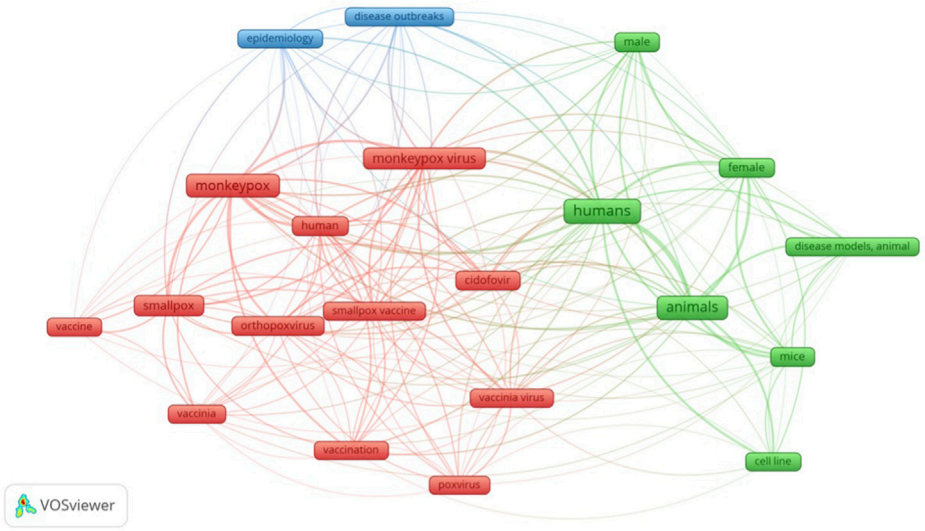

FIGURE 1
Visual network of keywords in published literature pertaining to treatment of monkeypox infection.

concerns pertaining to bioterrorism, the United States has stockpiled around two million doses to TPOXX and other drugs acting against mpox (Hoy, 2018).

The mpox virus enters the cytoplasm of human cells. After pre-processing, it replicates its DNA in Guarnieri inclusion bodies (Pauli et al., 2010). Then, the synthesised protein particles are assembled to form an intracellular virion. This undergoes maturation followed by enveloping. This prepares the mature virion to now exit the cell. The membranes of the virion and the cell fuse, and the extracellular mature virion exits the cell. It goes on to infect other human cells, thus amplifying the disease. The VP37 protein of the mpox virus is responsible for this enveloping of mature extracellular virions. Golgi-derived membrane is used for this envelopment. Tecovirimat inhibits this VP37 protein. Thereby, this enveloping of the mature viral particle is inhibited even though DNA synthesis and viral maturation progresses normally. The absence of enveloping ensures that the matured virion cannot properly exit the infected cells, systemically spread in the body, and infect other cells. Thus, TPOXX inhibits the propagation of mpox virus disease to other human cells and hinders disease progression (Hudu et al., 2023). VP37 also interacts with human proteins like TIP47 and Rab9. TPOXX inhibits this, further diminishing propagation of mpox virus in the human body (Grosenbach et al., 2011). Cross-resistance to cidofovir or brincidofovir is not expected (Duraffour et al., 2007).

TPOXX has several stereoisomeric forms. The monohydrate form has low solubility but considerable permeability. Amongst the several polymorphic forms available, the first form of Tecovirimat monohydrate crystal is packed in capsules after recrystallizing using water and ethyl acetate. This is preferred due to the higher thermodynamic stability compared to other forms (European Medicines Agency, 2021). It is usually administered as capsules

containing 200 mg of the active drug. But for those weighing less than 13 kg, the intravenous formulation is administered as a slow infusion. One crucial difference between the two dosage forms is that only the latter is contraindicated in patients with renal disease (creatinine clearance of 30 mL/min or less). Even in cases with mild-to-moderate renal impairment (creatinine clearance ranging from 30 to 89 mL/min) or in the pediatric population aged less than 2 years, caution is required. Thus, clinicians should routinely test this in all patients before starting Tecovirimat infusion. The reason behind this selective toxicity is the presence of an excipient hydroxypropyl-β-cyclodextrin in the parenteral preparation. This excipient is added due to the poor water solubility of Tecovirimat. Caution is needed in comorbid or multimorbid patients, especially in those with diabetes or history of seizures. It is also known to have drug-drug interactions with midazolam and repaglinide. When co-administered with Repaglinide or Midazolam, monitoring for hypoglycemia or Midazolam effectiveness is recommended. Drugs metabolized by CYP3A and CYP2B6 may show diminished therapeutic activity due to induction of these hepatic enzymes. A synergistic drug-drug interaction has also been reported. An *in silico* study has shown better efficacy against mpox virus with combination of atovaquone and TPOXX (Akazawa et al., 2022). Some of the common associated adverse events are headaches, gastrointestinal disturbances, and injection site complaints like pain, swelling, erythema and extravasation (De Clercq et al., 2022; Food and Drug Administration, 2022; Siegrist and Sassine, 2022). Amongst food-drug interactions, administration of this drug is with milk or yogurt is recommended. Though teratogenicity was observed in mice, but the results have not been replicated in rabbits. Thus, future studies should consider this gap in literature. It might be less effective in immunocompromised individuals. Amongst other pharmacokinetic parameters, it has a half-life of four to 6 h and a volume of distribution of 1,030 L following an oral dose of 600 mg. 77%–82% of the drug is protein-bound. Elimination is primarily by hepatic conjugation followed by renal excretion. Some portion is also excreted unchanged via faeces. It has a clearance of 31 L per hour (European Medicines Agency, 2021; Food and Drug Administration, 2022).

There are several studies on the use of Tecovirimat in monkeypox disease in humans. It has been effective in arresting the aggravation of the disease. A female in her 30 s developed mpox virus lesions over her thorax. On testing positive, she was started on Tecovirimat. New lesions stopped cropping up within a day, and lesions turned PCR negative within 2 days (Adler et al., 2022). A man with an extensive facial pustular lesion was administered Tecovirimat and improved (Rao et al., 2022). Another male patient developed genital lesions that gradually spread throughout the body. After starting Tecovirimat, the development of new lesions stopped within 2 days, and the patient recovered. In an HIV-positive patient, oedema over palatine tonsil and pain while ingesting food benefitted from Tecovirimat. Another patient did not improve with initial empirical treatment. With Tecovirimat, lesions started crusting and improved by the second day itself (Matias et al., 2022). Progressive oral symptoms not responding to several lines of treatments started improving within 2 days of initiating Tecovirimat (Ajmera et al., 2022). In this uncontrolled cohort study, with 25 participants receiving Tecovirimat, 23 recovered as per the reported 21-day outcome. In one individual, there were no new lesions. However, in another patient, new lesions were still developing despite having received a 21-day course of Tecovirimat, unlike the 14-day course prescribed to others (Desai et al., 2022). A report describes two cases of severe proctitis. Both had lesions spread throughout the body. However, the rectal lesions were very prominent and caused enough pain to require opioids. In both cases, rapid improvement was seen within 36–48 h (Lucar et al., 2022). Another male patient complaining of proctitis recovered with a course of TPOXX after empirical treatment with doxycycline, valacylovir, and benzathine penicillin G failed (37065384).

A patient presented with ulcerative lesions over the tip of the tongue and over the anterior aspect of its ventral surface. Lesions later spread throughout his body. Though he is still symptomatic, as per the last update, he has been improving with Tecovirimat (Peters et al., 2022). An attendee of a pride festival reported macules, papules, and pustules across the body. He also tested positive for Herpes Simplex Virus—2 alongside the mpox virus. He was co-treated with Tecovirimat and Valacyclovir (for Herpes Simplex Virus—2). He continued developing new lesions and was febrile for the first 2–3 days. He was then discharged as he turned afebrile and improved (Shaw et al., 2022). An uncontrolled cohort study in the Democratic Republic of Congo reports 14 patients with monkeypox virus infection and treated with Tecovirimat. Patients started showing signs of improvement, like suppression of active lesions from day 2 of Tecovirimat administration. All patients were better by the end of 2 weeks. Some lesions and lymphadenopathy persisted, but all 14 participants improved symptomatically (Mbrenga et al., 2022). This patient with multiple comorbidities like syphilis, Kaposi sarcoma, HIV, and hypertension reported papules, vesicles, and pustules throughout the body. The patient was started on Tecovirimat, and he improved rapidly (Hernandez et al., 2022). Two patients with mpox virus infection had encephalomyelitis. They presented with progressive hemiparesis, paraparesis, bowel and urinary abnormalities, and altered mentation, among other manifestations. Tecovirimat was administered orally to both patients. They improved and were subsequently discharged (Pastula et al., 2022). A 35-year-old female with encephalitis and transverse myelitis. Empirical acyclovir was stopped, and tecovirimat was initiated after the patient turned out to be positive for the mpox virus. The neurological pathology continued to progress, and she was started on methylprednisolone and cidofovir in the fourth week of the illness. She gradually improved. This could be attributed to the synergistic action of tecovirimat and cidofovir (Cole et al., 2022). A patient with progressive generalized mpox virus dermatological lesions and watery diarrhea was started on Tecovirimat. All the issues started improving within two to 3 days (Viguier et al., 2022). This case series reports three participants with various comorbidities like ulcerative colitis and syphilis. Treatment was initiated with Tecovirimat. In two of the patients, this led to fading away of existing lesions, no appearance of newer lesions, along with an immediate benefit seen clinically. In the third patient, clinical response was seen but was slow. However, C-reactive protein and viral DNA load were reduced in the first week itself (Nörz et al., 2022). This study reports twenty participants who received tecovirimat. All the patients recovered. Participants reported improvement within two to 3 days (Wu et al., 2022). Two patients with concomitant HIV and anogenital and rectal mpox virus infection recovered with

tecovirimat (Beatty et al., 2022). A case with disseminated ocular involvement was administered tecovirimat (Rimmer et al., 2023). All fifteen patients started on tecovirimat improved, and no new lesions developed (Mondi et al., 2022). This 35-year-old man with multiple swollen facial pustules. He was started on tecovirimat and improved (Manoharan et al., 2022). This patient presented with multiple lesions throughout the body, including eyes. All the lesions improved with tecovirimat (Rai et al., 2022). This study reports on the usage of tecovirimat under an investigational new drug protocol. 230 of 317 patients recovered, while most of the remaining 87 had not yet completed the 14-day course of tecovirimat (O'Laughlin et al., 2022). A recent case report on a patient with HIV not responding adequately to TPOXX have given rise to concerns regarding emerging resistance to TPOXX and the need to consider immune reconstitution inflammatory syndrome in such cases (36992234).

It has been associated with serious adverse events only in one study conducted in the Democratic Republic of Congo. Here, one patient developed severe anemia, with his hematocrit dropping to as low as 18%. He later recovered. Another patient was discharged after 2 weeks of treatment as his lesions were improving, and he turned out PCR negative. However, he died 3 days after that. In both cases, they considered the event unrelated to or unlikely to be related to this drug (Mbrenga et al., 2022). Some other studies have reported a few adverse events too. One patient reported loose stool after every dose. Another developed elevated alanine aminotransferase on the sixth day of treatment. This reduced over the next 2 days returning back to normal values without discontinuation of treatment. The derangement of hepatic enzyme resolved independently (Matias et al., 2022). Two more patients developed a transient rise in hepatic enzymes. Alanine aminotransferase and aspartate aminotransferase were elevated to 97 IU/L and 86 IU/L, respectively. This resolved on its own (Viguier et al., 2022). Another patient was quite different because he developed elevated gamma-glutamyl transferase levels of 277 U/L. However, his transaminases remained within range. Despite this different pattern of hepatic enzyme derangement, this was also only a transient rise (Nörz et al., 2022). Fatigue, headache, backache, and nausea are some of the other reported adverse events (Desai et al., 2022; O'Laughlin et al., 2022; Wu et al., 2022). Summing up, Tecovirimat is a promising option in terms of both efficacy and safety in worsening mpox virus disease.

Two clinical trials are going on to evaluate the efficacy and safety of Tecovirimat in mpox virus infection (NCT05534984, NCT05534165). The multinational randomized controlled trial has started recruiting. The second trial, which is based in Canada, is yet to start recruiting. Both are expected to be completed by the following year, i.e., 2023 (Ortiz-Saavedra et al., 2022).

3.2 Cidofovir (brand name: Vistide®)

Cidofovir is used in diseases due to cytomegalovirus (CMV), herpesviruses, and several DNA viruses. It is indicated in certain CMV diseases in immunocompromised people (Cherrington et al., 1998; Kendle and Fan-Havard, 1998) and has been used off-label in several conditions caused due to DNA viruses (Razonable, 2011).

Cidofovir is a nucleoside analogue. Using intracellular metabolism, it is activated into cidofovir-diphosphate, which competitively inhibits viral DNA polymerase, thereby interfering with viral DNA synthesis. It inserts into the viral genomic material, inhibiting further prolongation (Lea and Bryson, 1996). Unlike substances acting on A48R, cidofovir inhibits human DNA polymerases also. However, its activity here is eight to six hundred times less than for the viral enzyme (https://pubmed.ncbi.nlm.nih.gov/30397065/). Pharmacologically, it is an example of a hit-and-run drug. Administered intravenously, the serum concentration of the drug falls rapidly following the infusion, and it has a short plasma half-life of 2 h. However, the intracellular half-life of the active form is as high as 65 h. Cidofovir undergoes renal elimination, and this involves a critical drug-drug interaction. Probenecid blocks tubular secretion of this drug, thereby reducing its excretion and increasing its serum level (Cundy, 1999; Wolf et al., 2003). Nephrotoxicity is a common clinical concern with this drug, and hydration and probenecid are recommended to reduce its incidence (Lea and Bryson, 1996; Kazory et al., 2007). Relevant monitoring is recommended during therapy due to the risk of ocular complications (like hypotony, uveitis, and iritis) and myelosuppression (Ambati et al., 1999; Tseng et al., 1999).

In treating mpox, cidofovir has been used clinically in at least four reports. In three of the cases, it was only due to the unavailability of tecovirimat (Mailhe et al., 2022; Moschese et al., 2022; Raccagni et al., 2022). One patient developed vesicles over his nose along with suspected bacterial superinfection. He improved with cidofovir and antibiotics (Moschese et al., 2022). In this case of atypical presentation of mpox with ophthalmic manifestations like the involvement of cornea, conjunctiva, and eyelids, cidofovir was administered. However, the report says that the lesions are still evolving in spite of two intravenous injections (these are administered weekly (Mailhe et al., 2022). Another similar ophthalmic presentation of mpox was administered cidofovir. However, he reported improvement in 3 days (Scandale et al., 2022). Raccagni et al. (2022) describe four patients in Italy using cidofovir. They had varying clinical presentations ranging from pharyngeal and ocular involvement to rectal and genital involvement. They were given single-dose cidofovir along with hydration and probenecid, and they improved. In this cohort study, 12 patients were administered add-on topical cidofovir while others were on standard care. Topical cidofovir was associated with quicker clearance of lesions and higher resolution of lesions as per PCR testing (Sobral-Costas et al., 2022). A patient with co-infection with HIV and mpox virus had severe disease requiring hospitalisation. The administration of cidofovir was followed by rapid improvement (Fabrizio et al., 2022). All four patients on cidofovir recovered completely (Mondi et al., 2022).

Transient elevation in a hepatic enzyme was seen in one patient (Mondi et al., 2022). The other studies on cidofovir did not report any adverse events (Fabrizio et al., 2022; Mailhe et al., 2022; Moschese et al., 2022). The study employing topical cidofovir reported local adverse events, but there were not any systemic adverse events (Mondi et al., 2022; Sobral-Costas et al., 2022).

3.3 Brincidofovir (alternative name: CMX001, brand name: TEMBEXA®)

Brincidofovir (BCV) is a nucleotide analogue DNA polymerase inhibitor. It is a pro-drug composed of cidofovir conjugated to a lipid molecule. The lipid component resembles an endogenous lipid called lysophosphatidylcholine, allowing the molecule to enter the infected cells by taking on the natural lipid absorption mechanisms. Following absorption, the lipid molecule is broken down, releasing cidofovir for additional intracellular kinase phosphorylation to form cidofovir diphosphate, the active form of the drug. In contrast to cidofovir, brincidofovir does not act as a substrate for Organic Anion Transporter 1, which makes BCV less harmful to the kidneys. Therefore, brincidofovir has a higher safety profile for nephrotoxicity compared to cidofovir. Coming to preventive measures and adverse reactions of Brincidofovir, its administration requires continuous monitoring of hepatic function tests as it increases the serum transaminase and bilirubin levels. Other adverse effects seen are gastrointestinal side effects like diarrhea and vomiting. Pregnancy is ruled out before administering this drug as it is teratogenic in animal studies. Contraception is advised throughout the treatment and for 4 months after that. Brincidofovir is also known to have carcinogenic potential, so safety with handling is necessary. Brincidofovir is taken on an empty stomach or with a low-fat meal to increase the bioavailability of the drug. Drug-drug interactions are seen when used concomitantly with inhibitors of OATP1B1 and 1B3 (Organic Anion Transporting Polypeptide) like rifampin, erythromycin, and protease inhibitors like ritonavir as they increase its peak serum concentration, increasing the adverse events due to Brincidofovir (Das and Hong, 2019; National Center for Biotechnology Information, 2022).

Results with Brincidofovir have not been promising. In the solitary study on human patients, all three patients had to discontinue treatment because hepatic impairment led to hospitalization prolonging. Other issues encountered were conjunctivitis, lower limb abscess, and neuropsychiatric symptoms (Adler et al., 2022). They were hospitalized for 26–35 days. Thus, safety remains a key concern with this drug.

3.4 Vaccinia immune globulin (brand name: CNJ-016®)

Many medical countermeasures are kept on hand in case of orthopoxviruses as mpox emerges. Although most instances of mpox are minor and self-limited, supportive treatment is often enough to treat them. Most patients have moderate sickness and recover without medical help, but in very unwell or immunocompromised people, antivirals or vaccinia immune globulin (VIG) may be utilized. According to the Centre for Disease Control and Prevention (CDC), supportive care is often sufficient for people with a mpox virus infection because no particular medicines are available. Mpox virus disease can be prevented and treated similarly to other orthopoxvirus infections, and unless proven differently, all confirmed orthopoxvirus cases should be managed as though they are mpox (Centers for Disease Control and Prevention, 2022c; Rizk et al., 2022; UK Health Security Agency, 2022).

In immunosuppressed patients exposed to mpox for whom ACAM2000 vaccination is contraindicated, VIG, an injectable preparation of hyperimmune globulin made from the pooled blood of smallpox vaccine recipients, may be considered. These people's acquired antibodies against the smallpox vaccine are removed and purified. Additionally, VIG can treat vaccinia virus-related aberrant infections brought on by autoinoculation, eczema vaccinatum, or severe generalized or progressive vaccinia (Hopkins and Lane, 2004; Weinstein et al., 2005). VIG is used to treat some vaccine-related side effects like infections due to the vaccinia virus in people with pre-existing skin disease and aberrant infections brought on by the vaccinia virus. VIG is not advised to treat post-vaccine encephalitis or encephalomyelitis, myopericarditis following smallpox vaccination, moderate cases of widespread vaccinia, erythema multiforme, or isolated vaccinia keratitis. Its use has not been evaluated in people with mpox or smallpox, even though it is a potential treatment. Data on its efficiency against these conditions are mostly sparse. Clinicians should use an Investigational New Drug (IND) application to administer VIG treatments. If tecovirimat is unavailable, the current Australian recommendations reserve VIG as a backup treatment for mpox infection (Australian Government Department of Health, 2022).

3.5 Trifluridine

Trifluridine is a fluorinated structural analogue of the DNA constituent thymidine. It acts by inhibiting DNA synthesis. It inhibits the enzymes involved in this process and may itself get incorporated into DNA. However, its action may lack selectivity. It has only been used as a topical preparation for the eye in cases of mpox virus infections. It is said to be safe when applied topically as eye drops. This is because it does not penetrate the intact cornea. However, in cases with corneal pathologies disrupting its structure, trifluridine may penetrate the cornea and be detectable in aqueous humor. Mild adverse events have been noted. These include transient local burning sensation, oedema of the eyelids, inflammation of the cornea, and allergy. It may be dosed at a 2-h interval till there is complete regeneration of epithelium in the cornea. Then, it may be administered once every 4 h for another 7 days. However, it is not prescribed for prolonged durations. In such cases where it has to be given beyond 3 weeks, alternative pharmacological options may be explored (Carmine et al., 1982).

Trifluridine has been used in mpox virus infection. However, its use in both the studies has been as an add-on agent. Overall, five patients received both Tecovirimat and local administration of Trifluridine. Though all these four patients varied greatly in their presentation, they were common in that all had ophthalmological manifestations of mpox virus disease too. Four of them recovered promptly and were discharged. One of them continues to develop worsening ocular symptoms and decreased visual acuity. No adverse events were reported with this drug (Cash-Goldwasser et al., 2022; Perzia et al., 2023).

3.6 New discoveries

Researchers are exploring several new potential drug targets and therapeutic options for the treatment of mpox amidst concerns regarding both supply shortages and drug resistance (Hudu et al., 2023; Rabaan et al., 2023). Mutations have been reported in both the F13L and D13L genes with potential for resistance (Garriga et al., 2018). This study has reported a frameshift mutation in the currently prevalent strain, sparking fears of drug resistance (Zhang et al., 2022). Thus, discovery of newer options is critical.

The genetic material of all orthopoxviruses show similarity. The open reading frames responsible for viral protein synthesis are well-conserved in orthopoxviruses. This is especially manifested in the case of the VP37 protein. Tecovirimat targets this protein and has shown positive results in mpox. Therefore, novel drugs can be developed focusing on the VP37 protein (Hudu et al., 2023). There are several other patents involving TPOXX. This includes US11433051B2 which comprises of simethicone and several other pharmaceutical excipients to enhance its action. US8642577B2 involves combining TPOXX with other antiviral drugs, and can be used for a wide range of orthopoxvirus diseases. Since TPOXX doesn't inhibit viral nucleic acid synthesis, combining an envelope-formation inhibitor with DNA synthesis like Cidofovir targets mpox virus at two different levels can have synergistic action. CN115141136A proposes combining TPOXX with another crystalline ligand and can again be used for several orthopoxvirus infections. We have compiled the patents we feel are especially clinically relevant. A more comprehensive list can be found here (Almehmadi et al., 2022).

NIOCH-14 is another potential drug that has already cleared phase 1 clinical trial (Hudu et al., 2023). It is a prodrug of TPOXX, and is also administered as a capsule. Once inside the body, it quickly metabolises to TPOXX. It has shown similar or somewhat better efficacy and bioavailability compared to TPOXX in several *in-vitro* and *in-vivo* studies involving orthopoxviruses including mpox virus (Titova et al., 2015; Mazurkov et al., 2016; 2020; Sergeev et al., 2016). Thus, it is a promising medicine and the drug development process is expected to be complete by the next year (2024) (World Health Organization, 2022b).

Another drug inhibiting VP37 protein is N(1)-isonicotinoyl-N(2)-3-methyl-4-chlorobenzoylhydrazine. It has a similar mechanism of action, and inhibits envelopment, extracellular release and consequent propagation of disease in the human body. Though beneficial in *in-vitro* studies, the same results were not replicated in animal models (Prichard and Kern, 2005; Prichard and Kern, 2012). To add to all of this, there has been further exploration of the detailed structure of the VP37 protein. There has been greater insight into the allosteric site of the target protein, and how the inhibitor is dynamically flipped and it's strong binding energy (Sen Gupta et al., 2023). This can lead to development of more optimally designed drugs acting on the VP37 protein.

Other potential drug targets could be E9L and A24R to arrest viral nucleic acid replication. These inhibit DNA polymerase and RNA polymerase respectively. While the former is required for mpox virus to replicate its own double stranded DNA, the latter is needed for protein synthesis. Drugs acting on A48R can act as nucleoside analogues to terminate chain prolongation. As discussed earlier, this is different to the case of Cidofovir. Though Cidofovir also acts as a nucleoside analogue, it inhibits human polymerases too (Chamberlain et al., 2019). Instead, a drug acting on A48R would inhibit phosphorylation of thymidine monophosphate which is structurally quite different from its human counterpart thereby imparting specificity of action. North-Methanocarbathymidine has also shown promising action (Smee et al., 2007). Aciclovir and KAY-2-41 were effective in *in-vitro* studies on orthopoxviruses (Sauerbrei et al., 2005; Duraffour et al., 2014). Nucleic acid replication can also be inhibited by targeting topoisomerases. Targeting H5R, B1R, and F10L can prevent phosphorylation thereby inhibiting tyrosine kinase. This approach has been successful previously in cytomegalovirus (Piret and Boivin, 2019). Similarly, the ErbB-1 kinase can be inhibited by epidermal growth factor signal transduction inhibitors. This again inhibits phosphorylation. Viral entry can be reduced by designing drugs acting on E8L and A6R. Interferons act on B8R and inhibit the terminal step of protein synthesis. Drugs acting on I7L and D13L can target preparation of viral core and membrane. Other drugs that can be used as a reference to design new drugs for mpox can be found here (Rabaan et al., 2023). Nanotechnology based drug administration and nanomedicines are also being explored (Dash and Kundu, 2023).

A detailed *in silico* study screened over 1000 approved drugs for activity against mpox viral proteins. Routinely used in oncology, fludarabine showed the best results with a high stability and docking score for A6R, a protein concerned with viral replication. On top of that, it also demonstrated activity against D8L involved in viral entry into human cells. Moreover, Fludarabine acts against F13L that codes for the VP 37 protein responsible for envelopment of mature virion particles as discussed earlier. Fludarabine also inhibits viral attachment to human cells by blocking an asparagine residue (Altayb, 2022). Fludarabine and its analogues can be studied *in-vitro* to generate more evidence regarding its activity in mpox. Norov-29 and Bemnifosbuvir have also demonstrated promising results with high binding free energies (Abduljalil and Elfiky, 2022). Another high throughput virtual screening has identified Naldemedine and Saquinavir to form stable complexes with mpox viral targets (Srivastava et al., 2023). A study has explored protein-protein interactions across the whole genome of several mpox strains and the human proteome. It identified several drugs including Fostamatinib and Tamoxifen (Kataria et al., 2023). This study identified 11 possible compounds for inhibition of thymidylate kinase, after screening hundreds of thousands of compounds (Sib Tul Hassan Shah and Naeem, 2023). Several small molecule inhibitors have also been studied shortlisting drugs like imatinib, conivaptan, lumacaftor, betulinic acid, and fluspirilene (Dutt et al., 2023; Khan et al., 2023).

Repurposing of drugs, and especially herbal formulations has long been seen as a practical solution in case of emerging and upcoming diseases with limited known therapeutic options. A study incorporating opinions of close to 300 herbal medicine practitioners found several formulations that are not studied enough for diseases like mpox. It includes Moringaceae, African palm oil, and Acacia pod extract. These can be studied for their antiviral efficacy, and depending on the results, taken up for further research (Abubakar et al., 2022). Several substances derived from curcumin have shown promising actions along with good pharmacokinetic properties and physiological stability (Akash et al., 2023). Traditional Chinese

medicine is also being explored to find other options (Rong et al., 2023). There are several other studies focusing on repurposing of existing drugs for mpox (Arasu et al., 2023).

3.7 Vaccines

When it comes to the prevention of mpox virus infection, two vaccines are mainly considered. These are ACAM 2000, and JYNNEOS (also known as IMVANEX, IMAMUNE, and MVA). There are no approved vaccines specifically for mpox virus infection. The mpox virus and the variola virus (that causes smallpox) belong to the same genome of orthopoxvirus. Orthopoxviruses are known to share immunological cross-protection between them, but the evidence is not very strong. Immunity developed against smallpox may help protect against mpox virus infection too. Thus, smallpox vaccines are being repurposed for use in mpox virus infection (Huang et al., 2022; Akter et al., 2023).

JYNNEOS (or Imvamune) is a third-generation vaccine. It is modified vaccine Ankara (MVA), manufactured in Denmark. It comprises a virus that is incapable of replicating. It is approved in the United States for both smallpox and mpox virus infection. 0.5 mL of the vaccine is delivered subcutaneously 4 weeks apart. Then, the person is said to have been vaccinated 2 weeks after the second dose. It is also approved by the World Health Organization and medical agencies in Europe and Canada for post-exposure prophylaxis. There are several trials that are undergoing to test this vaccine further. Intradermal administration has also been practiced (Bloch et al., 2022; Huang et al., 2022; Jeyaraman et al., 2022). However, there are conflicting reports on its efficacy (Chakraborty et al., 2022). Breakthrough infection several weeks after vaccination has also been reported (Hazra et al., 2022).

ACAM 2000 is a second-generation vaccine. It is a derivative of the first-generation Dryvax vaccine (Nalca and Zumbrun, 2010). It differs from JYNNEOS in retaining the ability to replicate. Thus, it may cause severe adverse events like progressive vaccinia, eczema, cardiac injury and pericardial injury and can be unsafe in the immunocompromised population. Another disadvantage is that a bifurcated needle is used to puncture the skin at multiple places. Pustule formation at the site of vaccination indicates successful immune response and is labelled “take” of mpox vaccine. It is reserved for use in cases wherein JYNNEOS is contraindicated. It is only approved for smallpox and has not yet been approved for mpox virus infection. It has shown a protective action in monkey and dog models (Bloch et al., 2022; Huang et al., 2022; Jeyaraman et al., 2022). This has replaced the earlier Dryvax vaccine, which is the oldest smallpox vaccine globally (Chakraborty et al., 2022; Katamesh et al., 2023). LC16m8 and NYVAC are other third-generation vaccines. LC16m8 has considerably lesser replicative property, and is not given in immunosuppressed and those below 18 years of age. There have been several first-generation vaccines that are not in use currently. These are active replicating vaccines with varying reactogenicity. Some examples are Dryvax, Lister, EM-63, and Tian-Tian (Reina and Iglesias, 2023).

These vaccines have been associated with several adverse events including myocarditis and pericarditis (Food and Drug Administration, 2007; Voigt et al., 2016). Imvamune is considered unsafe for more than 15% of people living in the United States due to concerns over immunogenic adverse events (Rabaan et al., 2023). And it is considered safer than ACAM 2000. Both TPOXX and NIOCH-14 have been claimed to beneficial in avoiding vaccine side effects. However, drug-vaccine interactions should be carefully assessed and researched comprehensively before recommending it for routine use (Grosenbach et al., 2011).

Several other vaccines for mpox virus infection are under development or under study. Aventis Pastuer smallpox vaccine is being used under an investigational new drug protocol for smallpox. This may be later developed for use in mpox too (Rizk et al., 2022). A Japanese vaccine by the name of LC16m8 is of the replicating subtype and was used for smallpox. It has shown protective action against mpox virus infection in several animal models, including mice, rabbits, and non-human primates. A novel vaccine by the name of TNX-801 has been patented. It has also shown benefits in animal models like mice and macaques (Huang et al., 2022). mRNA vaccines with four to six antigens have been tested in mice. These have shown potent immune response (Zeng et al., 2023). Harnessing the potential of bioinformatics in designing new molecules for prevention of diseases including mpox virus infection, several studies have introduced designs for vaccines with multiple epitopes (Aiman et al., 2022; Akhtar et al., 2022; Aziz et al., 2022; Singh et al., 2023; Zaib et al., 2023). These researchers have developed two vaccine candidates targeting A35R, B6R, and H3L. Both the candidates have shown promising docking and dynamics for toll-like receptors 2 and 4, and major histocompatibility complexes (Tan et al., 2023). Another group developed two mRNA vaccine candidates with four to five components that have demonstrated immune response in mice (Zhang et al., 2023). These may be further tested along the process of drug development, like in the case of other novel molecules.

4 Discussion

This review elaborates on the different pharmacological treatment options for mpox virus infection. A bibliometric analysis was also carried out across several databases like PubMed, Scopus, and Embase. We observed that five drugs are mainly used for specific management of mpox disease in humans: tecovirimat, cidofovir, brincidofovir, trifluridine, and Vaccinia Immune Globulin. Tecovirimat has emerged as an exciting option with efficacy in progressive disease. No signals for safety concerns have been detected either. All other options are infrequently used. Cidofovir and its related compound brincidofovir are also used. The latter is linked with hepatic impairment, and treatment had to be discontinued in all three cases in a study. Vaccinia immune globulin has not been used much and is mainly preferred for other indications like post-vaccine complications. Trifluridine is successfully used as an add-on treatment option in patients with ocular manifestations of the mpox virus.

According to the interim guidelines by CDC for treating mpox virus infection, treatment should not be considered across all cases. It should only be considered based on clinical features and individual baseline risk. Clinically, severe disease and involvement of areas of the body that can potentially cause serious complications are conditions for treatment consideration.

Pharyngeal lesions may lead to dysphagia and lack of control of secretions. Rectal involvement may lead to severe proctitis and pain. Treatment should also be considered in high-risk individuals like immunocompromised, pregnant, lactating, children, and those with dermatological diseases that affect cutaneous integrity (Centers for Disease Control and Prevention, 2022a; Gandhi et al., 2023; Rao et al., 2023).

These drugs still need to be adequately tested in well-designed studies on patients with monkeypox infection. The main reason behind the lack of such studies might be feasibility issues. Further studies, including randomized controlled trials like these (NCT05534984, NCT05534165), must confirm the results and optimize the dosage range.

Author contributions

Conceptualization: MS, PS, BP, PD, AM, AR, RS; Methodology: MS, PS, BP, AM, AR, RS; Analysis: MS, BP; Writing: MS, BP, SV, NA, AP, AA; Review and editing—all the authors. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 09 May 2023
DOI 10.3389/fpubh.2023.1153136

OPEN ACCESS

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RECEIVED 28 January 2023
ACCEPTED 29 March 2023
PUBLISHED 09 May 2023

CITATION
Swed S, Alibrahim H, Bohsas H, Jawish N, Rais MA, Nasif MN, Hafez W, Sawaf B, Abdelrahman A, Fathey S, Atef Ismail Ahmed Ibrahim I, Almashaqbeh SHA, Yousef Aljawarneh RM, Rakab A, EL-Shafei EHH, Hurlemann R, Elsayed MEG and Data Collection Group (2023) A multinational cross-sectional study on the awareness and concerns of healthcare providers toward monkeypox and the promotion of the monkeypox vaccination. *Front. Public Health* 11:1153136. doi: 10.3389/fpubh.2023.1153136

A multinational cross-sectional study on the awareness and concerns of healthcare providers toward monkeypox and the promotion of the monkeypox vaccination

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Background: The aim of this study was to explore potential healthcare workers' (HCWs) concerns about the monkeypox virus in order to create practical solutions to manage this disease.

Methods: Online cross-sectional research was conducted in 11 Arabic countries (Egypt, Saudi Arabia, Yemen, Syria, Libya, Algeria, Tunisia, Iraq, Palestine, Jordan, and Sudan) from 2 August 2022 to 28 December 2022.

Results: Approximately 82% of respondents felt the need to acquire further information. The acceptability of the vaccine against monkeypox has been indicated by more than half of the participants (54.5%). Furthermore, we state that 45% of the participants are knowledgeable about the monkeypox virus, and 53.1% of the participants have never been affected with COVID-19 before are more worried about COVID-19 than about monkeypox. Participants diagnosed with COVID-19 were 0.63 times less likely to worry about monkeypox than those who were not diagnosed with COVID-19. A greater willingness to get the monkeypox vaccination was seen among the age group 21–30 years (42.4%) compared to the other age groups.

Conclusion: Most healthcare professionals have a moderate knowledge of the monkeypox virus. Furthermore, they demonstrated a low willingness to get the vaccination against the monkeypox virus.

KEYWORDS

monkeypox, COVID-19, anxiety, vaccination, multi-national cross-sectional study

1. Introduction

Health experts are worried about the emergence of a new epidemic caused by the monkeypox virus, and they believe monkeypox virus may pose a new threat to human health when the world seems to be in the late stages of the coronavirus disease 2019 (COVID-19) pandemic (1, 2). Monkeypox virus is a DNA virus with two strands that belong to the genus *Orthopoxvirus*, which also contains variola, cowpox (CPX), and vaccinia viruses (3, 4). Since the Democratic Republic of the Congo (DRC) reported the first human cases of monkeypox in 1970, the disease has spread to other parts of Africa and, more recently, instances of spread of monkeypox outside of Africa have been reported (5). According to the World Health Organization (WHO), there have been more than 13,069 instances of monkeypox worldwide, as on 18 July 2022, with 80% of these cases occurring in the European Union (6). Sexual transmission of infections or diseases has been identified as a major factor associated with greater spreading of the current epidemic, particularly among males who have been identified as homosexual or bisexual (7). Moreover, the virus may get spread by sharing beds or clothes and through direct exposure to infected sores, scabs, or bodily fluids. While the symptoms of monkeypox are comparable with those of smallpox, the lesser extent of severity of the symptoms of monkeypox such as fever, rash, and lymphadenopathy characterize the clinical condition (8, 9). On the contrary, it is characterized by many complications, the most important of which are secondary bacterial infections, keratitis that threatens vision, encephalitis, and pneumonitis. As of late May 2022, many cases of monkeypox have been discovered in various countries in the Middle East (10).

As a result of the extraordinary success achieved by the World Health Organization (WHO) in smallpox eradication 40 years ago, smallpox vaccination is no longer used, with about 70% of the population worldwide have not been vaccinated. The smallpox vaccine is delineated as a prevention method for the monkeypox virus as well since it is effective against orthopoxvirus infections; however, most cases of monkeypox infection have occurred in non-vaccinated individuals (11). Healthcare workers are at high risk of contracting infectious diseases like the monkeypox virus. That increased risk stems from close contact between infected patients and healthcare staff, especially when personal protective equipment is unavailable. The third generation of the smallpox vaccine has shown high efficacy in healthcare workers (12): however, healthcare workers may decline vaccination because of emotional and personal considerations rather than scientific knowledge of this particular situation, and if they are affected by vaccine hesitancy, they may convey this attitude to the patients they care for (13). Healthcare workers must deal with the growing number of human monkeypox virus cases worldwide through early detection, management, and prevention. According to the WHO statement, one of the reasons for the resurgence of the infection was poor knowledge of monkeypox among healthcare workers (10). Before the monkeypox virus spreads further, it is necessary to renovate healthcare facilities and prepare for future epidemics, particularly in low-income countries with limited healthcare system resources (14). During the current COVID-19 pandemic, low- and middle-income courtiers have more reasons to worry about monkeypox virus due to their lower socioeconomic level and limited access to healthcare.

Consequently, they must prepare to cope with another outbreak (15). In Syria, the outbreak of COVID-19 has been a major challenge added to the country's inhabitants who were also affected by the catastrophic effects of warfare (16). A previous study from Jordan revealed that healthcare workers had limited knowledge of the monkeypox virus and confirmed that practitioners lacked confidence in their abilities to diagnose and treat infected patients (17). The monkeypox virus has been a source of rising worry among scientists for various circumstances, including the fact that the disease does not have a definitive treatment or vaccine until now, and the current treatment management depends on improving symptoms and preventing complications. Furthermore, after the monkeypox outbreak in many countries, concerns about the possibility of virus phenotype changing by different mutations have increased (18). The objective of this study is to assess the concerns of healthcare workers in the Arabic countries about the monkeypox virus and the factors associated with good knowledge, in addition to examining vaccine advocacy among them.

2. Methods

2.1. Study design and setting

An online cross-sectional study was conducted from 2 August 2022 to 28 December 2022 to assess worries and concerns among HCWs toward the monkeypox virus and the factors associated with good knowledge, as well as to examine monkeypox virus vaccine advocacy among them. The inclusion criteria were healthcare workers, such as physicians, nurses, pharmacists, and undergraduate medical students, from the Arabic countries. The countries involved in this study were Egypt, Saudi Arabia, Yemen, Syria, Libya, Algeria, Tunisia, Iraq, Palestine, Jordan, and Sudan. All participants were informed of the aim of the study, the work team identity, their right to withdraw from the study, and the confidentiality of their personal information. The questionnaire

was developed based on a previous cross-sectional study conducted in the Arabic country, Saudi Arabia, which included validated scales (19). Furthermore, a professional translator translated the survey from English into Arabic to ensure the total comprehension of the questions. We performed convenience and snowball sampling strategies to perform a professional and non-biased data collection process as possible. We collected the data by creating a Google Forms survey and sending it to respondents through social media platforms such as Facebook, WhatsApp, and Telegram. Fourteen collaborators from each investigated Arabic country in our study were responsible for the data-gathering process. In addition, there was a lead collaborator in each involved study as a local investigator to monitor the data collection and investigate if there were any random, multi-auto, or illogical responses on the online questions, and to check the current job of each respondent to avoid including any person from non-medical staff.

2.2. Sample size calculation

The minimal sample size was computed by interrupting a single proportion of the population formula [$n = [(Z\alpha/2)2 \times P(1 - P)]/d^2$], with a 95% of confidence level (CI); $Z\alpha/2 = 1.96$; a 5% margin of error; P, the proportion of healthcare workers who were more concerned about Monkeypox disease compared to COVID-19 (35.7%); and the proportion of healthcare workers who accepted the vaccination (67.7%) (19). According to the formula, a sample size of 385 was required. The study questionnaire was sent to 3,902 participants through the Google Forms; however, 46 of them refused to participate, bringing the total number of responses to 3,856.

2.3. Measures

The questionnaire consists of 44 questions divided into five sections. The first section contains information about the participants' sociodemographic variables; the second evaluates HCWs' knowledge of the monkeypox virus and their sources of information; the third examines the perceptions and concerns of healthcare workers about the monkeypox virus; the fourth addresses questions regarding knowledge of HCWs monkeypox infection; and the final section of the questionnaire includes questions adapted from the Generalized Anxiety Disorder-7 (GAD-7) to assess HCWs anxiety about the monkeypox virus.

2.3.1. Sociodemographic variables and professional characteristics

To identify about the participants' demographic characteristics, such as their age, country of origin, gender, marital status, place of residence, chronic disease, number of family members, economic status, and educational background (including whether they are physicians, nurses, pharmacists, or medical students and their academic year), 14 questions were included in the questionnaire. Furthermore, there were questions about the participants' working hospital type (primary, secondary, or tertiary healthcare centers). The respondents' years of experience and their workplace within the hospital if they work in the hospital pharmacy, intensive care units, isolation departments, or elsewhere were included as additional information. The last question of this section asked the respondents if they had ever been diagnosed with COVID-19.

2.3.2. Healthcare workers' awareness and sources of information about monkeypox disease

This section consists of four questions about participants' awareness of the monkeypox virus, including whether the respondents had visited a monkeypox-endemic country (West or Central Africa, Europe, North America, the UAE, and Australia). Also, participants were asked to evaluate their current awareness of the monkeypox disease (low, high, or moderate), and they were asked how informed they were about monkeypox disease (international health websites, social media platforms, or scientific journals) and whether they needed to read more about monkeypox after participating in the survey.

2.3.3. Perceptions and worries of healthcare workers about monkeypox disease

This section contains eight questions designed to measure the concerns and perceptions of healthcare workers regarding the monkeypox virus. Respondents were asked if they were concerned on whether the monkeypox virus will cause a global pandemic like COVID-19 and whether they believe that the monkeypox infection causes a more severe disease than monkeypox. In addition, they were asked to identify the cause of their monkeypox worries (such as their fear of being affected by the disease, concerns about developing another worldwide pandemic, or worries about national lockdown). Respondents were questioned on their acceptance of vaccination and their perceptions of which category should first get the monkeypox vaccine (older adult, children, college students, etc.).

2.3.4. Knowledge of the monkeypox virus among healthcare workers

Regarding assessing HCWs' knowledge of the monkeypox virus, we adopted questionnaire items from a study about knowledge of human monkeypox among students in various Jordanian health schools (20). In this section, with 11 questions about monkeypox, participants were asked: "is monkeypox common in the Middle East?" "is monkeypox common in Western and Central Africa?" "is there a global epidemic of human monkeypox?" "is monkeypox caused by a virus or another pathogen?" and "is spreading the disease from person to person a risk?" Participants were also asked "whether human monkeypox could be treated with antibiotics?" "whether diarrhea is one of the signs or symptoms of human monkeypox?" "whether pustules are one of the signs or symptoms of infection?" "whether skin rash is one of the signs or symptoms of human monkeypox?" "whether monkeypox has similar signs and symptoms to smallpox?" and "whether vaccination is available to prevent human monkeypox?" The possible answers to each knowledge item were "yes," "no," and "I do not know"). Correct replies were given a score of 1,

wrong responses were assigned a score of −1, and "I do not know" was given a score of 0. These scores represented the participants' monkeypox knowledge score (MPX K-score). An adequate degree of knowledge was determined as a score of 70% correct replies or above as we depended on the published studies.

2.3.5. Generalized anxiety disorder toward monkeypox

This scale contains seven items that measure participants' GAD regarding the monkeypox virus (20, 21). Participants were asked to rate how often they had felt symptoms such as worry, concern, restlessness, impatience, and dread over the past 2 weeks. We assigned values from 0 to 3 for the four frequency levels of never, sometimes, often, and very frequently. There were four levels of severity determined by the GAD7 score: minimum (1–4), mild (5–9), moderate (10–14), and severe (0–14). (15–21).

2.4. Pilot study

To make sure the survey questions were clear before launching the online survey on social media platforms, we sent the questions to 45 randomly selected Arabic healthcare providers from specific countries. Then we modified the survey depending on the feedback and suggested adjustments. Although we have used the scales from a published study of an Arabic country, we ran a pilot study in which we sent the questionnaire to 50 volunteers, who were healthcare providers from those countries involved in our study to confirm the reliability of the used scales, for which we determined the Cronbach's α to each involved scale. Then, we confirmed that the scales we used in our cross-sectional study had high internal consistency levels (Cronbach's α was above 7.0).

2.5. Ethical consideration

The Syrian Ethical Society for Scientific Research at Aleppo University, Syria provided the ethical approval for conducting the study (IRB: SA-1087). In addition, we ordered at least one printed ethical approval from the clinical and educational institutions (Hospitals and Medical Colleges) of the lead collaborators from each investigated country of our study. The first question in the online survey was about the respondent's acceptance to complete the survey. We also ensured that all methods in our online cross-sectional were according to the Declaration of Helsinki developed by World Medical Association (WMA). The survey takes 5–12 min to complete, and for security purposes, all data is saved in an online database.

2.6. Statistical analysis

The data were examined using the Statistical Package for the Social Sciences (IBM SPSS V. 28.0). Statistical significance was defined as a p-value of ≤ 0.05. The quantitative data were given with a mean and standard deviation, while the categorical data were presented with frequency and percentages. After validating the data and distribution that were non-parametric using the Shapiro–Wilk test, we used the Kruskal–Wallis test to compare how much each subgroup differed from others in terms of their awareness of monkeypox, desiring to vaccinate themselves against monkeypox, and worrying toward the new pandemic that will arise due to monkeypox. Finally, using the cutoff points from the Saudi Arabian research (22), we conducted a binary logistic regression to calculate the odds ratios (ORs) between the dependent variables (awareness of monkeypox and desire to vaccinate themselves against monkeypox) and independent variables (sociodemographic factors) for having an appropriate awareness of monkeypox and a desire to vaccinate themselves against monkeypox.

3. Results

3.1. Demographic characteristics

The questionnaire was distributed to 3,902 participants; however, 46 among the participants declined to participate, resulting in a final sample size of 3,856. Most of the participants were aged between 21 and 30 years (78%), and more than half of the sampling participants (56.3%) were of females. Participants residing in the city comprised 82.3%, and most of the participants (50.2%) had a moderate financial condition. Students involved in the study were 50.1%, while practitioners involved in the study remained 30.7%. The majority (63.6%) of participants were employed by the hospital's central wards, while 16.5% worked in the outpatient department, and 12.1% were employed by the hospital's pharmacy or laboratory (Table 1).

3.2. HCWs' monkeypox disease perceptions and COVID-19 status

Participants with previous diagnoses of COVID-19 comprised 35.7%; however, 8.8% of participants were concerned that monkeypox might generate an epidemic like COVID-19, whereas 43.5% of participants were uncertain about the severity of monkeypox compared to smallpox. Respondents concerned more about monkeypox than about COVID-19 were 18.1%, and 82.3% of the respondents felt that they needed to learn more about it after reading the survey. More than half of the participants (54.5%) have expressed acceptance of the vaccination against monkeypox. Participants reported social media (58.1%), websites of the WHO/Centers for Disease Control and Prevention (CDC) (31.1%), and the Internet (30.2%) as a source of information about monkeypox (Table 2).

3.3. HCWs' sources of worries from monkeypox disease

We found that 61.7% of participants were concerned about being infected themselves or their family, while 54.6% were worried about the number of monkeypox cases increasing to the level that might force a national lockdown. Less than half of the participants

10.3389/fpubh.2023.1153136

TABLE 1 Participants' baseline sociodemographic and professional characteristics.

Statement		Frequency	Percentage
Country	Jordan	602	15.6%
	United Arab Emirates	14	0.36%
	Algeria	23	0.59%
	Saudi Arabia	264	6.8%
	Sudan	555	14.4%
	Somalia	9	0.2%
	Iraq	93	2.4%
	Kuwait	10	0.3%
	Morocco	8	0.2%
	Yemen	1,041	27.0%
	Tunisia	56	1.5%
	Oman	3	0.1%
	Syria	351	9.1%
	Palestine	40	1.0%
	Qatar	10	0.3%
	Lebanon	6	0.25%
	Libya	79	2.0%
	Egypt	692	17.9%
Sex	Female	2,171	56.3%
	Male	1,685	43.7%
Age (years)	<20	451	11.7%
	21–30	3,006	78.0%
	31–40	260	6.7%
	41–50	102	2.6%
	51–60	26	0.7%
	>60	9	0.2%
Marital state	Never married	3,107	80.6%
	Married	749	19.4%
Households (family) size	1–3 members	466	12.1%
	4–6 persons	1,873	48.6%
	7–10 persons	1,299	33.7%
	More than 10 persons	218	5.7%
Households' monthly income	Bad	248	6.4%
	Moderate	1,937	50.2%
	Good	1,341	34.8%
	Excellent	330	8.6%
Working hospital type	Primary healthcare center	1,569	40.7%
	Secondary healthcare hospital	1,134	29.4%
	Tertiary healthcare hospital	1,153	29.9%
Clinical role	Medical student	1,932	50.1%
	Technicians/lab workers and pharmacists	404	10.5%
	Nurses	337	8.7%
	Physicians	1,183	30.7%
Study year	First year	100	4.6%
	Second year	224	10.2%
	Third year	339	15.4%
	Fourth year	445	20.3%
	Fifth year	554	25.2%
	Sixth year	533	24.3%
Experience duration	<5 years	2,054	84.7%
	More than 5 years	372	15.3%
Living place	Village	684	17.7%
	City	3,172	82.3%
Chromic disease	Don't have	3,559	92.3%
	Have	297	7.7%
Hospital working area/covering service	Pharmacy and laboratory	468	12.1%
	Critical care units	221	5.7%
	Infectious disease/isolation wards	81	2.1%
	General wards	2,451	63.6%
	OPD	635	16.5%

OPD, outpatient department.

reported being anxious about the sickness progressing to the level of a global pandemic (45.9%) (Table 3).

3.4. The level of human monkeypox knowledge among HCW

Approximately half of the participants (55%) were unaware of the monkeypox virus (Figure 1), and 23.8% of respondents believe that the monkeypox virus is expected in the Arabic countries. In comparison, 35.3% of respondents do not know whether there is a global epidemic of monkeypox. Regarding the resemblance of symptoms between monkeypox and smallpox, 58.4% of participants thought the symptoms were similar, while 23.3% of participants agreed that antibiotics might be used to treat monkeypox. Only 27.1% of respondents believe that monkeypox immunization is available (Table 4).

TABLE 2 Descriptive analysis of the HCWs' monkeypox disease perceptions and COVID-19 status.

Statement		Frequency	Percentage
Have you been previously diagnosed with COVID-19?	Yes	1,375	35.7%
	No	2,481	64.3%
Have you traveled in the last month to a country where monkeypox was recently reported?	I don't travel	3,665	95.1%
	Europe, North America, and Australia	54	1.4%
	UAE	63	1.6%
	West or Central Africa	24	0.6%
	Other (far Asia, India, Spain, France, and countries of Middle East)	50	1.3%
How would you rate your awareness of Monkeypox in the meantime?	Low	2,019	52.4%
	Moderate	1,656	42.9%
	High	181	4.7%
How worried are you that monkeypox can cause a worldwide pandemic like COVID-19?	None/less worried	1,991	51.6%
	Moderate worry	1,526	39.6%
	Worried a lot	339	8.8%
Do you think Monkeypox causes a more severe disease compared to Smallpox?	Disagree	666	17.3%
	Unsure	1,679	43.5%
	Agree	1,511	39.2%
Which is more worrisome to you, COVID-19 or monkeypox disease?	Unsure/equally worried	1,492	38.7%
	I am more worried about COVID-19	1,665	43.2%
	I am more worried about monkeypox	699	18.1%
Healthcare workers should apply more infection control measures than the current ones, with the new monkeypox outbreaks	Agree	3,069	79.6%
	Neither agree nor disagree	536	13.9%
	Disagree	251	6.5%
Please rate your worry level about traveling abroad with the new monkeypox outbreaks in some countries	Not worried at all	1,375	35.7%
	Somewhat worried	2,124	55%
	Extremely worried	357	9.3%
After Receiving this survey, did you perceive the need to read more about monkeypox disease?	No	682	17.7%
	Yes	3,174	82.3%
Your sources of information about monkeypox disease	Official local statements	1,163	30.1%
	International health authorities' websites (WHO or CDC)	1,202	31.1%
	Social media	2,244	58.1%
	Scientific journals	652	16.9%
	Other Internet-based sources	1,166	30.2%
Do you want to receive the monkeypox vaccine?	No	1,754	45.5%
	Yes	2,102	54.5%

3.5. HCWs' odds ratios of high worry of monkeypox compared to COVID-19

Our results show that females were more concerned about COVID-19 (44.6%) than monkeypox (11.7%), as well as participants who had not been diagnosed with COVID-19 were concerned more about COVID-19 (53.1%) than about monkeypox (11.2%). Among respondents who felt that monkeypox symptoms are like smallpox, 10.8% were more concerned about monkeypox than about COVID-19. Among anxious individuals, 18.4% are more concerned about COVID-19 than about monkeypox virus. Notably 7 out of 15 predictor factors were significantly linked with greater worry from monkeypox than from COVID-19 ($p < 0.05$). Participants with more than 5 years of work experience were less likely to be concerned more about monkeypox than about COVID-19 (OR = 0.59; 95% CI: 0.374–0.931), comparable to

TABLE 3 HCWs' sources of worries from monkeypox disease.

Statement	Frequency	Percent
Worried monkeypox might surge to cause national lockdown	2,107	54.6%
Me or my family being affected by the monkeypox	2,383	61.7%
Another worldwide pandemic	1,774	45.9%
International flight suspension	515	13.3%
Other	399	10.3%

those with <5 years of work experience. Participants diagnosed with COVID-19 were less likely to worry about monkeypox (OR = 0.63; 95% CI: 500–807) than those without. A higher likelihood of worrying about monkeypox than COVID-19 was anticipated among participants who worried more about monkeypox causing a widespread epidemic like COVID-19 (OR = 2.87; 95% CI: 1.962–4.212). Concern about monkeypox was expected to be higher than COVID-19 (OR = 4.47; 95% CI: 2.852–7.020) among participants who believed that monkeypox produces more severe symptoms than smallpox (Table 5).

3.6. HCWs' odds ratios of supporting vaccinations against monkeypox disease

Our analysis shows that 29.4% of females, 42.4% of participants aged between 21 and 30 years, 32.9% of participants diagnosed with COVID-19, and 46.6% of participants with <5 years of experience accepted receiving the vaccine. However, 13.7% of participants with good economic status, 3.6% of participants aged between 31 and 40 years, and 35.3% of participants who were not anxious about monkeypox refused to receive the vaccine.

Notably, 7 of the 15 predictor factors were statistically associated with HCWs' support for immunizations against monkeypox ($p < 0.05$). Male MHWs were more likely to accept immunization against monkeypox (OR = 1.3; 95% CI: 1.168–1.668) than female MHWs. Participants aged between 21 and 30 years were 2.36 times more likely to receive the vaccine than those aged under 20. Participants who were not diagnosed with COVID-19 infection have a lower probability of accepting the vaccine than participants who were diagnosed with COVID-19 infection (OR = 0.64). Regarding the GAD-7 scale, anxious participants were more likely to endorse immunizations against monkeypox (OR = 1.48) than those without anxiety (Table 6).

3.7. HCWs' odds ratios of supporting the implementation of tighter infection control measures against monkeypox compared to the currently applied during COVID-19

Regarding adherence to monkeypox disease control measures, participants who showed no adherence were 53.7% of females, 73.1% of participants aged between 21 and 30 years, 38.9% of participants worried more about COVID-19, and 23.6% of respondents had anxiety about monkeypox. In comparison, participants revealed adherence to control measures were 4.9% of participants aged between 31 and 40 years, 4.8% of participants who do not have anxiety about monkeypox, and 5.9% of participants with <5 years of experience.

Notably, five of the 14 predictor factors were substantially linked to HCWs' probability of backing more stringent infection control measures against monkeypox ($p < 0.05$). Females have shown greater adherence to disease control measures than males (OR = 1.67). Participants with anxiety were more likely to adhere to disease control measures than that of the participants without anxiety (OR = 1.79). Participants worried more about COVID-19 have a greater probability of disease control measures adherence (OR = 1.64) compared to participants who expressed equal concern about both illnesses (Table 7).

3.8. HCWs' odds ratios of monkeypox knowledge score

Good knowledge about the monkeypox virus was shown by 25.4% of females and 34.8% of individuals aged 21–30 years. However, only 21.3% of medical students and 7.7% of clinicians with more than 5 years of experience show adequate knowledge of monkeypox. Only 22.2% of the participants agreed that monkeypox develops a more severe illness than smallpox, and 27.6% of respondents who agreed to receive the monkeypox vaccination had good knowledge of monkeypox. Only 12.1% of individuals with anxiety disorders have a good knowledge of monkeypox. In a multivariate logistic regression analysis, we found that family size, study year, participants' ratings of their awareness of monkeypox, participants' worry that monkeypox will cause a pandemic like COVID-19, and whether healthcare workers should apply more infection control measures were all significantly associated with HCWs' odds ratios of knowing about monkeypox ($p < 0.05$). Participants concerned about monkeypox developing a similar pandemic like COVID-19 have greater knowledge than participants who did not concern about monkeypox (OR = 1.82). Respondents who disagreed that HCWs should adhere more to the disease control methods were less likely to be knowledgeable about monkeypox than participants who agreed that HCWs should adhere more to the disease control methods (OR= 0.38) (Table 8).

4. Discussion

Monkeypox is an infectious disease caused by orthopoxvirus characterized by a rash that may be isolated, preceded, or accompanied by fever or lymph nodes (23). Since 14 May 2022, confirmed cases of the virus have been reported or confirmed in several countries in Europe and North America, and the situation is evolving rapidly. In the UK, 16 cases of infection have been detected (as of 17 May 2022). Except for the first infected person, returning from Nigeria, all appear to have been infected in the UK, according to the local health safety agency (24). For fear of a possible new pandemic, health authorities worldwide have boosted their efforts to ensure the control of its spread by studying the means of transmission and early clinical signs. During the current increase of the reported infected cases of monkeypox, the knowledge, concern,

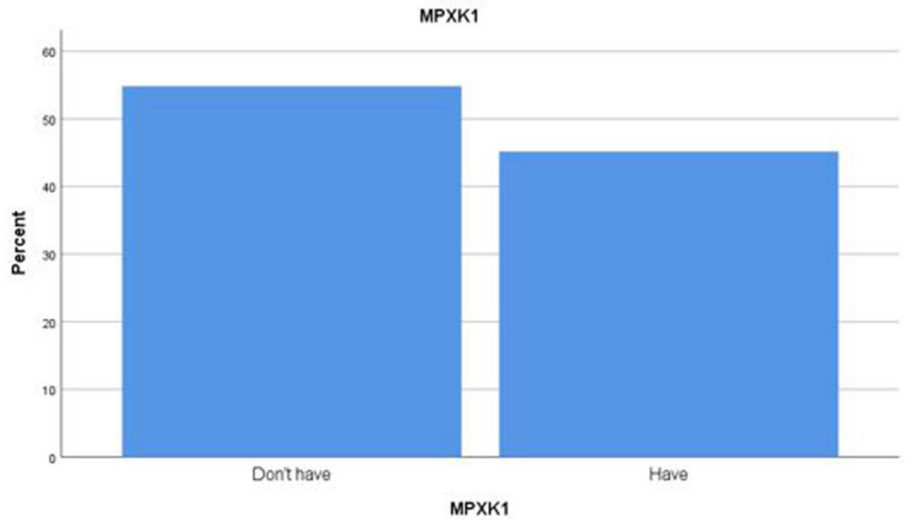

FIGURE 1
The prevalence of human monkeypox knowledge among HCW.

and perception of the available vaccines are concerning factors, especially among healthcare providers and medical staff persons (25). Our study was conducted in the Middle East and North Africa (MENA) region with a final sample size of 3,856 participants, of which 1,375 had a history of COVID-19 diagnosis. Results reported that about 9% of participants considered that monkeypox might generate as an epidemic and a tremendous burden on human health scenario might occur like COVID-19, while 51.6% had no worries about monkeypox. These findings are similar to a Saudi Arabian study where only 25.3% of the study population were very worried, and 48.7% had no or less worries about a further monkeypox pandemic (22). Also, for almost the quarter, 18.1% of participants were concerned more about monkeypox than about COVID-19. These findings are also concomitant with another Saudi Arabian study published in August 2022 carried out by Mohamad et al. among the general population, where results reported a higher worry (62%) about COVID-19 than monkeypox (26). Concerning HCWs' sources of worries toward monkeypox disease, the majority (61.7%) of participants were concerned about being infected themselves or their relatives, and slightly more than half (54%) were afraid of a possible future lockdown. Similarly, these findings are concomitant with results found in a Saudi Arabian study by Temsah et al. (26).

About monkeypox knowledge level, slightly more than half (55%) of the participants were unaware of the monkeypox virus and had no sufficient information about it, and 58.4% of respondents could not make a difference between monkeypox and smallpox symptoms. Also, only 27.1% of participants reported positively that monkeypox immunization is already available. Knowledge findings were concomitant overall with Indonesian research conducted by Harapan et al., where monkeypox knowledge level was evaluated as low at 63.5% and insufficient among 432 general practitioners (27).

Male HCWs in the MENA region were less predicted to worry about monkeypox than female HCWs (6.4 and 11.7%, respectively), which was also reported toward COVID-19 worries. This was similarly found in a Saudi Arabian study by Ajman et al. (22). In addition, results reported that participants diagnosed with COVID-19 were less likely to worry about monkeypox (OR = 0.63 times) than those who had not been infected with COVID-19 virus. Findings also reported higher acceptance for the monkeypox vaccine by participants who had not been diagnosed with COVID-19 ($n = 1{,}268$, 32.9%) than those had been diagnosed with previous COVID-19. This incomprehensible and unpredictable finding could only be justified by a drop in healthcare workers' confidence level in vaccine protection after COVID-19 infection following administration of vaccines. This should be adjusted and corrected as approved vaccines have proven to be effective in preventing fatal complications of COVID-19 infection, reducing the number of people hospitalized and admitted to the intensive care unit, and reducing the number of infections without preventing it (28).

The knowledge of monkeypox infection, attitude toward its possible spread among healthcare practitioners in the MENA region, and vaccine advocacy must be improved urgently. This will prevent a possible pandemic because a good knowledge of the symptoms, confidence in diagnosis, modes of transmission, physiopathology, and comorbidities will help to avoid the maximum number of cases. Also, in case of a further pandemic, it will pave way to control the situation efficiently and professionally, based on the previous COVID-19 experience (15). These human monkeypox concerns among Arabic healthcare professionals can be corrected and improved through several approaches and by multiple means such as (29–32) (a) continuing medical education and scientific improvement on the infection process, which makes it less contagious than COVID-19 and, therefore, same rapid spread and a sudden pandemic scenario like COVID-19 are not expected; (b) more data about available vaccines and their efficiency—currently, only two vaccines, ACAM2000 vaccine and JYNNEOS, known as Imvanex, are available, and (c) involvement in research of international

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TABLE 4 The level of human monkeypox knowledge among HCWs.

Human monkeypox knowledge item	Response	Frequency	Percent
Monkeypox is prevalent in the Arabic countries	Incorrect	970	25.2%
	Do not know	1,967	51.0%
	Correct	919	23.8%
Monkeypox is prevalent in Southeast Asia	Incorrect	368	9.5%
	Do not know	2,201	57.1%
	Correct	1,287	33.4%
There is an outbreak of human monkeypox in the world	Incorrect	824	21.4%
	Do not know	1,363	35.3%
	Correct	1,669	43.3%
Monkeypox is caused by a virus	Incorrect	175	4.5%
	Do not know	908	23.5%
	Correct	2,773	71.9%
Human-to-human transmission of monkeypox occurs easily	Incorrect	778	20.2%
	Do not know	1,346	34.9%
	Correct	1,732	44.9%
Monkeypox and smallpox have similar signs and symptoms	Incorrect	284	7.4%
	Do not know	1,319	34.2%
	Correct	2,253	58.4%
Skin rash is one of the signs or symptoms of human monkeypox	Incorrect	193	5.0%
	Do not know	917	23.8%
	Correct	2,746	71.2%
Pustule is one of the signs or symptoms of human monkeypox	Incorrect	252	6.5%
	Do not know	1,630	42.3%
	Correct	1,974	51.2%
Antibiotics are used to treat human monkeypox	Incorrect	1,362	35.3%
	Do not know	1,595	41.4%
	Correct	899	23.3%
Diarrhea is one of the signs or symptoms of human monkeypox	Incorrect	554	14.4%
	Do not know	2,310	59.9%
	Correct	992	25.7%
Vaccination is available to prevent human monkeypox	Incorrect	876	22.7%
	Do not know	1,936	50.2%
	Correct	1,044	27.1%

(Continued)

monkeypox network and patients' sensitivity and education on preventive measures.

5. Limitations and strengths

To examine the present degree of opinions of healthcare professionals concerning the characteristics of the monkeypox epidemic, next to COVID-19, our international cross-sectional survey includes a large sample size from various countries in the Arabic region. Additionally, we utilized scales that were getting better with its effectiveness, developed by Arabic scholars. We checked their validity to ensure if the questions they were using accurately represented the subject being investigated. Nevertheless, even though cross-sectional research may be carried out in a short amount of time and at no cost, the research needs to consider the specific and valid causal link and the generality of monkeypox. In addition, concerning the online cross-sectional research, it is difficult to get answers from those who do not have surplus time for attempting the questionnaire, those who do not have Internet access and a mobile phone, or those who are having trouble completing the survey due to technical challenges. This is particularly the case in connection with older adult individuals who are not familiar with the use of mobile phones.

6. Conclusion

Our results showed that healthcare professionals in the Arabic countries seemed to be less concerned about the monkeypox virus compared with their concern about the COVID-19 virus. Moderate knowledge of the monkeypox virus was noticed, and less tendency to receive vaccination against the monkeypox virus was also noticed. Furthermore, negative attitudes toward the monkeypox virus protection methods were observed. As a result, we recommend further regulations for the medical staff and precautionary measures. Furthermore, adequate awareness programs should be implemented for medical staff to teach them about the risks of monkeypox infection.

TABLE 5 Multivariate binary logistic regression analysis of the HCWs' odds ratios of high worry from monkeypox compared to COVID-19.

Variables	Categories	A high worry from monkeypox compared to COVID-19				P-value	Non-adjusted odds ratio (non-AOR)	Lower	Upper	P-value	Multivariate adjusted odds ratio (AOR)	Lower	Upper
		Worry more about COVID-19 or equal worrying		Worry from monkeypox									
		Frequency	Percentage	Frequency	Percentage								
Age (years)	<20	348	9.0%	103	2.7%					1			
	21–30	2,453	63.6%	553	14.3%	0.025	0.762	0.600	0.967	0.190	1.309	0.876	1.956
	31–40	226	5.9%	34	0.9%	0.002	0.508	0.333	0.775	0.547	1.225	0.633	2.370
	41–50	98	2.5%	4	0.1%	0.000	0.138	0.050	0.384	0.236	0.475	0.138	1.629
	51–60	24	0.6%	2	0.1%	0.089	0.282	0.065	1.211	0.967	0.965	0.184	5.074
	>60	7	0.2%	2	0.1%	0.965	0.965	0.197	4.718	0.351	2.266	0.406	12.639
Sex	Female	1,720	44.6%	451	11.7%					1			
	Male	1,437	37.3%	248	6.4%	0.000	0.658	0.555	0.780	0.216	0.859	0.676	1.093
Marital state	Not married	2,515	65.2%	592	15.4%					1			
	Married	642	16.6%	107	2.8%	0.000	0.658	0.555	0.780	0.781	1.049	0.748	1.472
Households (family) size	1–3 members	392	10.2%	74	1.9%					1			
	4–6 persons	1,551	40.2%	322	8.4%	0.499	1.100	0.835	1.449	0.277	1.235	0.844	1.809
	7–10 persons	1,036	26.9%	263	6.8%	0.040	1.345	1.013	1.784	0.024	1.581	1.063	2.351
	More than 10 persons	178	4.6%	40	1.0%	0.420	1.190	0.779	1.818	0.571	1.174	0.674	2.043
Clinical role	Medical student	1,566	40.6%	366	9.5%					1			
	Technicians/lab workers and pharmacists	323	8.4%	81	2.1%	0.608	1.073	0.820	1.404	0.234	1.276	0.854	1.906
	Nurses	282	7.3%	55	1.4%	0.253	0.834	0.612	1.138	0.674	0.897	0.540	1.489
	Physicians	986	25.6%	197	5.1%	0.107	0.855	0.706	1.034	0.411	1.120	0.855	1.468
Experience duration	<5 years	1,669	68.8%	385	15.9%					1			
	More than 5 years	333	13.7%	39	1.6%	0.000	0.508	0.358	0.720	0.024	0.590	0.374	0.931

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TABLE 5 (Continued)

Variables	Categories	A high worry from monkeypox compared to COVID-19				P-value	Non-adjusted odds ratio (non-AOR)	Lower	Upper	P-value	Multivariate adjusted odds ratio (AOR)	Lower	Upper
		Worry more about COVID-19 or equal worrying		Worry from monkeypox									
		Frequency	Percentage	Frequency	Percentage								
Chromic disease	Don't have	2,916	75.6%	643	16.7%	1							
	Have	241	6.3%	56	1.5%	0.735	1.054	0.778	1.427	0.240	1.272	0.852	1.898
Have you been previously diagnosed with COVID-19?	Yes	1,109	28.8%	266	6.9%								
	No	2,048	53.1%	433	11.2%	0.144	0.881	0.744	1.044	0.000	0.635	0.500	0.807
Have you traveled in the last month to a country where monkeypox was recently reported?	I didn't travel	2,995	77.7%	670	17.4%	1							
	Europe, North America, and Australia	43	1.1%	11	0.3%	0.694	1.144	0.587	2.229	0.480	0.692	0.249	1.922
	UAE	56	1.5%	7	0.2%	0.149	0.559	0.254	1.231	0.110	0.437	0.158	1.207
	West or Central Africa	21	0.5%	3	0.1%	0.469	0.639	0.190	2.147	0.672	0.752	0.201	2.813
	Other (far Asia, India, Spain, France, and middle eastern countries)	42	1.1%	8	0.2%	0.679	0.851	0.398	1.822	0.537	0.705	0.232	2.139
How would you rate your awareness of Monkeypox at the meantime?	Low	1,641	42.6%	378	9.8%	1							

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TABLE 5 (Continued)

Variables	Categories	A high worry from monkeypox compared to COVID-19				P-value	Non-adjusted odds ratio (non-AOR)	Lower	Upper	P-value	Multivariate adjusted odds ratio (AOR)	Lower	Upper
		Worry more about COVID-19 or equal worrying		Worry from monkeypox									
		Frequency	Percentage	Frequency	Percentage								
	Moderate	1,374	35.6%	282	7.3%	0.183	0.891	0.752	1.056	0.024	0.757	0.595	0.963
	High	142	3.7%	39	1.0%	0.353	1.192	0.822	1.729	0.720	1.100	0.653	1.853
How worried are you that monkeypox can cause a worldwide pandemic similar to COVID-19?	Unsure/equally worried	1,778	46.1%	213	5.5%					1			
	I am more worried about COVID-19	1,173	30.4%	353	9.2%	0.000	2.512	2.087	3.024	0.000	1.706	1.311	2.221
	I am more worried about monkeypox	206	5.3%	133	3.4%	0.000	5.389	4.154	6.991	0.000	2.875	1.962	4.212
Do you think Monkeypox causes a more severe disease compared to smallpox?	Disagree	627	16.3%	39	1.0%					1			
	Unsure	1,436	37.2%	243	6.3%	0.000	2.721	1.915	3.864	0.002	2.094	1.322	3.318
	Agree	1,094	28.4%	417	10.8%	0.000	6.128	4.351	8.632	0.000	4.475	2.852	7.020
Healthcare workers should apply more infection control measures than the current ones, with the new Monkeypox outbreaks	Agree	2,444	63.4%	625	16.2%					1			
	Neither agree nor disagree	485	12.6%	51	1.3%	0.000	0.411	0.304	0.556	0.016	0.576	0.367	0.903

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TABLE 5 (Continued)

Variables	Categories	A high worry from monkeypox compared to COVID-19				P-value	Non-adjusted odds ratio (non-AOR)	Lower	Upper	P-value	Multivariate adjusted odds ratio (AOR)	Lower	Upper
		Worry more about COVID-19 or equal worrying		Worry from monkeypox									
		Frequency	Percentage	Frequency	Percentage								
	Disagree	228	5.9%	23	0.6%	0.000	0.394	0.255	0.611	0.198	0.689	0.391	1.215
Please rate your worry level about traveling abroad with the new monkeypox outbreaks in some countries	Not worried at all	1,215	31.5%	160	4.1%	1							
	Somewhat worried	1,705	44.2%	419	10.9%	0.000	1.866	1.533	2.271	0.097	1.270	0.957	1.686
	Extremely worried	237	6.1%	120	3.1%	0.000	3.845	2.922	5.060	0.007	1.753	1.169	2.628
GAD-7	Don't have	2,448	63.5%	432	11.2%	1							
	Have anxiety	709	18.4%	267	6.9%	0.000	2.134	1.793	2.540	0.004	1.452	1.130	1.865

The logistic regression model was statistically significant, X2(31) = 284.591, $p = 0.000$. Hosmer and Lemeshow test 5.712 ($p = 0.679$). The model explained 18.3% (Nagelkerke R^2) of factors associated with high worry from monkeypox compared to COVID-19.

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TABLE 6 Multivariate binary logistic regression analysis of the HCWs' odds ratios of supporting vaccinations against monkeypox disease.

		Do you want to receive the monkeypox vaccine				*p*-value	Non-adjusted odds ratio (OR)	Lower	Upper	*p*-value	Multivariate adjusted Odds Ratio (OR)	95% C.I.for EXP(B)	Upper
		No		Yes								Lower	
		Frequency	Percentage	Frequency	Percentage								
Sex	Female	1,038	26.9%	1,133	29.4%								
	Male	716	18.6%	969	25.1%	0.001	1.240	1.091	1.409	0.000	1.396	1.168	1.668
Ageo	<20	189	4.9%	262	6.8%	0.008		0.004					
	21–30	1,372	35.6%	1,634	42.4%	0.137	0.859	0.703	1.050	0.173	0.807	0.593	1.098
	31–40	140	3.6%	120	3.1%	0.002	0.618	0.455	0.841	0.130	0.689	0.426	1.115
	41–50	34	0.9%	68	1.8%	0.112	1.443	0.918	2.268	0.021	2.362	1.136	4.911
	51–60	13	0.3%	13	0.3%	0.418	0.721	0.327	1.591	0.602	0.764	0.277	2.103
	>60	4	0.1%	5	0.1%	0.879	0.902	0.239	3.403	0.729	0.777	0.187	3.224
Marital state	Not married	1,409	36.5%	1,698	44.0%								
	Married	345	8.9%	404	10.5%	0.725	0.972	0.828	1.140	0.382	0.901	0.713	1.139
Working hospital type	Primary healthcare center	711	18.4%	858	22.3%	0.950				0.449			
	Secondary care hospital	514	13.3%	620	16.1%	0.996	1.000	0.857	1.165	0.962	1.005	0.816	1.238
	Tertiary care hospital	529	13.7%	624	16.2%	0.770	0.977	0.839	1.139	0.272	0.889	0.722	1.096
Experience duration	<5 years	928	38.3%	1,126	46.4%								
	More than 5 years	163	6.7%	209	8.6%	0.627	1.057	0.846	1.320	0.522	0.901	0.655	1.240
Have you been previously diagnosed with COVID-19?	yes	541	14.0%	834	21.6%								
	No	1,213	31.5%	1,268	32.9%	0.000	0.678	0.593	0.775	0.000	0.642	0.534	0.773

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TABLE 6 (Continued)

		Do you want to receive the monkeypox vaccine				*p*-value	Non-adjusted odds ratio (OR)	Lower	Upper	*p*-value	Multivariate adjusted Odds Ratio (OR)	95% C.I.for EXP(B)	Upper
		No		Yes								Lower	
		Frequency	Percentage	Frequency	Percentage								
Healthcare workers should apply more infection control measures than the current ones, with the new Monkeypox outbreaks	Agree	1,296	33.6%	1,773	46.0%	0.000				0.000			
	Neither agree nor disagree	293	7.6%	243	6.3%	0.000	0.606	0.504	0.729	0.001	0.627	0.479	0.820
	Disagree	165	4.3%	86	2.2%	0.000	0.381	0.291	0.499	0.000	0.408	0.288	0.579
Households' monthly income	Bad	130	3.4%	118	3.1%	0.000				0.003			
	Moderate	944	24.5%	993	25.8%	0.275	1.159	0.889	1.510	0.096	1.348	0.948	1.917
	Good	530	13.7%	811	21.0%	0.000	1.686	1.284	2.213	0.013	1.586	1.102	2.283
	Excellent	150	3.9%	180	4.7%	0.098	1.322	0.950	1.839	0.001	2.121	1.359	3.311
After receiving this survey, did you perceive the need to read more about monkeypox disease?	No	466	12.1%	216	5.6%								
	Yes	1,288	33.4%	1,886	48.9%	0.000	3.159	2.649	3.768	0.000	3.068	2.427	3.877
Gad7	Don't have	1,360	35.3%	1,520	39.4%								
	Have anxiety	394	10.2%	582	15.1%	0.000	1.322	1.141	1.531	0.000	1.482	1.222	1.797

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TABLE 6 (Continued)

		Do you want to receive the monkeypox vaccine				*p*-value	Non-adjusted odds ratio (OR)	Lower	Upper	*p*-value	Multivariate adjusted Odds Ratio (OR)	95% C.I.for EXP(B)	Upper
		No		Yes								Lower	
		Frequency	Percentage	Frequency	Percentage								
Your sources of Information about monkeypox disease	Official local statements	112	2.9%	116	3.0%	0.000				0.073			
	International health authorities' websites (WHO or CDC)	122	3.2%	154	4.0%	0.271	1.219	0.857	1.733	0.565	1.138	0.733	1.765
	Social media	602	15.6%	570	14.8%	0.536	0.914	0.688	1.214	0.169	0.777	0.543	1.113
	Scientific journals	38	1.0%	48	1.2%	0.435	1.220	0.741	2.008	0.958	1.017	0.545	1.897
	Other internet-based sources	207	5.4%	215	5.6%	0.986	1.003	0.727	1.384	0.687	0.919	0.609	1.386
	more than one source	673	17.5%	999	25.9%	0.011	1.433	1.086	1.891	0.788	1.049	0.739	1.489
Constant		0.043	0.537										

The logistic regression model was statistically significant, X2(23)= 254.087, $p = 0.000$. Hosmer and Lemeshow test 8.258 ($p = 0.408$). The model explained 13.3% (Nagelkerke R^2) of factors associated with supporting vaccinations against monkeypox disease.

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TABLE 7 Multivariate binary logistic regression analysis of the HCWs' odds of supporting the implementation of tighter infection control measures against monkeypox compared to the currently applied during COVID-19.

Variable	Subgroups	Tighter infection control measures				P-value	Non-adjusted OR	Lower	Upper	P-value	Multivariate adjusted OR	Lower	Upper
		Not doing		Doing tighter control measures									
		Frequency	Percentage	Frequency	Percentage								
Sex	Female	2,071	53.7%	100	2.6%								
	Male	1,534	39.8%	151	3.9%	0.000	2.039	1.570	2.647	0.002	1.678	1.200	2.347
Age (years)	<20	432	11.2%	19	0.5%	0.002				0.716			
	21–30	2,817	73.1%	189	4.9%	0.086	1.525	0.942	2.471	0.282	1.487	0.721	3.066
	31–40	229	5.9%	31	0.8%	0.000	3.078	1.701	5.570	0.318	1.659	0.614	4.479
	41–50	94	2.4%	8	0.2%	0.131	1.935	0.822	4.553	0.968	0.972	0.244	3.866
	51–60	22	0.6%	4	0.1%	0.017	4.134	1.296	13.190	0.591	0.516	0.046	5.749
	>60	9	0.2%	0	0.0%	0.999	0.000	0.000	.	0.999	0.000	0.000	.
Marital state	Not married	2,914	75.6%	193	5.0%								
	Married	691	17.9%	58	1.5%	0.128	1.267	0.934	1.719	0.448	0.824	0.499	1.359
Experience duration	<5 years	1,912	78.8%	142	5.9%								
	More than 5 years	335	13.8%	37	1.5%	0.041	1.487	1.017	2.175	0.084	1.642	0.936	2.882
After receiving this survey, did you perceive the need to read more about Monkeypox disease?	No	570	14.8%	112	2.9%								
	Yes	3,035	78.7%	139	3.6%	0.000	0.233	0.179	0.304	0.000	0.360	0.253	0.513
GAD-7	Don't have	2,696	69.9%	184	4.8%								
	Have anxiety	909	23.6%	67	1.7%	0.603	1.080	0.808	1.443	0.003	1.791	1.218	2.633

(Continued)

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TABLE 7 (Continued)

Variable	Subgroups	Tighter infection control measures				P-value	Non-adjusted OR	Lower	Upper	P-value	Multivariate adjusted OR	Lower	Upper
		Not doing		Doing tighter control measures									
		Frequency	Percentage	Frequency	Percentage								
Your sources of information about Monkeypox disease	Official local statements	203	5.3%	25	0.6%	0.000				0.031			
	International health authorities' websites (WHO or CDC)	242	6.3%	34	0.9%	0.638	1.141	0.659	1.975	0.077	1.883	0.933	3.798
	Social media	1,117	29.0%	55	1.4%	0.000	0.400	0.244	0.656	0.560	0.831	0.445	1.550
	Scientific journals	79	2.0%	7	0.2%	0.462	0.719	0.299	1.730	0.999	1.000	0.345	2.899
	Other Internet-based sources	392	10.2%	30	0.8%	0.094	0.621	0.356	1.085	0.835	0.925	0.446	1.922
	More than one source	1,572	40.8%	100	2.6%	0.005	0.517	0.325	0.820	0.352	0.753	0.415	1.367
Clinical role	Medical student	1,822	47.3%	110	2.9%	0.010				0.232			
	Technicians/lab workers and pharmacists	386	10.0%	18	0.5%	0.321	0.772	0.464	1.287	0.105	0.499	0.215	1.155
	Nurses	307	8.0%	30	0.8%	0.025	1.619	1.062	2.467	0.553	1.224	0.628	2.387
	Physicians	1,090	28.3%	93	2.4%	0.018	1.413	1.062	1.881	0.277	0.809	0.552	1.185
Chromic disease	No	3,334	86.5%	225	5.8%								
	Have	271	7.0%	26	0.7%	0.104	1.422	0.930	2.173	0.967	1.011	0.584	1.751
How worried are you that monkeypox can cause a worldwide pandemic similar to COVID-19?	None/less worried	1,833	47.5%	158	4.1%	0.000				0.862			
	Moderate worry	1,444	37.4%	82	2.1%	0.003	0.659	0.500	0.868	0.697	0.928	0.637	1.352
	Worried a lot	328	8.5%	11	0.3%	0.003	0.389	0.209	0.725	0.802	1.099	0.525	2.300

(Continued)

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TABLE 7 (Continued)

Variable	Subgroups	Tighter infection control measures				P-value	Non-adjusted OR	Lower	Upper	P-value	Multivariate adjusted OR	Lower	Upper
		Not doing		Doing tighter control measures									
		Frequency	Percentage	Frequency	Percentage								
Please rate your worry level about traveling abroad with the new Monkeypox outbreaks in some countries	Not worried at all	1,241	32.2%	134	3.5%	0.000				0.303			
	Somewhat worried	2,020	52.4%	104	2.7%	0.000	0.477	0.366	0.622	0.143	0.761	0.528	1.097
	Extremely worried	344	8.9%	13	0.3%	0.000	0.350	0.196	0.626	0.344	0.685	0.313	1.499
Which is more worrisome to you, COVID-19 or monkeypox disease?	Unsure/equally worried	1,429	37.1%	63	1.6%	0.000				0.022			
	I am more worried about COVID-19	1,500	38.9%	165	4.3%	0.000	2.495	1.850	3.365	0.010	1.642	1.124	2.397
	I am more worried about monkeypox	676	17.5%	23	0.6%	0.296	0.772	0.475	1.255	0.947	1.021	0.551	1.891
Do you think monkeypox causes a more severe disease compared to smallpox?	Disagree	544	14.1%	122	3.2%	0.000				0.000			
	Unsure	1,599	41.5%	80	2.1%	0.000	0.223	0.166	0.301	0.000	0.315	0.215	0.463
	Agree	1,462	37.9%	49	1.3%	0.000	0.149	0.106	0.211	0.000	0.224	0.141	0.357
Households (family) size	1–3 members	435	11.3%	31	0.8%	0.880				0.995			
	4–6 persons	1,753	45.5%	120	3.1%	0.847	0.961	0.638	1.445	0.850	1.051	0.627	1.761
	7–10 persons	1,216	31.5%	83	2.2%	0.843	0.958	0.625	1.468	0.989	1.004	0.582	1.731
	more than 10 persons	201	5.2%	17	0.4%	0.585	1.187	0.642	2.194	0.929	1.035	0.485	2.211

The logistic regression model was statistically significant, X2(30) = 200.97, $p = 0.000$. Hosmer and Lemeshow test 3.89 ($p = 0.866$). The model explained 19.4% (Nagelkerke R^2) of factors associated with the supporting implementation of tighter infection control measures against monkeypox compared to the currently applied during COVID-19.

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TABLE 8 Multivariate binary logistic regression analysis of the HCWs' odds ratios of monkeypox knowledge score.

Variable	Subgroups	Monkeypox knowledge				*p*-value	Non-adjusted OR	Lower	Upper	*p*-value	Multivariate adjusted OR	Lower	Upper
		Don't have		Have									
		Frequency	Percentage	Frequency	Percentage								
Sex	Female	1,191	30.9%	980	25.4%					1			
	Male	923	23.9%	762	19.8%	0.959	1.003	0.883	1.140	0.127	0.807	0.613	1.063
Age	<20	258	6.7%	193	5.0%					1			
	21–30	1,664	43.2%	1,342	34.8%	0.461	1.078	0.883	1.317	0.558	1.154	0.715	1.861
	31–40	142	3.7%	118	3.1%	0.502	1.111	0.817	1.510	0.755	1.219	0.351	4.233
	41–50	30	0.8%	72	1.9%	0.000	3.208	2.015	5.107	1.000	0.000	0.000	
	51–60	16	0.4%	10	0.3%	0.664	0.835	0.371	1.882	1.000	0.000	0.000	
	>60	3	0.1%	6	0.2%	0.168	2.674	0.660	10.825	0.999	0.000	0.000	
Marital state	Not married	1,764	45.7%	1,343	34.8%					1			
	Married	350	9.1%	399	10.3%	0.000	1.497	1.276	1.757	0.183	1.410	0.851	2.337
Households (family) size	1–3 members	260	6.7%	206	5.3%					1			
	4–6 persons	1,013	26.3%	860	22.3%	0.507	1.072	0.874	1.314	0.292	1.293	0.802	2.087
	7–10 persons	739	19.2%	560	14.5%	0.682	0.956	0.773	1.184	0.230	1.352	0.826	2.212
	More than 10 persons	102	2.6%	116	3.0%	0.028	1.435	1.039	1.982	0.011	2.220	1.197	4.118
Working hospital type	Primary healthcare center	861	22.3%	708	18.4%					1			
	Secondary care hospital	591	15.3%	543	14.1%	0.156	1.117	0.959	1.302	0.460	0.886	0.644	1.220
	Tertiary care hospital	662	17.2%	491	12.7%	0.187	0.902	0.774	1.051	0.527	0.896	0.638	1.259
Clinical role	Medical student	1,111	28.8%	821	21.3%					1			
	Technicians/lab workers and pharmacists	204	5.3%	200	5.2%	0.010	1.327	1.070	1.645	0.533	0.813	0.425	1.557
	Nurses	172	4.5%	165	4.3%	0.027	1.298	1.030	1.637	0.150	1.452	0.873	2.414
	Physicians	627	16.3%	556	14.4%	0.014	1.200	1.037	1.388	0.572	0.853	0.491	1.481

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TABLE 8 (Continued)

Variable	Subgroups	Monkeypox knowledge				*p*-value	Non-adjusted OR	Lower	Upper	*p*-value	Multivariate adjusted OR	Lower	Upper
		Don't have		Have									
		Frequency	Percentage	Frequency	Percentage								
Study year	First year	56	2.6%	44	2.0%					1			
	Second year	119	5.4%	105	4.8%	0.632	1.123	0.699	1.804	0.770	1.110	0.550	2.240
	Third year	206	9.4%	133	6.1%	0.393	0.822	0.523	1.290	0.088	0.548	0.274	1.094
	Fourth year	256	11.7%	189	8.6%	0.780	0.940	0.607	1.455	0.140	0.572	0.272	1.202
	Fifth year	365	16.6%	189	8.6%	0.059	0.659	0.428	1.015	0.012	0.383	0.181	0.812
	Sixth Year	284	12.9%	249	11.3%	0.617	1.116	0.726	1.715	0.392	0.721	0.340	1.526
Experience duration	<5 years	1,122	46.2%	932	38.4%					1			
	More than 5 years	185	7.6%	187	7.7%	0.082	1.217	0.976	1.518	0.121	0.645	0.371	1.122
Chromic disease	Don't have	1,940	50.3%	1,619	42.0%					1			
	Have	174	4.5%	123	3.2%	0.175	0.847	0.666	1.077	0.723	1.088	0.683	1.734
Hospital working area/covering service	Pharmacy and laboratory	253	6.6%	215	5.6%					1			
	Critical care units	129	3.3%	92	2.4%	0.288	0.839	0.607	1.160	0.369	0.698	0.318	1.530
	Infectious disease/isolation wards	32	0.8%	49	1.3%	0.016	1.802	1.114	2.915	0.688	1.291	0.371	4.489
	General wards	1,356	35.2%	1,095	28.4%	0.614	0.950	0.779	1.159	0.549	1.182	0.684	2.041
	OPD	344	8.9%	291	7.5%	0.970	0.995	0.783	1.265	0.651	0.863	0.457	1.632
Have you been previously diagnosed with COVID-19?	Yes	709	18.4%	666	17.3%					1			
	No	1,405	36.4%	1,076	27.9%	0.002	0.815	0.714	0.931	0.456	1.121	0.830	1.516

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TABLE 8 (Continued)

Variable	Subgroups	Monkeypox knowledge				*p*-value	Non-adjusted OR	Lower	Upper	*p* -value	Multivariate adjusted OR	Lower	Upper
		Don't have		Have									
		Frequency	Percentage	Frequency	Percentage								
Have you traveled in the last month to a country where monkeypox was recently reported?	I didn't travel	1,988	51.6%	1,677	43.5%					1			
	Europe, North America, and Australia	37	1.0%	17	0.4%	0.039	0.545	0.306	0.971	0.062	0.213	0.042	1.082
	UAE	42	1.1%	21	0.5%	0.052	0.593	0.350	1.005	0.060	0.365	0.128	1.044
	West or Central Africa	19	0.5%	5	0.1%	0.021	0.312	0.116	0.837	0.435	0.558	0.129	2.414
	Other (far Asia, India, Spain, France, and countries from Middle East)	28	0.7%	22	0.6%	0.804	0.931	0.531	1.634	0.766	1.169	0.418	3.273
How would you rate your awareness of Monkeypox at the meantime?	Low	1,290	33.5%	729	18.9%					1			
	Moderate	722	18.7%	934	24.2%	0.000	2.289	2.004	2.615	0.000	1.828	1.390	2.405
	High	102	2.6%	79	2.0%	0.045	1.371	1.008	1.864	0.924	0.969	0.508	1.849
How worried are you that monkeypox can cause worldwide pandemic similar to COVID-19?	None/less worried	1,198	31.1%	793	20.6%					1			
	Moderate worry	749	19.4%	777	20.2%	0.000	1.567	1.370	1.793	0.003	1.577	1.171	2.124
	Worried a lot	167	4.3%	172	4.5%	0.000	1.556	1.235	1.960	0.047	1.627	1.006	2.632

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TABLE 8 (Continued)

Variable	Subgroups	Monkeypox knowledge				*p*-value	Non-adjusted OR	Lower	Upper	*p*-value	Multivariate adjusted OR	Lower	Upper
		Don't have		Have									
		Frequency	Percentage	Frequency	Percentage								
Do you think monkeypox causes more severe disease compared to smallpox?	Disagree	399	10.3%	267	6.9%					1			
	Unsure	1,059	27.5%	620	16.1%	0.154	0.875	0.728	1.052	0.626	0.903	0.600	1.360
	Agree	656	17.0%	855	22.2%	0.000	1.948	1.618	2.344	0.299	1.254	0.818	1.921
Which is more worrisome to you, COVID-19 or monkeypox disease?	Unsure/equally worried	858	22.3%	634	16.4%					1			
	I am more worried about COVID-19	898	23.3%	767	19.9%	0.044	1.156	1.004	1.331	0.138	1.256	0.929	1.699
	I am more worried about monkeypox	358	9.3%	341	8.8%	0.006	1.289	1.076	1.544	0.509	1.132	0.783	1.636
Healthcare workers should apply more infection control measures than the current ones, with the new monkeypox outbreaks	Agree	1,605	41.6%	1,464	38.0%					1			
	Neither agree nor disagree	332	8.6%	204	5.3%	0.000	0.674	0.558	0.813	0.002	0.464	0.289	0.746
	Disagree	177	4.6%	74	1.9%	0.000	0.458	0.346	0.607	0.001	0.389	0.220	0.689
Please rate your worry level about traveling abroad with the new monkeypox outbreaks in some countries	Not worried at all	894	23.2%	481	12.5%					1			
	Somewhat worried	1,053	27.3%	1,071	27.8%	0.000	1.890	1.644	2.174	0.720	0.945	0.695	1.286

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TABLE 8 (Continued)

Variable	Subgroups	Monkeypox knowledge				*p*-value	Non-adjusted OR	Lower	Upper	*p*-value	Multivariate adjusted OR	Lower	Upper
		Don't have		Have									
		Frequency	Percentage	Frequency	Percentage								
	Extremely worried	167	4.3%	190	4.9%	0.000	2.115	1.671	2.676	1	0.764	0.469	1.245
Do you want to receive Monkeypox vaccine	No	1,078	28.0%	676	17.5%								
	Yes	1,036	26.9%	1,066	27.6%	0.000	1.641	1.443	1.866	0.370	1.133	0.862	1.488
GAD-7	Don't have	1,605	41.6%	1,275	33.1%					1			
	Have anxiety	509	13.2%	467	12.1%	0.052	1.155	0.999	1.336	0.172	0.813	0.605	1.094

The logistic regression model was statistically significant, X2(45) = 147.186, $p = 0.000$. Hosmer and Lemeshow test 5.554 ($p = 0.697$). The model explained 16.8% (Nagelkerke R^2) of factors associated with monkeypox knowledge score.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The Syrian Ethical Society for Scientific Research at Aleppo University, Syria, provided the ethical approval for conducting the study (IRB: SA-1087). In addition we ordered at least one printed ethical approval from the lead collaborator from each inquired country in our study that was given by the clinical, and educational institutions (Hospitals, Medical Colleges). To confirm that participating in our study was voluntary, the first question in the online survey was about the respondent's acceptance to complete the survey.

Author contributions

SS: study conception and design. HA, HB, NJ, MR, MN, WH, BS, AA, and SF: writing and reviewing the manuscript. MN and SS: formal analysis. IA, SA, and RY: draft manuscript. AR and ME: interpretation of results and revising. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

BS was employed by Hamad Medical Corporation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1153136/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Brief Research Report
PUBLISHED 26 October 2023
DOI 10.3389/fphar.2023.1159377

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Pharmacology of Infectious Diseases, a section of the journal Frontiers in Pharmacology

RECEIVED 05 February 2023
ACCEPTED 30 March 2023
PUBLISHED 26 October 2023

CITATION
Satapathy P, Goel K, Sharma V, Sarkar S, Kang M, Dhingra S, Bora I, Kaur K, Arora N, Aggarwal A and Ratho RK (2023), Outbreak investigation of acute febrile illness from the Himalayan foothills: Solving the puzzle of fever.
Front. Pharmacol. 14:1159377.
doi: 10.3389/fphar.2023.1159377

Outbreak investigation of acute febrile illness from the Himalayan foothills: Solving the puzzle of fever

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In September 2022, Panchkula Civil Hospital reported an outbreak of acute febrile illness (AFI) in Pinjore, located in the Himalayan foothills, Haryana, North India. There was an upsurge of fever cases. Blood samples were taken from suspected patients (*n* = 58) with AFI and subjected to serology of dengue, chikungunya, Japanese encephalitis, *leptospira* and scrub typhus. The samples were also screened for West Nile & Zika virus RNA using real-time PCR. Viral strains were characterized by sequencing. Of the 58 cases of AFI, Dengue could be identified in 45 (77.58%) followed by JE and Chikungunya in 2 cases each (3.44%), respectively. Among Dengue positive cases, 44 had monoinfection (97.77%) and 1 patient had dengue and JE. None were positive for Zika, West Nile, Scrub typhus, and *Leptospira* with the testing protocol. Four patients developed dengue with warning signs, such as abdominal pain in one patient and recurrent vomiting in the remaining three. The dengue serotype could be determined in 17 samples and revealed serotype 2. Molecular evolution analysis based on the complete envelope gene revealed that all DENV-2 strains (*n* = 13) circulated in the outbreak area belonged to the DENV-2 cosmopoliton genotype. In the early stages of infection, relying only on clinical manifestations is ineffective, so both molecular and serological assays along with clinical diagnosis are noteworthy for determining the aetiology of AFI.

KEYWORDS

acute febrile illness, dengue fever, DENV-2, cosmopoliton genotype, outbreak

Introduction

Infection remains the common cause of febrile illness in developing countries (Mulders-Manders et al., 2015), where most primary investigations fail to ascertain a specific etiology. Thus, they are categorized into the group of AFI with a fever lasting less than 2 weeks duration. Due to limited resources in diagnosis, these illnesses remained as poorly characterized (Chao, 2012). Over the years, dengue, chikungunya, Japanese encephalitis (JE), *leptospira*, scrub typhus, and malaria are being considered as the known aetiologies of AFI cases in South Asia countries. However, in recent decades, dengue has rapidly emerged as a foremost cause of AFI

in Southeast Asia (Wangdi et al., 2019). Most AFI cases can be diagnosed if the investigation is based on the clinical findings supported with laboratory investigations. Because of improper diagnosis, many a times, clinicians are forced to administer unnecessary antibiotics. Thereby leading to misuse of antimicrobials and poor patient outcomes (Long, 2016). It is crucial to determine the prevalence and epidemiology of the causative pathogens to develop protocols for therapeutic interventions of AFI.

In September 2022, Panchkula Civil Hospital reported an outbreak of fever in Pinjore, located in the Himalayan foothills of Haryana. There was an upsurge of fever cases in September 2022, (n = 842). Most of the patients had the acute febrile disease, body aches, and arthralgia; on investigation, the patients revealed thrombocytopenia. The present study was carried out to investigate the outbreak by characterization of the virus strains isolated from the patients.

Methods

On the request of Haryana health authorities, the Department of Community Medicine and School of Public Health and the Department of Virology, Post Graduate Institute of Medical Research (PGIMER), Chandigarh, investigated the outbreak from 20 September to 30 September 2022. Blood samples were taken from suspected patients (n = 58) with AFI, serology, molecular diagnosis, and viral strain sequencing were performed at the Regional Virus Research and Diagnostic Laboratory, Department of Virology, PGIMER. Demographic and clinical details were obtained from the patients. Ethical clearance was obtained from the institutional ethics committee.

Serological diagnosis

Blood samples were tested for Dengue NS1 antigen Elisa (Abbott, Panbio), DENV IgM capture ELISA (NIV, Pune), Chikungunya IgM ELISA (NIV, Pune), JE IgM ELISA (NIV/Pune), Scrub Typhus IgM ELISA (Detect™, Inbios) and *Leptospira* IgM ELISA (CTK). Bio Teck according to the manufacturer's instructions. The presence of DENV IgG was detected by dengue IgG Capture ELISA (Panbio™, Abbott).

Molecular detection

Viral RNA was extracted from serum samples using a commercial Qiagen Viral Mini Kit (Qiagen, Hiedelberg/Germany). The preparation of the first-strand cDNA was done using the high-efficiency cDNA kit (Invitrogen/United States). One step Real-Time PCR (RT-PCR) was done for the detection of dengue, chikungunya and zika viruses using CDC (Centre for Disease Control and Prevention) Trioplex RT-PCR assays (Centers for Disease Control and Prevention, 2017). Further real-time dengue PCR positive samples were subjected to serotype determination using conventional hemi-nested multiplex dengue serotype RT-PCR following the method of Lanciotti et al. (1992). The samples were also screened for West Nile virus RNA using in-house standardized real-time PCR.

Conventional PCR method was utilized for the amplification of the complete Envelope gene of DENV strains using two overlapping sets of primer pairs (Warrilow et al., 2012). The amplified E gene of representative DENV strains was purified and sanger di-deoxy sequencing was performed utilizing both forward and reverse primers. Sequences generated with forward and reverse primers were made consensus using DNASTAR Lasergene software. Phylogenetic trees based on the complete E gene coding region were constructed for the present isolates together with other reported isolates of DENV-1, DENV-2, DENV-3, and DENV-4 originating from various geographic regions. Phylogenetic trees were constructed with the help of MEGA 7.0.26 (Kumar et al., 2016). The confidence values for the branches of the phylogenetic tree were provided by bootstrap analysis of 1,000 replicates. The trees generated with several molecular algorithms were evaluated for the log-likelihood value, and the highest log-likelihood was chosen for the display.

Results

Among the 58 recruited AFI patients selected from the outbreak location, 44.82% (26/58) patients suffered from fever. Among other clinical presentations along with fever, 13.79% (8/58), 18.96% (11/58), 10.34% (6/58) patients had myalgia, arthralgia, and skin rashes, respectively. Thrombocytopenia was detected in 12.06% (7/58) of the patients. The maximum number of cases (63.8%) was in the 19–40 years age group and 67.25% were female (Table-1). Of the 58 cases of AFI, Dengue could be identified in 45 (77.58%), followed by JE and chikungunya in 2 cases each (3.44%), respectively. Among dengue-positive cases 44 had mono-infection (97.77%) and one patient had dengue and JE. Of the 45 dengue-positive cases, 37 blood samples could be obtained within 5 days after the onset of the fever, where Dengue NS1 Ag was positive in 29 samples (78.38%), Dengue viral RNA positive in 25 samples (67.57%) and Dengue IgM antibodies in 17 samples (45.94%). However, 7 out of 8 samples (87.5%) collected after 5 days of fever had Dengue IgM antibody and NS1 Ag in 2 cases (Table-2). None were positive for Zika, West Nile, Scrub typhus, and *Leptospira* with the testing protocol. Dengue IgG was detected in 18 of the 44 (40.90%) Dengue positive patients.

Of 26 RTPCR Dengue positives (Table 2), serotype could be determined in 17 samples, revealing serotype 2. Representative samples of DENV-2 (n = 13) were subjected to sequencing of the DENV-2 E gene. The aligned DNA sequences were searched with BLAST and submitted to the global gene bank with accession numbers OP808344 to OP808356. The sequences of the study strains along with the reference sequences of the DENV-2 strains belonging to the American genotype, the American/Asian genotype, Asian genotype I, Asian genotype II, and the cosmopoliton genotypes were aligned with Clustal X. To deduce the phylogenetic analysis of the study strains evolutionary analyses were performed in MEGA7. The evolutionary history was inferred using the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest logarithmic likelihood (−2,238.53) is shown in Figure 1. The percentage of trees in which the associated taxa cluster together is shown next to the

TABLE 1 Etiological agents identified in association with socio demographic and clinical features of study participants (*N* = 58).

	Total no. of patient	DENV POS	CHKV POS	JEV POS
	N = 58	45/58 (77.58%)	2/58 (3.44%)	2/58 (3.44%)
Gender				
Male	19/58 (32.76%)	15/45 (33.33%)	0	0
Female	39/58 (67.24%)	30/45 (66.77)	2/2	2/2
Age group				
1–18 years	9/58 (15.52%)	6/45 (13.33%)	0	0
19–40 years	37/58 (63.79%)	28/45 (62.22%)	½	2/2
40–60 years	12/58 (20.68%)	11/45 (24.44%)	½	0
Duration of Symptoms				
≤ 5 days	45/58 (77.59%)	37/45 (82.22%)	½	½
> 5 days	13/58 (22.41%)	8/45 (17.78%)	½	½
Clinical features				
Fever	26/58(44.82%)	20/45(44.44%)		½
Fever with Rashes	6/58 (10.34%)	3/45 (6.66%)	½	
Fever with Myalgia	8/58(13.79%)	7/45(15.55%)		
Fever with Myalgia & Arthralgia	11/58 (18.96%)	8/45 (77.77%)	½	½
Fever with Thrombocytopenia	7/58 (12.06%)	7/45 (15.55%)	0	0

TABLE 2 DENV positivity spectrum according to the duration of fever.

	Total (45/58)	Patients with fever duration ≤ 5 days (37)	Patients with fever duration > 5 days (8)
DENV NS1 Ag ELISA positive	31/58 (53.4%)	29/37 (78.37%)	2/8 (25%)
DENV IgM ELISA positive	24/58 (41.3%)	17/37 (45.94%)	7/8 (87.5%)
DENV RT-PCR positive	26/58 (44.82%)	25/37 (67.56%)	1/8 (12.5%)

branches. The initial tree(s) for the heuristic search were obtained automatically by applying the neighbour-join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model and then selecting the topology with a superior log-likelihood value. Evolutionary analysis revealed that all DENV-2 strains (n = 13) circulating in the outbreak area belong to the DENV-2 cosmopoliton genotype.

Discussion

The spread of arboviral diseases in tropical countries has become a major public health problem. The vector density increases during the post-monsoon period, thereby augmenting disease transmission. Climatic factors (temperature, rainfall, precipitation, humidity), human behavior trends, and immunity status are the main determining factors for the seasonal incidence of arboviral diseases such as dengue, chikungunya, and zika (Oki and Yamamoto, 2012; Sippy et al., 2019; Mohapatra et al., 2022). With monsoon onset, AFI cases increase and persist until winter. Scrub typhus, dengue, malaria, and chikungunya are the important etiology of AFI in India and other tropical countries (Abhilash et al., 2016; Mørch et al., 2017).

Our study confirmed that dengue is the main etiological agent (77.58%) of the fever outbreak in Panchkula, India, in the Himalayan foothills on 20 September 2022, during the post-monsoon period with an average rainfall of 426 mm. Similar observations were also found in AFI outbreak investigation studies from Arunachal Pradesh and Thailand (Khan et al., 2014; Luvira et al., 2019). Studies have reported that scrub typhus is the most prevalent among cases of AFI during the post-monsoon months (Bithu et al., 2014; Raina et al., 2018). Interestingly, in spite of the hilly area with unplanned urbanization, scrub typhus infection could not be documented in this outbreak.

In this study, 53.4% of cases were detected using the NS1 dengue antigen test, 41.3% using the IgM antibody test, while RT-PCR was positive in 44.82% (Table 2). IgG antibodies were detected in 18/44 DENV-positive cases. Samples during acute phase were positive for the NS1 antigen; however, the early appearance of IgM antibody has been reported in secondary dengue infections compared to primary dengue infection (Vazquez et al., 2010). Out of 17 cases

FIGURE 1
Maximum likelihood method based on the JTT matrix-based model. There were 495 amino acid positions of the 61 DENV-2 sequences in the final dataset. Evolutionary analyzes were conducted in MEGA7.

positive for dengue IgM antibody in the acute phase of fever, 7 patients had pre-existing IgG antibodies.

Secondary infection with a different serotype or multiple infections with different serotypes may lead to severity. Of the 7 cases of fever with thrombocytopenia, all are positive for DENV. Four patients developed dengue with warning signs, such as abdominal pain in one patient and recurrent vomiting in the remaining three.

The study spanning 1994–2006 in Thailand postulated the association of DENV-2 infection with severe dengue manifestations (Fried et al., 2010). The same was corroborated by Huy et al. (2013), through a systematic review suggesting DENV-2 as a risk factor for dengue shock syndrome. Kumaria, (2010) from India documented elevated levels of liver enzymes in DENV-2 infected patients. Therefore, the sudden increase in fever cases, and high morbidity, could possibly be explained by the presence of DENV-2 strains in the locality, as revealed in the current study.

The non-sylvatic DENV-2 genotypes are classified into five genotypes (Asian I, Asian II, American, Asian-American and Cosmopoliton). The American genotype is present in Central and the South America, and Asian I and Asian II genotypes circulate on the Asian continent. Asian-American genotypes are identified in South East Asian countries as well as South American countries. The Cosmopoliton genotype is the most widespread DENV-2 found in Africa, the Middle East, and Asia-Pacific countries, including India (Weaver and Vasilakis, 2009; Yenamandra et al., 2021). The DENV-2 American genotype strains isolated from Kerala, Tamil Nadu, and New Delhi were considered the first strains circulated in 1950–1971. The Asian-American genotype was identified in Maharashtra in 2017. The cosmopolitan DENV-2 genotype was the predominant strain circulated in India from 1980 to date (Kumar et al., 2010; Kasirajan et al., 2019). Zhang et al. (2021) reported that DENV-2 cosmopoliton strains enhance pathogenicity and delay in viral clearance compared to other DENV subgenotypes. Phylogenetic tree analysis of complete envelope gene amino acids through the Maximum Likelihood method based on the JTT matrix-based model revealed that the strains of the current study belong to DENV-2 cosmopoliton genotypes, which could be the factor for a large number of fever cases and hospitalizations.

Conclusion

Our findings demonstrate that DENV was the primary etiological agent in the fever outbreak in Panchkula, Haryana,

North India. The timely diagnosis of AFI is crucial in preventing mortality and morbidity. Overlapping clinical signs, symptoms, and cross-reacting results of serological tests are the main concerns in diagnosing AFI. Serological detection accompanied by a molecular assay is helpful in the identification of sporadic outbreaks of AFI. Active syndromic surveillance and point-of-care testing should be implemented to avert AFI mismanagement. Early identification, accurate diagnosis, and early administration of proper treatment, along with enhanced global health security through appropriate control measures, would be the mainstay for controlling high-risk pathogens.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study involving human participants were reviewed and approved by Institutional ethics committee PGIMER, Chandigarh (No. PGI/IEC/2023/EIC00627). As the study was an outbreak investigation and the department of virology, PGIMER, Chandigarh has no direct contact with the patients the institutional ethics committee reviewed the study protocol and approved waiving off the written patient consents for this outbreak investigation study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgments

The authors acknowledge the Regional Virus Research and Diagnostic Laboratory (RVRDL), Department of Health Research (DHR), Ministry of Health and Family Welfare (MoHFW), Government of India (GoI) for providing infrastructure and consumables, NVBDCP for ELISA kit to carry out the study. The authors thank the patients and hospital staff of the area for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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