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PROBLEMS OF INFECTIOUS AND PARASITIC DISEASES VOLUME 53, NUMBER 1/2025

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CONTENTS

A REVIEW OF GENOME ORGANIZATION, EVOLUTION, TRANSMISSION, CIRCULATION,	
AND CLINICAL MANIFESTATION OF MONKEYPOX VIRUS	5
S. Krumova, D. Ivanov, I. Christova	
ATTITUDE AND KNOWLEDGE OF HIV INFECTION AMONG HEALTH PERSONNEL IN BULGARIA	.15
R. Emilova, Y. Todorova, M. Nikolova	
Α CASE OF MYCOTIC SEPSIS DUE TO TRICHOSPORON COREMILEORME	
	22
	25
S. voycneva, E. Bozova, I. Filipova, L. Boyanova	
BEYOND MOSQUITO BITES: ANALYZING MALARIA RISK FACTORS IN SOUTHERN NIGERIA	.28
S. Baadel, H. Sabi, K. Attai, N. Ekpenyong, D. Asuquo, M. Baadel,	
J. Obi, Sh. Benson, Ch. Akwaowo, F. Uzoka	
IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX SPECIES IN BULGARIA	34
S. Yordanova, Y. Atanasova, A. Baykova, E. Bachiyska, M. Evtimov	
INSTITUTIONAL APPROACHES TO HEALTH CRISES - A REVIEW OF GLOBAL PRACTICES	.39
Zh. Getsova	

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A REVIEW OF GENOME ORGANIZATION, EVOLUTION, TRANSMISSION, CIRCULATION, AND CLINICAL MANIFESTATION OF MONKEYPOX VIRUS

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ABSTRACT

Mpox is an illness caused by the monkeypox virus (MPXV, genus Orthopoxvirus), which infects animals and humans. Genetically, there are two MPXV clades: The Central (1) and West (2), with two reported subclades for each. MPXV can be transmitted between animals, from animals to humans, and humans to humans. Since May 2022, a multi-country outbreak of mpox has been registered in non-endemic regions. After a decrease in the number of confirmed cases in 2023, a re-emerging spread of mpox clade I in Africa and various EU/EEA countries has been registered since mid-2024, and into 2025. According to available genomic data, nonsense or frameshift mutations of MPXV resulting in loss of protein-coding genes and noncoding genes or regulatory regions observed in endemic regions of Central Africa have been associated with human-to-human transmission of the virus. Urbanization caused by population growth in West Africa may increase the risk of human MPXV infection. The infection spread, especially among

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Assoc. Prof. Stefka Krumova, PhD National Reference Laboratory "Measles, Mumps, Rubella National Centre of Infectious and Parasitic Diseases 44A Stoletov Blvd., 1233 Sofia, Bulgaria phone: +359 878 854 203 email: stefka.krumova@gmail.com the countries of the European continent, has led to increased research on mpox prevention and therapy, with data being continuously updated. Monitoring of potential animal reservoirs and exploring new transmission routes are important. Over time, the MPXV has evolved by accumulating genome mutations, contributing to its adaptability and easier human-to-human transmission.

1. Classification

Mpox is an illness caused by the monkeypox virus (MPXV), which infects animals and humans. MPXV is a member of the genus Orthopoxvirus, subfamily Chordopoxvirinae, family Poxviridae [1]. The genus Orthopoxvirus has 13 identified representatives. Among them, important human pathogens include variola, vaccinia, cowpox and rabbitpox viruses. MPXV was first isolated in monkeys in 1958 at the Primate Research Institute in Denmark [2]. In 1970, an outbreak study in the Democratic Republic of Congo (DRC) proved its importance in human pathology [3]. Since then, MPXV has been considered endemic to DRC and was detected in other 11 African countries [1,4]. Based on laboratory data, various small mammals are a reservoir of MPXV, animals infect the human population, whereby the chain of human to human transmission is relatively short in case of close contact. [5, 6] Genetically, there are two MPXV clades: The Central African (Congo Basin - DRC, Republic of Congo, Gabon, Cameroon and Central African Republic) and the West African (Nigeria, Benin, Côte d'Ivoire, Liberia, and Sierra Leone) and [7-9].

Central Africa's viruses are more virulent than those from West Africa [10, 11]. Studies show that infections caused by the Central African clade tend to be more severe, with a higher fatality rate (10%), in contrast to the West African strain, which has a fatality rate of 4% [12, 13]. These variations in virulence are linked to differences in genome structure, including deletions in gene regions and fragmentation of genes within open reading frames [14]. The genetic similarity between the two clades is at most ~95%, while the homology of viruses within the clade is on the order of 99% [9]. In addition to geographic distribution, they differ in clinical pattern, severity, and transmission [15-17]. The study of Happi et al.

Probl. Inf. Parasit. Dis.

[18], discussed a change in the nomenclature of the MPXV in three clades and separation of isolates from the Congo Basin in clade 1 and those from West Africa in clades 2 and 3. Clades 2 and 3 thus defined are less virulent and less likely to infect humans, unlike clade 1. This explains the zero reported deaths in the 2003 outbreak in the USA [19], where 90% of reported cases were from the Congo Basin [20].

2. Structure

Morphologically, MPXV has an oval or rectangular brick shape visible by electron microscopy, a characteristic of poxviruses. It measures 200 × 250 nm and has membranous surface tubules or filaments, a double-stranded core disc with linear double-stranded DNA (dsDNA), and a lipoprotein envelope [14]. The immature virion has spherical shapes, whereas the mature virion can be seen on negative staining in two forms: smaller ink (M) or larger capsular (C).

3. Genomic organization and evolution

MPXV's dsDNA genome is approximately 197 kb long and encodes around 180 proteins, which posed a significant challenge during de novo assembly of the entire genome [21, 22].

The MPXV genome is linear and features covalently closed ends, meaning it lacks free 3' and 5' ends. Each end of the genome contains 10 kb of inverted terminal repeats (ITRs) [22]. The genes are compactly arranged, with few intergenic regions longer than 100 bp. The central region of the genome contains genes crucial for transcription, replication, and the assembly of the virus, while the terminal, which varies between different poxviruses, encodes proteins that influence clinical symptoms and the virus's host range. The first fully sequenced MPXV genome from the 2022 outbreak (isolated as MPXV_U.S._2022_MA001) was deposited in the GenBank with accession ID ON563414 [118] on May 30, 2022 [23]. According to Oxford Nanopore sequencing, the genome of MPXV spans 197,205 base pairs of linear ds DNA.

The reported rate of poxviruses mutations per replication cycle is 10^{-5} to 10^{-6} . Genome analysis of the 2022 U.S. MPXV outbreak indicates a notable accumulation of mutations as opposed to MPXV isolates from previous years. These mutations

primarily occur at the 5' GA-to-AA sites within Apolipoprotein B mRNA Editing Catalytic Polypeptidelike 3 (APOBEC3), a protein with cytosine deaminase activity, and these G-to-A mutations are typically for MPXV 2 clade [23, 24]. The APOBEC3 proteins, known for their activity on single-stranded DNA, are studied in RNA viruses [24]. Their role in DNA viruses, however, has also been demonstrated [25-27]. APOBEC3 proteins play a crucial part in the innate immune defense of vertebrates by inhibiting virus replication through their cytosine uracil deaminase activity [24, 28].

In a study by Jones et al. [29], 47 MPXV genomes from Berlin, Germany, collected between May 20 and July 4, 2022, were analyzed. several nonsynonymous amino acid changes compared to the previous outbreak were revealed. Notably, the original 5' gene was duplicated in sequences isolated from two lesions one of the same patient. Additionally, four genes near the 3' end of the genome were either disrupted or completely deleted due to an 856-nucleotide translocation between the genome's endpoints. Such genomic rearrangements in orthopoxviruses could confer host-specific advantages, potentially enabling better adaptation and facilitating virus transmission between humans during the current mpox epidemic [29].

The greatest genome variability in orthopoxviruses is observed in the two inverted terminal repeat (ITR) regions at the genomeends, where immune escape factors predominantly reside and play a role in virulence [30, 31]. Mutations in these regions are believed to be the key mechanism for the rapid adaptability of orthopoxviruses following host switching [32, 33]. MPXV genomes from both West and Central Africa show mutation in the ITR regions [29, 34]. The evolution of MPXV genome stimulates its virulence, and ability to evade immune responses.

4. Viral replicative cycle

Poxviruses are obligate cellular parasites and their replication is realized only in the cytoplasm. MPXV has aerosol or intradermal transmission, can penetrate damaged skin or mucous membranes, where initial replication occurs and spreads to local lymph nodes [35]. In aerosol infection, the virus enters the respiratory tract. It has been established that MPXV can enter the body through sexual contact [36, 37]. In addition, direct contact with materials contaminated with the virus, such as clothing, utensils, and furniture, is a prerequisite [36, 37].

Viral penetration occurs through micropinocytosis, viral endocytosis, and fusion with the cell membrane, enabling entry via various routes, including airborne and contact-based pathways. Upon inoculation, MPXV replication triggers generalised infection with organ involvement via the bloodstream. The virus replication occurs entirely in the cytoplasm of the host cell, under the control of two antigenically distinct virion forms: mature (MV) and enveloped (EV). Following the transcription and translation of MPXV mRNA, intracellular mature virions (IMVs) containing viral DNA are produced. IMVs, encapsulated in a membrane derived from the Golgi apparatus, form intracellular enveloped virions (IEVs). These IEVs then fuse with the host cell membrane, producing cellassociated virions (CEVs), which are subsequently released into extracellular spaces as extracellular enveloped virions (EEVs) [38]. Similar to other viruses, the members of Orthopoxvirus genus have evolved various mechanisms to avoid host defences, which facilitates their entry. and Orthopoxviruses may impair the pattern recognition receptors (PRRs) expressed by innate immune cells. PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-1-like receptors (RLRs), C-type lectin receptors (CLRs) etc. responsible for recognizing molecules of infectious agents and released from damaged cells [39]. Once PRRs bind to microbial ligand [40]. , activation of inflammation-related transcription factors such as nuclear factor kappa B (NF-κB), interferon regulatory factors (IRFs), and activator protein-1 (AP-1) follows

Signal transduction of TLRs involves several types of intracellular adaptor proteins, such as MyD88, MAL, TRAM, TRIF, and SARM, which are crucial for triggering I immune responses [39, 41]. Any disturbance in those adaptor proteins can lead to an inadequate and incapacitated immunological response against viral infections. At this stage, orthopoxviruses contain genes encoding proteins that can interact with and disrupt the the physiological functions of adaptor proteins , followed by inhibition of transcription factors associated with inflammation, i.e. NF- κ B [40].

Furthermore, MPXV and other members of Orthopoxviridae can affect cellular apoptosis, particularly the function of enzymes caspase-1, caspase-8, and caspase-9 [40, 42]. MPXV genome encodes a protein that mimics the activity of B-cell lymphoma-2 (Bcl-2) proteins, which are known to play an important role in the regulation of apoptosis [40, 43] and NF-κB activation [40, 44, 45]. Orthopoxviruses can also affect the immune response against viral agents by inhibiting the production of interferon regulatory factors (IFRs), thereby blocking the synthesis of interferon [40].

Orthopoxviruses and MPXV possess many genes encoding proteins that can disrupt different stages of host's inflammatory cascadecomprising cytokine and chemokine production, complement system activity, ubiquitin-proteasome pathway activity, and several other targets [40, 46].

Once able to evade the host immune system, MPXV can attack many systems of the host. Mpox and smallpox can have similar clinical manifestations. A specific symptom for mpox which is not detected in smallpox cases is lymphadenopathy (most often in submandibular, cervical, or inguinal region) [36, 47]. An important role in the development of lymphadenopathy plays the vaccinia complement control protein (VCP) [40], which consists of four short consensus repeats (SCRs). VCP can bind to some complement components (C3b, C4b and other), thus disrupting th esubsequent complement cascades [40]. Ultimately, VCP suppresses the inflammatory response [48, 49].

unchanged similar structure of the VCP protein has been found in other orthopoxviruses (variola, vaccinia, cowpox viruses). In contrast, , the VCP structure of MPXV is either shortened (clade 1) or deleted (clade 2) [40].

5. Epidemiology and transmission

Mpox primarily spreads in Africa regions, with MPXV transmission occurring through several routes: between small mammals, from animals to humans and within the human population [50]. The most common mode of animal-to-human transmission is through direct contact with an infected animal or its fluids [51]. Initially, human infections were linked to animal exposure, but in regions with high rodent infestations and where hunting or preparing meat from wild animals is common, deciphering the source of infection is difficult [52]. Human-to-human transmission was observed in Nigeria and other parts of West Africa [53]. Close living conditions or shared use of utensils are potential sources of contamination, which could increase transmission risk within households. The first reported case of human mpox in the U.S. occurred in 2003, when the outbreak was linked to infected prairie dogs. Despite this, there was still a risk of human-to-human transmission [54]. In September 2018, MPXV was transmitted to a healthcare worker in the UK through contaminated bed linens [55]. Viral penetration can also occur through close contact or by respiratory secretions from an infected individual [52]. Vivancos et al. [56] reported an ongoing MPXV outbreak in the UK since May 2022, noting that all previously documented cases in the country were either imported or involved healthcare workers in contact with international cases [55]. In Israel and Singapore, cases of mpox were reported, with epidemiological data indicating the importation of the infection associated with travel to Nigeria [57]. Regarding the current mpox epidemic in Europe, there are no initial epidemiological links to sites in West and Central Africa, raising the possibility of long-term undetected transmission in the region [58]. The incidence of mpox was reported to range from 0.64 per 100,000 in 2001 to 50 per 10,000 in 2016 [59]. The death ratio ranged from 1 to 10%, with clade 1 being more virulent and deadly compared to clade 2. A major proportion of deaths due to MPXV are concentrated in Africa. After the year 2000 a significant demographic manifestation of mpox and increase in deaths primarily among children under 10 years and young adults was documented [59].

The reported epidemic from May 2022 to December 31, 2024, covered 122 countries, with only 7 being part of the previously endemic countries, and the confirmed mpox cases were 102,000 [60]. Globally, the cases of mpox peaked in August 2022. Althoughsignificantly fewer cases are reported presently, the transmission of clade 2b MPXV continues globally. Confirmed cases in the European Union/European Economic Area (EU/EEA) countries, including Bulgaria, by 12 February 2025 are 23 882 (reported from 29 EU/EEA countries) [61]. The current epidemic in non-endemic for the virus countries, is spreading mainly among men who have sex with men (MSM) and multiple partners. However, there is a potential for spread to other population groups. Clinical manifestations of mpox reported in the EU/EEA are mild to moderate. The severity may be higher in young children, pregnant women, and immunocompromised individuals. Case isolation, contact tracing, and rapid laboratory diagnosis form the core of the current mpox epidemic control strategy in most EU/EEA countries. Collaboration between public health and clinicians, especially sexual health professionals, is critical to identify as many close contacts as possible are. Early diagnosis, isolation, and effective contact tracing are key to epidemic control [3]. Based on the available data, ECDC assesses the likelihood of further spread of mpox in EU/EEA countries and globally in the coming months as medium-high, resulting in a moderate risk for the general population. The inability to rapidly contain the epidemic situation determines the WHO's change of strategy and designation of the disease. Mpox infection was defined as one of 'international concern and public health urgency' in 2022, after which it was specified again as 'moderate' in 2023 and since August 2024 mpox outbreak and rapid spread of a new virus strain in DRC, clade 1b, is defined as a public health emergency of international concern.

The current risk of further spread of MPXV among persons with multiple sexual partners in linked groups (including some MSM groups) is defined as high.

Transmission to healthcare workers from mpox patients (e.g., face-to-face contact for prolonged periods, contact with open lesions without gloves, intubation, or other invasive medical procedures), including nosocomial spread, is possible given the risk of transmission of other orthopoxviruses. In parallel, thousands of clinically compatible mpox cases are being reported in Africa, where the access to laboratory diagnostics remains limited. Measures have been developed for rapid detection and prevention of the virus. Currently, the primary alert is from the DRC, where the epidemic spread of mpox genetic lineage 1 has been observed. In 2024, DRC reported over 40,000 cases, over 9,000 confirmed cases, and 40 deaths. Burundi reported over 3 000 confirmed cases, and Uganda reported over 1 500 confirmed cases. Mpox clade 1 cases were reported also in Rwanda, Kenya, Zambia, and Zimbabwe. Outside of the African continent, only travel-associated cases due to MPXV clade 1 and/ or sporadic cases with epidemiological links to travel-associated cases have been reported. No wider community transmission and no deaths have been reported due to MPXV clade 1 in any country outside of Africa [62].

The CDC has investigated recent MPXV outbreaks to assess possible transmission routes, such as hugging, kissing, and sexual contact (oral, anal, and vaginal). These modes of transmission may be connected to genetic mutations that enhance virus's ability to spread between individuals [63-65].

The 2022 epidemic indicates a divergence from the original two MPXV clades, especially regarding human-to-human transmission. This divergence is marked by the branching of clade 2, which is now referred to as clade 3 or "human MPXV" (hMPXV). The most notable difference between clades 1, 2, and 3 appears to be in the terminal inverted terminal repeats (ITR), which harbor genes responsible for encoding host response modifier (HRM) proteins. One of these is the mpox analogue of the poxviral inhibitor of complement enzymes (PICEs) or MOPICE protein, which was once thought to be a differential virulence factor between clade 1 and 2, with the absence of MOPICE in clade 2 contributing to its lower pathogenicity [9]. However, a solid study in rhesus macaques showed the opposite. Eliminating MOPICE increased replication in vivo and attenuated the adaptive immune system response [66]. The virulence determinants distinguishing the two clades seem more likely to be influenced by numerous genetic factors within the vast MPXV genome, including the open reading frames of D10L, B10R, B14R, and B19R [4].

The mpox outbreak in 2022 suggests that the MPXV virulence correlates with the genetic variability in the virus genome. All reported MPXV isolates are phylogenetically related to clade 2, and the estimated mortality rate is below 1% [67].

6. Clinical manifestation of MPOX, pathogenesis and immune response

Mpox has an incubation period ranging from 5 to 21 days, with an average of 6 to 13 days. The illness typically begins with symptoms such as fever, muscle aches, fatigue, and headache. Within three days of symptoms onset, a maculopapular rash develops, spreading centrifugally from the primary infection site to other areas of the body. In cases of disseminated rash, the palms and soles are affected. The skin lesions progress over about 12 days, evolving from macules to papules, then to vesicles and pustules before fading. Secondary bacterial infections may occur as a result of itching and subsequent skin damage. Lesions may also appear on the oral or ocular mucosa (enanthema). Lymphadenopathy is commonly observed in many patients before and during the rash phase. It is important to note that clinical manifestations in travel-related cases are generally mild, often with only a few lesions. The appearance of the rash marks the beginning of the infectious period, which typically lasts between two and four weeks [68, 69].

In most infected individuals, the symptoms are mild to moderate. However, clinical complications can include encephalitis, secondary bacterial skin infections, conjunctivitis, keratitis, pneumonia and dehydration. There is limited information on how mpox affects immunocompromised patients. In a 2017 outbreak in Nigeria among individuals coinfected with HIV, the disease was more severe, with more extensive skin lesions and genital ulcers compared to those who were HIV-negative.

7. Laboratory diagnosis of mpox

Laboratory confirmation of MPXV infection is based on a molecular assay detecting a unique viral DNA sequence in appropriate clinical material. PCR analysis can be used alone or in conjunction with sequencing. Molecular protocols to detect OPXV, particularly MPXV, some of which include differentiation of the Congo Basin and West African clade have been quickly validated. Some protocols involve two steps where the first PCR reaction detects OPXV but does not identify the species. The assay can then be followed by a second step, which is PCR-based or uses sequencing to detect MPXV species precisely [70]. Electron microscopy can be used as a laboratory approach to evaluate a sample for the presence of a potential poxvirus, but the high technical skills and facilities required, and the advent of modern molecular assays , has precluded this method from routine poxvirus diagnostic.

Virus isolation in Vero cell cultures is a relatively simple approach but is not recommended as a routine diagnostic procedure and should only be performed in laboratories with specially trained laboratory workers and BSL Class III biosecurity facilities.

A wide range of infectious agents cause skin rashes with similar clinical course to MPXV, making a diagnosis based on clinical presentation alone challenging, especially in cases with atypical presentation. Therefore, it is important to consider other potential causes of discrete skin lesions or disseminated rash. Examples of etiologic agents causing similar-appearing skin lesions at various stages of development include herpes simplex virus 1 and 2 (HSV 1 and 2), varicella zoster virus (VZV), molluscum contagiosum virus, enteroviruses, measles, scabies, Treponema pallidum (syphilis), bacterial skin infection, drug allergies, and parapoxviruses (causing orf and related conditions) [71].

In case case of clinical suspicion for mpox in human, skin lesions serve as a source material for etiological identification of the causative agent. In those clinical specimens, the demonstration of the virus occurs with the greatest frequency. In addition, naso- and oropharyngeal swabs can be examined in the first days of clinical manifestation (days 1-4). It is advisable to process at least two lesions similar in type from two different anatomical regions [70]. The material from those is placed in one container. When the lesions are different in type, the different specimens are not mixed and are placed in separate containers. Many studies, have detected viral DNA with varying frequency in a range of other clinical materials (saliva, ejaculate, urine, and faeces).

8. Therapy and prevention of mpox infection

Studies have shown that the smallpox vaccine offers cross-protection against poxviruses in 85% of those immunized. Although post-vaccinal immunity decreases over time, it is believed that the smallpox vaccine still provides some level of protection for

adults over the age of 50. Following the official declaration of smallpox eradication by the WHO in 1980, vaccination ceased in many countries. In Bulgaria, the last individuals to receive the smallpox vaccine were those born between 1976 and 1978, and vaccination was not administered to all children. A scarification scar on the right arm, typically located in the deltoid muscle area, is considered a reliable indicator of smallpox vaccination [71].

Given the epidemic situation that has developed and the large number of mpox cases in nonendemic countries, the effects of various antiviral therapeutics and medications are beginning to be studied. A smallpox vaccine, ImvanexTM (Modified Vaccinia Ankara), is available in Europe and has been authorized for use by the European Medicines Agency under "emergency circumstances" [72]. A new mpox-specific treatment with Tecovirimat, an inhibitor of the VP37 assembly protein, was approved by the European Medical Association (EMA) in 2022, and a newer vaccine based on the Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) platform was introduced in 2019 for prevention of the viral disease [72].

9. State of research of the problem

Although smallpox was eradicated over 40 years ago, infection with another Orthopoxvirus, the monkeypox virus, can produce a clinical presentation similar to that of smallpox [73]. Early theories suggested that MPXV was an extremely rare virus, not easily transmitted, and confined to the rainforest regions of Africa [74]. The ongoing MPXV epidemic in 2022, which spans over 100 countries worldwide, belies this claim.

In the 1980s, some authors put forward the opinion that t variola virus could be easily derived from the monkeypox virus [75], but that was not confirmed [76] and was even refuted subsequently [77]. Although sufficient differences between the MPXV and variola genomes have been demonstrated to rule out simple interconversion, the possibility remains that MPXV is a more ancient ancestor of the variola virus. Ultimately, sequence analysis of the two agents indicated the presence in MPXV DNA of only a 1,065bp homolog that is part of the open reading frame of the variola genome but with many accumulated deletions. This is an irrefutable evidence that MPXV is not a variola virus precursor but a distinct orthopoxviral agent that strengthens confidence in smallpox eradication's long-term success [73].

The cessation of variola vaccination has likely contributed to the rise in mpox cases, as the unvaccinated population serves as a key factor in the ongoing incidence increase . [78].

The largest mpox outbreak in West Africa's history began in Nigeria in September 2017 [79]. For the first 11 and a half months, no cases were exported; however, between September 2 and 23, 2018, three unrelated individuals infected with MPXV left Nigeria and traveled to two different countries [80]. Seven months later, a person of Nigerian nationality fell ill with mpox in Singapore [81]. These cases marked the first documented instances of MPXV being carried from the African continent by a human host. Meanwhile, several mpox outbreaks were also reported in laboratory and zoo animals, with no identified source of infection [82]. In 2003, the United States experienced an mpox outbreak, traced back to the shipment of rodents from West Africa [83]. The index case of the current mpox outbreak in Europe was confirmed in a UK resident on May 6, 2022, and was linked to a travelling to Nigeria. Sequencing of the first isolate from a patient in Portugal, with clinical material collected on 4 May 2022, suggested that the MPXV isolates were homologous to those imported into the UK in 2018-2019 and ere genetically related to the Nigerian MPXV strain belonging to the West African MPXV clade [84]. Other available MPXV sequences from patient isolates from the USA and Belgium also showed a closer genetic relationship with West African MPXV isolates and those from the UK in 2018 and 2019 [85]. The earliest date of symptoms onset in patients has been reported as April 3, 2022. Most reported cases were aged between 31 and 40 years (42%), of whom were male (99.5%). Among cases with known HIV status 43% were HIV positive. The majority of patients presented with rash (95.2%) and systemic symptoms such as fever, fatigue, mylagias, vomiting, diarrhoea, chills, sore throat or headache (64%). Just over 10.2% were hospitalized. Healthcare workers (over 40) were reported sick, and investigations are ongoing to determine whether the infection was due

to occupational exposure. The highest proportion of viral DNA was detected in skin lesions (46.7%), followed by oropharyngeal swabs (19.1%) and rectal swabs (15.4%) [86]. The presence of viral DNA is not an evidence of the detection of viable virus and infectious potential of the clinical material concerned. However, it is subject to thorough analysis as it may be a reservoir for virus shedding and contamination of household products, especially when it comes to contaminated faecal water. The basis of the disease and transmission is puzzling due to the unusually high incidence of person-to-person transmission, with studies focusing on possible genetic modifications of the virus.

According to available genomic data, nonsense or frameshift mutations of MPXV resulting in loss of protein-coding genes and noncoding genes or regulatory regions observed in endemic regions of Central Africa have been associated with human-tohuman transmission of the virus. The 2022 MPXV outbreak affecting multiple countries will likely have a single origin, with early signs of microevolution in the clusters of the outbreak. Urbanization caused by population growth in West Africa, especially in Nigeria, may increase the risk of human MPXV infection. The infection spread, especially among the countries of the European continent, has led to increased research on mpox prevention and therapy, with data being continuously updated. Monitoring potential animal reservoirs (e.g., rodents) and exploring new transmission routes are important. Over time, the MPXV evolved by accumulated genome mutations, contributing to its adaptability and easier human-to-human transmission. Studies suggest that variation in gene copy number may be a crucial factor in modulating the fitness of the virus [10].

In line with what has been discovered so far, the WHO Advisory Committee on Smallpox Virus Research at its twenty-fifth meeting (Geneva, 25 and 26 October 2023) reviewed reports from the two WHO Collaborating Centres on Smallpox Virus Conservation, taking into account the global implications of the COVID-19 pandemic and the global mpox epidemic since 2022. Variola virus research strategies need to be modified and updated about the threat of a global weakening of immunity to the virus, the spread of immunosuppressive conditions, and the ongoing evolution of orthopoxviruses leading to adaptation to more efficient human-to-human transmission, as is the case with MPXV. The Advisory Committee encourages efforts towards a rapid MPXV diagnosis and sequencing of all virus isolates. Regarding research on antiviral therapeutic agents approved for use against MPXV, cowpox and vaccinia virus (Vaccinia Virus) infection are the antiviral agents Tecovirimat (approved in Europe) and NIOCH-14 (approved in the Russian Federation). Research on therapeutics with complex activity against orthopoxviruses, including variola virus and MPXV, is encouraged.

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ATTITUDE AND KNOWLEDGE OF HIV INFECTION AMONG HEALTH PERSONNEL IN BULGARIA

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ABSTRACT

Background Addressing HIV-related stigma among healthcare workers is vital in the era of contemporary antiretroviral therapy (cART). It is equally important for stimulating early diagnosis, and for meeting the complex medical needs of people living with HIV (PLHIV).

Material and Methods. We analyzed the results of anonymous local survey comprised of 18 closedchoice questions on the knowledge and attitude towards HIV infection and PLHIV. The survey was conducted among 91 Bulgarian healthcare workers as a part of a large cross-national study in Europe and Central Asia, launched by ECDC and EACS in 2023.

Results. The respondents were predominantly women (65,7%) working mostly as medical doctors (34%), 23% - in a specialized unit for PLHIV, 42% - with over 20 years of experience.

The most important results were: lack of training on PrEP (in 62%), on stigma and discrimination (in 75%). Low level of knowledge on HIV transmission and prevention (in 40%) was associated with anxiety, exaggerated preventive measures and no professional experience with PLHIV. As compared to the mean EACS survey results, specific for Bulgaria were the high prevalence of the misconception "HIV

ADDRESS FOR CORRESPONDENCE:

Radoslava Emilova NRL of Immunology National Center of Infectious and Parasitic Diseases Yanko Sakazov boul. 26, 1504 Sofia, Bulgaria email: remilova@ncipd.org is a result of irresponsible behavior, the ignorance of PrEP, the low rate of administrative sanctions related to PLHIV discrimination, and the low awareness of the availability of PEP.

Conclusions There is an urgent need of targeted and tailored educational programs on HIV-related issues among the different healthcare workers groups. Those should be combined with legislative and administrative measures to assure the implementation of UNAIDS 2030 goals.

Key words: stigma, healthcare workers, people living with HIV

INTRODUCTION

In 40 years, HIV infection has evolved from death sentence to chronic infection. Timely started contemporary antiretroviral therapy (cART) successfully suppresses HIV replication, and provides satisfactory immune recovery for people living with HIV (PLHIV) (1). Still, the share of late presenters diagnosed with suboptimal CD4 absolute counts (CD4AC) remains considerable even in the EU/ EEC countries (2). Reducing stigma to less than 10% affected is one of the goals set by UNAIDS on the road of ending up with AIDS, and was included as strategic intervention in the National Program for control of HIV and STI in Bulgaria, 2021-2025 (3,4). Nevertheless the problem remains significant worldwide, our country included.

HIV stigma combined with lack of basic medical knowledge about HIV infection, the realistic risks of transmission, and the benefits of cART is a recognized barrier hampering HIV prevention, timely diagnosis and treatment efforts. While ignorance and discrimination towards PLHIV among the general population has primarily affective and moral consequences, stigma among healthcare workers has direct detrimental effects both on the spread of infection, and on the health of those living with HIV. Therefore, in the period September 15th - November 30th 2023, the European Center for Disease Prevention and Control (ECDC) and the European AIDS Clinical Society (EACS) launched a cross-national study in European and Central Asian countries to assess the level of knowledge and the attitude of healthcare personnel towards HIV infection and PLHIV (5). The present paper analyses the results of the local survey

conducted among healthcare workers in Bulgaria.

MATERIAL AND METHODS

The target population included any person working in a healthcare facility, regardless of profile, including medical doctors of any specialty, dentists, and nonclinical positions. Healthcare facilities were defined as any facility providing healthcare services. The survey was conducted online, after obtaining a written consent from the participant, by filling-in an anonymous card comprised of 18 closed-choice questions (**Table 1**). The sampling was of a nonrepresentative and snowball nature; the participants were reached through professional contacts, and were asked to further distribute the questionnaire using their own networks. None of the survey activities was remunerated or compensated.

In Bulgaria, 663 healthcare workers (doctors, nurses, dental specialists and others) from all administrative regions of the country were invited to participate in the survey.

Data analysis included assessment of the sociodemographic and professional characteristics of the respondents. The questions covered four

Table 1. Questions on HIV infection included in the survey*(5)

Have you ever worked in a clinic or department that specializes in HIV care and treatment?
• No
Yes, currently
 Yes, within the last 5 years (although not currently)
Yes, more than 5 years ago How many people living with HIV have you interacted with at work within the past 12 months?
None that I am aware of
• Less than 5
Between 5 and 20
More than 20 but less than 50
Between 50 and 100
More than 100
Don't know
Have you received training in the following subjects? Check all that apply. Equity, diversity and inclusion
Patients' informed consent, privacy, and confidentiality
Infection control
HIV stigma and discrimination
Did your training in infection control training include post-exposure prophylaxis (PEP)?
Do you agree or disagree with the following statements? (Agree/Disagree/Don't know)
People living with HIV who are on effective treatment and have an undetectable viral load cannot transmit
the virus sexually
Taking a short course of HIV medicines after a possible exposure to HIV prevents the virus from taking hold
in your body
 Someone who does not have HIV can take HIV medicines to prevent them from getting HIV How worried would you be about getting HIV if you did the following? (Not worried/A little worried/Worried/
Very worried/Not applicable)
Iouched the clothing of a patient living with Hiv
Dressed the wounds of a patient living with HIV
Drew blood from a patient living with HIV
 look the temperature of a patient living with HIV Do you typically use any of the following measures when providing care or services for a patient living with HIV?
(Yes/No/Not applicable)
Avoid physical contact
Wear double gloves
 Wear gloves during all aspects of the patient's care
d. Use any special infection-control measures with people living with HIV that you do not use with other
patients
In the past 12 months, now often have you observed the following in the place you work? (Never/Once or twice/
Unwillingness to care for people living with HIV or thought to be living with HIV
Poorer quality of care provided to a person living with HIV or thought to be living with HIV, relative to other
patients
Discriminatory remarks or talking badly about people living with HIV or thought to be living with HIV
Disclosure of a person's HIV status without their consent

ATTITUDE AND KNOWLEDGE OF HIV INFECTION AMONG HEALTH PERSONNEL IN BULGARIA

Please,	indicate if the following statements are correct in relation to your healthcare facility. (Yes/No/Don't know) In my facility it is not acceptable to test a patient for HIV without their knowledge
•	I will get in trouble at work if I discriminate against people living with HIV
•	My health facility has written guidelines to protect people living with HIV from discrimination
•	There are standardized procedures/protocols in my health facility that reduce my risk of acquiring HIV
•	My health facility has a Post-Exposure Prophylaxis (PEP) protocol in case of needle stick injury
•	My facility has a policy for scheduling people living with HIV on the end of an operating/procedure list
•	In my facility there are guidelines recommending wearing double gloves when caring for people living with
	HIV
Do you	agree or disagree with the following statements? (Strongly Agree/Agree/Neither agree nor disagree/Dis-
agree/S	strongly Disagree)
	Describe Britan with 1007 should be allowed to been a fulfilling accord Bfe
•	People living with HIV should be allowed to have a fulfilling sexual life
•	Women living with HIV should be allowed to have babies if they wish
•	Most people living with HIV have had too many sexual partners
•	People acquire HIV because they engage in irresponsible behaviours
•	HIV is punishment for bad behaviour
•	People living with HIV should feel ashamed of themselves
•	Most people living with HIV do not care if they infect other people
•	People living with HIV with detectable viral loads should not be parents
	a choice, I would prefer not to provide care or services to (Strongly Agree/AgreeNeither agree nor dis-
	People who inject prohibited drugs
•	Men who have sex with men
•	Sex workers
•	Transgender men and women
I prefer	not to provide care or services to people who inject prohibited drugs because Check all reasons that
apply.	They put me at higher rick for disease
	They put the at higher fish for disease
	Libra not received training to work with this group
I prefer	not to provide care or services to men who have sex with men because Check all reasons that apply. They put me at higher risk for disease
•	This group engages in immoral behaviour
•	I have not received training to work with this group
•	I prefer not to provide care or services to sex workers because Check all reasons that apply.
•	They put me at higher risk for disease
•	This group engages in immoral behavior
•	I have not received training to work with this group
l prefer •	not to provide care or services to transgender men and women because Check all reasons that apply. They put me at higher risk for disease
•	This group engages in immoral behaviour
•	I have not received training to work with this group
•	I have not received training to work with this group

*Questions on sex, age, professional position, professional experience not shown

thematic areas: knowledge and training related to the prevention and transmission of HIV infection; attitudes towards and behaviour of PLHIV; stigma and discrimination observed in health facilities; facilities' HIV-related policies and practices. The proportions for the different response categories were calculated, and if relevant further analyzed by background and other thematic characteristics.

RESULTS:

Of the 663 invited, only 91 (13.7%) returned a filled-in questionnaire. The average (min-max) age of the respondents was 43 (22 - 72) years, with a prevalence of women (65, 7%) over men (26, 29%).

The professional structure and experience of the respondents are shown in **Fig.1** The majority of respondents were medical doctors (34%) followed by non-clinical specialists – 19.7%, other clinical specialists – 9.8%, medical students and fellows – 9.9%, dentists – 7.7%, managers of health facilities – 7.7%, other non-clinical positions - 7.7%, nurses – 5.5%, and administrative personnel – 3% (**Fig.1A**). Clinical staff was mostly from infectious, surgery, pediatric, oncology and primary care departments. Notefully, 21 (23%) of the respondents worked in a specialized unit for treatment of people with HIV. Five (5.5%) of the participants were interns, students or fellows of foreign origin including 3 Germans,

Probl. Inf. Parasit. Dis.



Fig. 1. Professional characteristics of the participants.

A. Distribution according to the professional role. B. Distribution according to the experience.

one North Macedonian and one Latvian. A major part of the interviewed (42%) had over 20 years of professional experience; 17.5% – between 10 and 20 years; 15% – between 5 and 10y, and 25% - less than 5 years (Fig.1B).

Regarding the level of patients' knowledge on HIV, most respondents reported to be familiar with the methods for infection control (56, 6%), followed by training on the necessity of informed consent for HIV testing, and the importance of privacy, and confidentiality regarding PLHIV (53, 58%). Postexposure prophylaxis (PEP) was substantially less addressed as a method for infection control (35 or 38%). Importantly, only 23 respondents (25%) have received training on HIV stigma and discrimination.

Further on, knowledge about HIV transmission and prevention was checked by three correct statements (**Fig 2A**). The statement that 'People living with HIV who are on effective treatment and have an undetectable viral load cannot transmit the virus sexually (U=U) was confirmed by 49 (54%) of respondents. The definition of PEP: 'Taking a short course of HIV medicines after a possible exposure to HIV (PEP) prevents the virus from taking hold in your body' was familiar to 36 (39%). Only 27 (one third of the respondents) confirmed being familiar with PrEP by agreeing with: 'Someone who does not have HIV can take HIV medicines to prevent them from



Fig2. Evaluation of basic knowledge on HIV infection: **A.** Proportions of participants affirming to be familiar with (grey), or ignore (black) with the concepts of undetectable = untransmittable (U=U); postexposure (PEP), and preexposure prophylaxis (PrEP).

B. Overall knowledge on HIV infection: the individual's level of HIV knowledge was evaluated based on the sum of agreements with correct statements.



ATTITUDE AND KNOWLEDGE OF HIV INFECTION AMONG HEALTH PERSONNEL IN BULGARIA



□ Not worried □ Worried a little ■ Very worried

Fig.3 Worries are associated with the level of special knowledge. Participants that had agreed with all 3 correct statements on HIV infection were defined as having high knowledge (H), and those who had answered negatively to the 3 statements – as having low/no knowledge (L). Within these groups, the proportions of "not worried", "little worried" and "worried/very worried" about working with HIV+ patients are shown.

getting HIV (PrEP), The overall individual's level of HIV knowledge was evaluated based on the sum of agreements with correct statements, as "no", "low", "medium" and "high". According to this scale, 40% of the respondents had no basic knowledge on HIV infection (Fig.2B).

The group of questions related to worries and preventive measures associated with care for PLHIV is the most revealing about stigma in healthcare institutions. From those to whom the questions were applicable, 83.5% and 80% respectively were not worried about taking the temperature or touching the clothing of PLHIV. However, 77% were anxious about dressing wounds or drawing blood from such patients. Excessive preventive measures like avoiding any contact and wearing double gloves were envisaged by 21% and 51%, respectively. Noteworthy, low level of special knowledge about the infection was associated with concerns about working with HIV+ patients (Fig.3). At the same time, 100% of respondents with low/no knowledge on HIV infection reported having had no or less the 5 professional contacts with PLHIV, while 32% of respondents with high level of special knowledge had met more than 5 PLHIV during the last year.

Between 20% and 33% of the interviewed reported having noticed some form of discrimination at their working place during the past 12 months, including: refusal or poor quality of care, disclosure of positive status, testing without consent or discriminatory remark. As to the attitude to specific risk groups, the respondents were most often reluctant to take care of IDUs (31%), followed by 17.5% for sex workers, 16% for MSM, and 18% for transgender people. The most frequent reason was "I am not trained for that", followed by "This group engages in immoral behavior", and "They put me at higher risk of disease". Whenaskedaboutcertainstigmatizingmisconceptions about PLHIV, 98% of the respondents disagreed that "PLHIV should be ashamed of their behavior", 96% disagreed that PLHIV deserve punishment, and 89% did not accept that "PLHIV do not care if they infect other people". Still, 14% were persuaded that PLHIV should not have babies, almost 30% believed that most PLHIV had too many sexual partners, and 46% - that people acquire HIV as a result of irresponsible behavior.

Regarding the healthcare facilities' policies in the field of HIV infection, 68% of respondents were aware that HIV testing consent was obligatory at their working place; 43% were sure to be administratively punished in case of discriminating PLHIV, while only 21% reported the existence of written documents and procedures protecting PLHIV from discrimination.

In 59% respondents were aware of any standardized procedures/protocols reducing personnel's risk of acquiring HIV, and in 45% - of the availability of PEP. At the same time, discriminative measures, such as scheduling PLHIV on the end of an operating/ procedure list, and recommending wearing double gloves when caring for PLHIV were at place in 23% and 13% of the facilities.

DISCUSSION

Nowadays, thanks to contemporary ART, the life expectancy of PLHIV is similar to that of their HIVnegative peers (6). Therefore, the international guidelines recommend immediate start of ART after diagnosis (1). At the same time a significant share of PLHIV are still diagnosed with advanced immune deficiency (CD4 cell count < 350 cells / μ l), a nonnegligible number of them being in their sixties. The result is an increasing share of patients ageing with HIV, and experiencing the effects of long-term ART. The latest ECDC surveillance report indicated that a growing number of people are living with undiagnosed HIV, 52.4% of those diagnosed in 2023 were diagnosed late (2). In Bulgaria, the share of late presenters has varied between 41% and 57% during the period 2018 – 2023 with a tendency to increase (unpublished data).

Persisting immune activation in long-term treated PLHIV is a well-known problem associated with higher rates of comorbidities, hospitalizations, and other challenges relating to quality of life (7,8). This means that healthcare systems will need to adapt urgently in order to meet the complex and increasing needs of people ageing with the infection (9). In these settings, the problem of HIV stigma in society, and among healthcare workers at the first place, is clearly of critical importance (10).

The survey among healthcare personnel in Bulgaria on the attitude and knowledge about HIV infection in the context of the overall results of EACS study helps to explain in part the observed negative facts, to compare Bulgaria with other European and Central Asian countries, and to choose appropriate approaches for amelioration.

The results from the local survey revealed a significant share of respondents with negative attitude towards PLHIV, combined with important gaps in the specific knowledge about the infection. At the same time, for most of the questions, the share of negative answers was similar or slightly higher than the mean numbers reported by the EACS survey. This was valid for: infection control training including post-exposure prophylaxis (PEP), training on HIV stigma and discrimination, basic knowledge about HIV transmission and prevention. However, in the latter group of questions knowledge about

PrEP was demonstrated by only 27% of Bulgarian respondents vs. a mean of 41% in the EACS study. PrEP is a particularly sensitive theme closely related to stigmatizing misconceptions about MSM as a major risk group for HIV infection, and their behavior. Bulgaria is among the few European countries without implemented national policy and program on PrEP, together with other Central European countries as Romania, Serbia and Turkey. Noteworthy, the mean result for the Central European countries was 31% vs.41% for EU/EEC. (10,11) The identified particular gap in HIV-related knowledge is an important conclusion that might aid the further attempts for implementation of PrEP in Bulgaria.

Lack of basic knowledge correlated with professional fears, excessive preventive measures, and, importantly, admitting some form of discrimination at the working place, underlining again the crucial importance of educational campaigns for combating stigma, and addressing adequately the medical needs of PLHIV. Importantly, lack of special knowledge correlated with lack of professional contacts with PLHIV. This fact might indicate that educational programs on HIV are limited to HIV-dedicated staff, but also – that to a large extent stigma is based on prejudice, and not – on real experience.

Other important deviations in our local survey were the misconceptions about PLHIV having too many sexual partners, and PLHIV having irresponsible behavior (30% vs. 12%, and 46% vs. 22% for the EACS survey). The latter result was comparable to the mean for East European countries (ex-USSR republics), 41%, revealing the influence of traditional moral, religious and educational concepts on the overall attitude to PLHIV. These facts indicate the urgent need for more effective intersectional stigma interventions in CEE, including healthcare, community and legislative sectors (12).

A general flaw detected by both the local and EACS survey was the absence of written guidelines protecting PLHIV from discrimination (in 79% and 73% respectively). Specific for Bulgaria were the low rate of administrative sanctions related to PLHIV discrimination (41% vs. a mean of 66%) as well as the low awareness of the availability of PEP (45% vs. 69%). Unlike the previous group, this type of gaps are easily amendable through administrative measures.

The demographic and professional characteristics of the interviewed corresponded to the average European results, with a slightly lower prevalence of women (64% vs.75%), and a prevalence of the age group 45 - 54 (25%) vs. 34 - 45 (27%) for the EU/EEA. The predominance of female sex among the respondents might be a consequence of several factors: the nation-specific sex distribution among the participating professional categories, and women being more responsive and assiduous in general. At the same time, this prevalence might have impacted the results, especially the reported reasons for not providing services to certain risk groups. On the other hand, the professional structure of the interviewed in Bulgaria differed significantly as compared to EC/ EEA with a total share of medical doctors and nurses 39% vs. 61% at the expense of dentists (7.7% vs. 2%), and other non-clinical positions (20% vs.2.8%) which could explain some discrepancies in knowledge level. Importantly, the share of participants working in HIV clinics in the Bulgarian survey was 23% vs.7% for EACS. While it could be expected that working routinely with PLHIV might have a positive impact on the answers, that was not the case in our survey. It should be noted that another study on HIV stigma among healthcare workers in Washington, DC reported stigmatizing behaviors in up to 66% of participants in spite of the high HIV prevalence and the fact that most participants had been working with HIV+ patients, and required to periodically undergo HIV-related continuing medical education The authors concluded that the training/education related to HIV should be targeted, and specific topics may need to be addressed. (13).

This study has several general limitations: HIV 'knowledge' was focused on the modes of infection, and the ways to prevent HIV transmission. The participants had to approve or disapprove of correct statements, and not to choose between correct or incorrect, or answer to open questions. The survey had a non-random and a non-representative nature, thus limiting the generalisation of the findings.

CONCLUSIONS

HIV-associated stigma among healthcare personnel is an overwhelming fact in Bulgaria, as a part of a general tendency in Europe, and in particular, its Central and Eastern part.

The level of knowledge about the infection is the main determinant of the personal perceptions, and professional attitude. However, additional sociocultural, ethnical, and religious factors are also in play and may confront with formally existing policies.

The established deficiencies in the knowledge and instructions concerning HIV infection require targeted educational programs among the different healthcare professional groups. Those should be combined with legislative and administrative measures to assure the implementation of UNAIDS 2030 goals.

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A CASE OF MYCOTIC SEPSIS DUE TO *TRICHOSPORON COREMIIFORME* IN A TRAUMA PATIENT IN MHAT-SHUMEN

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ABSTRACT

Trichosporon spp. are yeast-like fungi,a rare cause of mycotic sepsis. We share a case report of a thirtythree year old man with polytrauma after a car accident, who died after a long hospitalization and treatment in intensive care unit. Four blood samples for blood culture were submitted to the Microbiology laboratory in MHAT-Shumen, which were all positive for fungi. The tests available and performed in our laboratory were insufficient for species identification. Pure culture was sent to National Reference Laboratory of Mycology and identified as *Trichosporon coremiiforme*. Mycotic sepsis remains a challenge in terms of diagnosis and therapy and often has a fatal outcome.

Keywords: Trichosporon spp., *Trichosporon coremiiforme*, mycotic sepsis, blood culture, intensive care

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INTRODUCTION

Mycotic sepsis is a life-threatening condition, which is often fatal. Prolonged hospitalization, prolonged treatment with corticosteroids and broad-spectrum antibacterial agents in intensive care units increase the risk of mycotic sepsis. Fungal isolates from blood culture are a challenge for clinicians as well as for microbiologists, due to species diversity and specific therapy needed. The described clinical case aims to expand our knowledge and outline the difficulties in identifying the causative agents of mycotic sepsis, considering Trichosporon spp.

Trichosporon spp. are widespread yeast-like fungi of the phylum Basidiomycota, a frequent part of human skin and gastrointestinal tract microbiota. The taxonomy of genus Trichosporon has changed over time, with an increasing number of species, seventeen of which are currently clinically significant. [1]

Species of the genus Trichosporon are characterized by the formation of radially symmetrical colonies on Sabouraud agar, which may be white to cream colored, with a smooth, velvety or rough surface. Microscopically rarely septate hyphae, abundant arthroconidia and blastoconidia are observed. A main characteristic of the genus is the ability of the species to hydrolyze urea. [2] This distinguishes them from the genus Geotrichum, whose species also form arthroconidia, but are urease test negative. [3]

Trichosporon spp. is known as the causative agent of the superficial infection "white piedra", but is also the second most common causative agent of invasive infections from phylum Basidiomycota, after Cryptococcus spp. [4]

Invasive trichosporonosis is an opportunistic infection observed in the settings of immune deficiency, in cancer patients, neutropenic patients, HIV-positive patients and those requiring prolonged intensive care. Due to the ability of Trichosporon to form a biofilm, cases of catheter-related fungemia have also been reported. [3, 5, 8] Despite the adequate treatment, invasive trichosporonosis is often fatal. [1, 2, 6]

Azoles are the recommended therapy for invasive Trichosporon spp infection according to Sanford Guide voriconazole 6 mg/kg i.v/p.os every 12 h on the first day, then - 4 mg/kg i.v/p.os every 12 h;

Amphotericin B, posaconazole or isavuconazole as an alternative), since the genus shows intrinsic resistance to echinocandins. [2, 6, 7, 8].

Until recently, *Trichosporon coremiiforme* was considered as a variety of *Trichosporon asahii*, due to their genetic relatedness. Therfore the reported cases of invasive trichosporonosis with this species are few. [7, 8]

CLINICAL CASE

A thirty-three year old man was transported to the emergency department of MHAT-Shumen, severely injured in a car accident. He was hospitalized in the intensive care unit (ICU) with intracranial and chest trauma. During the first seven days of hospitalization combined antibacterial therapy with ceftriaxone (4g/d) and metronidazole (1g/d) was started. A 12-day therapy with Dexamethasone was also administered for 12 days (12mg/d which were titrated down until discontinuation). The patient underwent weekly microbiological monitoring. At the end of the first week hospital strains of Acinetobacter baumannii complex and Klebsiella pneumoniae were isolated from the intubation cannula. The antibacterial therapy was changed to meropenem (3g/d), followed by colistin (9MU/d). During the therapy with colistin, antimycotic treatment with fluconazole (200 mg/d) was also started by the attending physician, and was discontinued in fourteen days.

During the first week of hospitalization, three blood samples for blood culture were taken as a part of the ICU monitoring, two of them were sterile and one was contaminated. During the third week of the ICU stay, Staphylococcus epidermidis MRS was isolated from another blood sample and treatment with teicoplanin (400mg/d) was administered. In the settings of ongoing treatment, on the twentyfifth day of his stay in the ICU, the patient presented with high temperature 38°C to 39°C. Subsequent laboratory tests revealed increased white cell count (13.34 x 10⁹/L) and C-reactive protein values (CRP; 90,4 - to 115,2 mg/L). A fungus was isolated from the following four blood samples taken for blood culture. Despite restarting treatment with the locally available antimycotic fluconazole (400 mg/d), fevers became more frequent, the patient's condition deteriorated and he died five days after the

first fungi positive blood culture.

The microbiological assessment was made in the Microbiology laboratory of MHAT-Shumen. Microscopic slides were prepared from the first positive for fungi blood culture showing elongated spores and hyphae. Therefore, in addition to standard blood agar (HiMedia, India) and MacConkey (HiMedia, India) agar, cultures on Sabouraud (BioLab, Hungary) and Chrom (HiMedia, India) agar were prepared. The same was done for the subsequent three blood cultures as well. After twenty-four hours, white colonies with a smooth surface and a creamy consistency were observed on Sabouraud agar, but forty-eight hours later they increased in size and took on a rough appearance. Substrate mycelium became creamy to brown. On microscope slides prepared from the colonies rare septate hyphae and numerous arthroconidia as well as spores were observed.

After twenty-four hours on Chrom agar for Candida (HiMedia, India), the colonies were white, and forty-eight hours laterbthey became pale pink with a velvety surface. Upon subsequent cultivation they became grayish-purple inside with a dirty gray back. In a liquid medium, a wide film on the walls of the tube above the solution was presented. The urease test performed was positive. Identification with Integral System Yeasts (Liofilchem, Italy) was and no resistance to the antimycotic dubitous agents available in the test was observed. The culture was further examined with VITEK 2 YST automated identification system (BioMerieux, France). The result was Cryptococcus laurentii (93% probability), which was not a convincing result given the macroscopic and microscopic morphology.

After a pure culture of the same species from the last four consecutive blood samples was obtained, the isolate was sent according to the standard protocol to the National Reference Laboratory of Mycoses at the NCIPD, Sofia. We received a confirmatory result for *Trichosporon coremiiforme*, based on validated laboratory methods (MALDI-TOF, Biotyper Bruker).

The isolate was tested for susceptibility to antimycotic agents. The minimum inhibitory concentration (MIC, Himedia, India) values were as follows: Voriconazole - 0.06 mcg/ml, Posaconazole and Itraconazole - 0.25

A CASE OF MYCOTIC SEPSIS DUE TO TRICHOSPORON COREMIIFORME IN A TRAUMA PATIENT IN MHAT-SHUMEN



Figure 1. Case progression – schematic presentation.

mcg/ml, Fluconazole - 4 mcg/ml, Caspofungin - 4 mcg/ml, Micafungin and Anidulafungin - 8mcg/ml, Amphotericin B – 4mcg/ml. There are no available criteria for interpretation for this species. The Sanford Guide recommendations for invasive infection are Voriconazole as primary regimen, and Amphotericin B or Posaconazole as alternative regimens. [9] DNA sequencing of the ITS region was performed as previously described. [10] The DNA sequence of the isolate was 100% identical with the type strain *T. coremiiforme* CBS2482.

The isolate was deposited at the National Center of Infectious and Parasitic Diseases culture collection as *T. coremiiforme* 454-23 and the DNA sequence



Figure 2. *Trichosporon coremiiforme*: Colony morphology on Sabouraud agar (1); Arthroconidia (2)



was submitted to Genbank (accession number: PP455503).

DISCUSSION

Hospitalization of patients in ICU is a risk factor for occurrence of mycotic sepsis. [11] Those are usually, polymorbid or trauma patients in a critical general condition who are subjected to broad-spectrum antibacterial therapy, intubation, catheterization and other invasive procedures. Some of them require a long corticosteroid treatment, which also increases the risk of mycotic sepsis. The most common causative agent of invasive mycotic infections are Candida spp., but given the specific therapy and high mortality from mycotic sepsis, microbiologists should consider a wider range of fungi. Despite the availability of new and diverse automated methods for accurate pathogen species identification, microscopic examination and other routine

microbiology methods retain their importance for adequate therapy choice.

In our case, species identification was not possible locally. As for Trichosporon coremiiforme, this would not have been possible without the use of MALDI-TOF MS technique and DNA sequencing due to its high similarity to Trichosporon asachii. There is a limited number of publications on Trichosporon coremiiforme as a causative agent of invasive infections. One possible reason is its misidentification as Trichosporon asachii. At the same time, due to advances in treatment, the survival rate of immunocompromised patients increases, which is a prerequisite for an increasing number of opportunistic infections. With the availability of modern identification methods, the knowledge about Trichosporon coremiiforme and its susceptibility to antimycotic agents is expected to expand, to which our clinical case aims to contribute.

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BEYOND MOSQUITO BITES: ANALYZING MALARIA RISK FACTORS IN SOUTHERN NIGERIA

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ABSTRACT

This study investigates how various risk factors affect the prevalence of febrile diseases, with a focus on malaria in the southern states of Nigeria. The study employed Pearson correlation and multilinear regression analyses to examine the relationships between risk factors and disease prevalence. Pearson

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Said Baadel Dept. of Mathematics and Computing Mount Royal University 4825 Mount Royal Gate SW Calgary, AB. Canada. T3E 6K6 phone: +1 (403) 440-5029 e-mail: Sbaadel@mtroyal.ca correlation analysis revealed that genetic conditions, high blood pressure, and direct contact with infected individuals were negatively correlated with malaria, while poor personal hygiene, substandard living conditions, and exposure to endemic areas had weak positive correlations. The strongest association was found with mosquito bites, which also had the highest unstandardized beta coefficient among the factors studied. Nevertheless, the study also highlights secondary risk factors, such as poor living conditions and hygiene, which are often overlooked in malaria intervention programs. These factors, although not as strong as mosquito exposure, can exacerbate the risk of infection, particularly in vulnerable populations living in impoverished areas. These results highlight the critical role of mosquito exposure in malaria and emphasize the need for targeted interventions in such areas. The findings can be beneficial to clinicians, general public, and all stakeholders involved in the fight against malaria.

Key words: Febrile disease; low-and-middle-income countries; malaria, tropical diseases

1.0 INTRODUCTION

Malaria continues to pose a major public health challenge in low- and middle-income countries (LMICs), particularly in Africa. In 2022, 233 million cases (94%), and 580,000 (95%) malaria deaths were reported in the World Health Organization (WHO) African Region report (WHO, 2023). Tropical and subtropical climates with heavy rainfall create ideal breeding conditions for mosquitoes, contributing to increased incidences of diseases such as malaria and dengue (Awosolu et al., 2021). Beyond their health impacts, febrile diseases such as malaria exert considerable socioeconomic burdens, hindering economic development, exacerbating poverty, and straining healthcare systems (Wilastonegoro et al., 2024). Malaria, transmitted by Anopheles mosquitoes and caused by Plasmodium parasites, is influenced by environmental, social, and individual factors. Southern Nigeria, with its tropical climate, has some of the highest malaria transmission rates globally. Previous research has mainly focused on vector control measures, such as insecticide-treated nets (ITNs) and antimalarial medications. However, there is a growing need for a thorough understanding of other

risk factors vital for the effective prevention of febrile diseases such as malaria (Uzoka et al., 2021a).

In Nigeria, malaria transmission occurs year-round, placing over 194 million people at risk of infection. In 2007, Nigeria recorded the highest malaria prevalence globally (FMOH, 2008). Among all other tropical diseases diagnosed in Nigeria, roughly 61.32% of patients were diagnosed with malaria, with almost 49.9% experiencing moderate to very high attack intensity, and only 11.4% presenting with low attack intensity (Uzoka et al., 2021a).

The aim of this paper is to explore the relationship between various risk factors and the prevalence of malaria in southern Nigeria. By identifying key predictors influencing malaria outcomes, the study seeks to inform public health strategies aimed at reducing the disease burden in LMICs. To achieve this, we posed the following research questions :

1. What is the influence of biological factors and genetically detemined conditions, including high blood pressure, elevated cholesterol levels, underlying chronic diseases etc., on the prevalence and confirmed diagnosis of malaria in LMICs?

2. How do socioeconomic factors, including street vendor occupations, overcrowding, travel to endemic areas, and direct contact with infected individuals, contribute to the prevalence and confirmed diagnosis of malaria in LMICs?

3. What is the influence of environmental factors, such as poor environmental conditions, exposure to mosquito bites, indoor pollution, and allergies, on the prevalence and confirmed diagnosis of malaria in LMICs?

4. Do behavioral factors, such as smoking, exposure to second-hand smoke, intravenous drug use, skin punctures, low fluid intake, and poor personal hygiene, affect the prevalence and confirmed diagnosis of malaria in LMICs?

To align with the core objectives of this research, we investigated the relationship between independent variables (i.e., risk factors) and their influence on the confirmed diagnosis of malaria. We hypothesixzed that a direct correlation exists between risk factors and the likelihood of being diagnosed with malaria in LMICs.

2.0 LITERATURE REVIEW

An earlier study by Gallup and Sachs (2001) examined the economic effects of malaria, revealing a strong correlation between malaria endemicity and poverty in African nations. Their findings indicated that countries with high malaria prevalence had income levels up to 33% lower than those with minimal malaria exposure, even after accounting for other factors. This research underscores the substantial economic burden malaria places on African countries and emphasizes the potential economic gains achievable through effective malaria control measures.

Bhatt et al. (2015) conducted an extensive review of malaria control efforts in Africa from 2000 to 2015, highlighting the limited understanding of the root causes of malaria in sub-Saharan Africa, despite its critical significance. Analyzing 32 highly endemic countries, which account for around 90% of the global malaria burden, the study found that national health records were inadequate for accurately tracking malaria trends. However, the authors reported a 50% reduction in malaria prevalence and a 40% decrease in clinical disease incidence in these countries over the 15-year period, attributing these improvements to targeted interventions such as bed nets among others.

In addressing the effects of climate change on malaria transmission, Caminade et al. (2014) utilized climate models to project future malaria risks in Africa. Their study indicated that while some regions may experience reduced transmission due to increased aridity, others, particularly highland areas, could face heightened risk from rising temperatures.

Nkumama et al. (2017) examined the evolving epidemiology of malaria in Africa amidst ongoing elimination efforts, observing a shift in the age distribution of clinical malaria cases towards older children and adults. This shift was linked to reduced transmission intensity and delayed immunity acquisition, highlighting the need for sustained vigilance and adaptive control strategies.

Monroe et al. (2019) carried out a systematic review of qualitative studies to examine the impact of human behavior on malaria transmission. They identified key behavioral factors affecting malaria risk, such as outdoor nighttime activities, sleeping arrangements, and varying perceptions of malaria risk.

Boyce and O'Meara (2017) reviewed the adoption of rapid diagnostic tests (RDTs) for malaria in Africa, noting a significant rise in their use. However, they identified ongoing challenges related to ensuring the quality of RDTs, their correct usage, and their effective integration into clinical decision-making. The authors stressed the importance of continuous training and robust quality assurance measures to fully realize the potential of RDTs in improving malaria case management.

Uzoka et al. (2021b) explored the use of mobile technology and artificial intelligence for malaria diagnosis, proposing a framework for cell phonebased testing that capitalizes on the widespread availability of mobile devices in LMICs. This approach aims to enhance access to rapid and accurate malaria diagnostics, particularly in remote areas with limited healthcare infrastructure. By incorporating image processing and machine learning algorithms on mobile platforms, these systems hold the potential to improve early detection and treatment, contributing to more effective malaria management and control efforts in endemic regions.

3.0 METHODOLOGY

Purposive sampling was employed to select the states and medical institutions where the study was conducted, based on accessibility and convenience. This method ensured the representation of diverse medical institutions, including large public hospitals, government facilities, private hospitals and clinics. Random sampling was then applied to select participants who expressed interest in the study. Invitations were advertised at the selected medical institutions, and interested patients submitted their names to designated officers. Participants were randomly chosen from this pool for data collection. Table 1 below highlights the study's participants.

The participation rates across the selected states were generally comparable, with the exception of Imo State, where fewer than 1,000 patients participated in the study. Children and adolescents under the age of 19 comprised 40% of the patient population, whereas only 7% were aged 60 years or older. Patients of female gender outnumbered those of the male gender (55% to 45% respectively).

The cross-sectional design of this study facilitated the collection of data from participants at a single point

	Number	%	
State			
Akwa	1223	25	
Ibom			
Cross	1531	32	
River			
Imo	882	18	
Rivers	1232	25	
	4868	100	
Age Group	p		
<19 yrs	1934	40	
19-24 yrs	424	9	
25-44 yrs	1557	32	
45-60 yrs	600	12	
>60 yrs	353	7	
	4868	100	
Gender			
Male	2175	45	
Female	2693	55	
	4868	100	

Table 1. Descriptive Statistics ofStudy Participants

Table 2. Risk factors used in the study

#	Risk Factors	Description		
	Biological Factors			
1	GNCN	Genetic condition		
2	HIBP	High blood pressure		
3	HICOL	High cholesterol		
4	UNCHRIL	Underlying chronic illness		
5	ALG	Allergies		
	Environmental	and Socioeconomic Factors		
6	STRVEN	Street vendor		
7	PPHYG	Poor personal hygiene		
8	PECON	Poor environmental conditions		
9	OVCRW	Overcrowding		
10	IVDRUS	Intravenous drug use		
11	TRVENRG	Travel to endemic region		
12	SKPUPR	Skin puncture procedure		
13	DRCOIFPS	Direct contact with infected person		
14	LWFLIN	Low fluid intake		
15	EXPMQBT	Exposure to mosquito bites		
16	SMEXSM	Smoking or exposure to smoking		
17	EXIDARPOL	Exposure to indoor air pollution		

in time and focused on assessing clinical diagnostic outcomes of malaria based on respondents' exposure to a range of risk factors. Seventeen risk factors ranging from genetic conditions, high blood pressure, and high cholesterol, to poor personal hygiene, intravenous drug use, travel to endemic areas, and exposure to mosquito bites—were examined for their impact on malaria diagnosis as shown in Table 2 below.

3.1 Research Instrument and Data Collection

A questionnaire utilizing a 5-point Likert scale, ranging from Absent, Mild, Moderate, Severe, to Very Severe, was employed to capture the presence of each risk factor for every participant. This data was then used to analyze the impact and correlation of the examined risk factors on the confirmed diagnosis of malaria based on participants' responses. The questionnaire was designed with reference to existing literature and previous studies investigating the relationship between risk factors and disease prevalence (Uzoka et al., 2021a).

Medical doctors at the selected medical institutions were enlisted to administer the questionnaire during routine consultations with patients who had opted in and were randomly selected to participate in the study. The data collection process spanned eight months, from May to December 2021, and covered four states in Southern Nigeria (Akwa Ibom, Cross River, Imo, and Rivers). A total of 4,870 patient consultation forms were distributed, yielding 4,868 usable responses, resulting in a remarkable response rate. The focus of this study was on non-clinical factors that may affect the patients. These were categorized as biological, environmental and socioeconomic factors as shown in Table 2 above.

The reliability of the study instrument was assessed using Cronbach's alpha, which yielded a value of 0.740. Since this value exceeds the 0.7 rule of thumb threshold (Nunally, 1978), it indicates that the instrument was reliable. The validity of the research tool was ensured through a pilot study, while content validity was confirmed via an independent review conducted by experienced colleagues who were not involved in the study.

3.2 Ethical Considerations

			Regressi	on Analy-	Beta coeffi-
	Correlation Analysis		sis		cients
Risk Factors	R-value	P-value	Т	P-value	β
GNCN	-0.06	0.000	-3.69	0.00	131
HIBP	-0.03	0.032	-1.86	0.06	063
HICOL	0.010	0.478	0.26	0.80	.014
STRVEN	0.087	0.000	0.59	0.56	.025
PPHYG	0.054	0.000	-5.51	0.00	185
PECON	0.126	0.000	-2.11	0.04	065
OVCRW	0.009	0.513	-3.09	0.00	104
IVDRUS	0.012	0.405	2.21	0.03	.156
TRVENRG	0.300	0.000	11.72	0.00	.275
SKPUPR	0.021	0.135	-6.60	0.51	045
DRCOIFPS	-0.054	0.000	-5.95	0.00	162
LWFLIN	0.111	0.000	5.13	0.00	.173
EXPMQBT	0.570	0.000	41.68	0.00	.807
SMEXSM	-0.026	0.072	-1.65	0.10	062
UNCHRIL	-0.025	0.086	-0.69	0.49	019
EXIDARPOL	0.015	0.303	-1.78	0.08	077
ALG	-0.027	0.062	-2.79	0.01	116

Table 3. Risk Factors for Malaria with $R^2 = 0.37$

Informed consent was obtained from participants who chose to participate in the study. They were thoroughly informed about the study's purpose and procedures prior to providing their consent. Participant confidentiality was maintained through data anonymization to protect their identities, and the data was stored securely. The Mount Royal University Ethics Committee a (Human Research Ethics Board # 102232) and the University of Uyo Teaching Hospital health research ethical committee (Ref # UUTH/AD/S/96/VOL.XX1/450) approved this research.

4.0 RESULTS AND DISCUSSIONS

SPSS version 20 was used to carry out the data analysis. The regional demographics, alongside with participants' gender and age were examines, utilizing frequency distributions. Additionally, multilinear regression analysis was carried out to investigate the influence of selected risk factors. With a sample size of 4,868, the analysis had adequate data to identify significant predictors. Table 3 below shows the correlations between malaria and the associated risk factors .

GNCN=Genetic condition, HIBP=High blood pressure, HICOL =High cholesterol, STRVEN=Street vendor, PPHYG=Poor personal hygiene, PECON=Poor environmental conditions, OVCRW=Overcrowding, IVDRUS=Intravenous drug use, TRVENRG=Travel to endemic region, SKPUPR=Skin puncture procedure, DRCOIFPS=Direct contact with infected person, LWFLIN=Low fluid intake, EXPMQBT=Exposure to mosquito bites, SMEXSM=Smoking or exposure to smoking, UNCHRIL=Underlying chronic illness, EXIDARPOL=Exposure to indoor air pollution, ALG=Allergies

Correlation coefficients highlighted in bold text indicate a significant association with malaria. Our analysis reveals that certain genetic conditions, high blood pressure, and direct contact with infected individuals were negatively correlated with malaria prevalence. Research on the association between genetic disorders and malaria susceptibility or resistance is growing (Tiwari et al., 2023; Gallego-Delgado et al., 2016).

Conversely, factors such as being a street vendor, poor personal hygiene, living in substandard

environmental conditions, traveling to endemic areas, and low fluid intake exhibited positive correlation with malaria prevalence, though the correlations are relatively weak. The most pronounced correlation was observed in patients exposed to mosquito bites (r = 0.570, p-value of < 0.001). This finding aligns with the results of Bhatt et al. (2013), who noted that rapid urbanization, inadequate infrastructure, and population growth created environments conducive to the spread of mosquito-borne diseases. This result also aligns with the findings of de-Souza and Weaver (2024), who linked vector-borne diseases to poor environmental conditions and stagnant water. Also, low fluid intake or dehydration increases the prevalence of malaria-associated diarrhea and children with dehydration are more likely to manifest malaria parasitaemia (Ibadin et al, 2000).

The various risk factors were plugged in as independent variables against the dependent variable (Malaria) to evaluate the impact and significance of those risk factors on the confirmed diagnosis of malaria in LMICs. The multilinear regression results on Malaria exhibited a high F-value of 166.91, p < 0.01 and an R² value of 0.37, indicating that 37% of the variance in malaria diagnoses can be explained by the risk factors included in this study.

The variance inflation factors (VIF) for all predictor variables (risk factors) were below 5, and all tolerance values were above 0.25, indicating minimal multicollinearity and a moderate, acceptable level of correlation among the predictors. Table 3 displays the statistical significance of each risk factor for the disease. The most significant factor was the impact of mosquito bites on the confirmed diagnosis of malaria, with a *t-value* of 41.68 and *p-value* < 0.01.

Furthermore, the unstandardized beta coefficients indicate the impact of a one-unit increase in each independent variable on the dependent variable (malaria). The results show that the highest coefficient is associated with the impact of mosquito bites on the confirmed diagnosis of malaria (β = 0.807, p < 0.001).

5.0 CONCLUSIONS

In summary, this research examined the hypothesis of a direct correlation between risk factors and the likelihood of being diagnosed with malaria in LMICs, specifically in Nigeria. With nearly 5,000 participants across four Nigerian states, our findings highlight a complex interplay of factors that influence malaria risk.

Firstly, a Pearson correlation analysis identified several risk factors: certain genetic conditions, high blood pressure, and direct contact with infected individuals negatively correlated with malaria prevalence. In contrast, risk factors such as poor personal hygiene, inadequate living conditions, exposure to endemic areas, and low fluid intake exhibited weak positive correlations with malaria. Among the various risk factors, mosquito bites were identified as the most significant predictor, both in terms of correlation and impact. The unstandardized beta coefficient for mosquito bites was notably high ($\beta = 0.807$, p < 0.001).

Secondly, the explained variance of 37% in malaria diagnoses underscores the role of environmental factors, particularly mosquito exposure, in malaria transmission. The strong association with mosquito bites supports vector control as the primary intervention, as outlined by Bhatt et al. (2013).

Lastly, our findings support the hypothesis and highlight that mosquito exposure is a predominant factor in malaria prevalence, emphasizing the need for targeted interventions to address this critical risk factor in the fight against malaria. Addressing mosquito exposure through preventive measures and improving living conditions in LMICs could significantly reduce malaria and especially in tropical African countries such as Nigeria. Understanding the risk factors associated with malaria within LMICs is crucial for developing targeted public health interventions and policies.

Conflict of Interest

The authors declare they have no conflict of interest to disclose.

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IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX SPECIES IN BULGARIA

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ABSTRACT

Tuberculosis is caused by closely related mycobacterial species, designated as M.tuberculosis complex (MTBC), which includes : *M.tuberculosis* sensu stricto, M.africanum, M.canettii, M.bovis, M.caprae, M. microti. There is an increase in human TB cases caused by *M.bovis* or *M.caprae* in the EU. Although Bulgaria is not a bovine TB-free country, the species identification inside the MTBC is not routinely performed for human isolates and the presumably animal related pathogens could not be distinguished. This study aimed to reveal the presence of *M.bovis/M.* caprae as an aetiological agent on the territory of Bulgaria.

For the period from 2022 to 2025, a total of 175 MTBC strains were further examined to differentiate the species. GenoType MTBC VER 1.X was used as a reliable identification tool.

Almost all cases were found to be *M.tuberculosis/M.* canettii (n=173; 98.85%). The prevalence of *Mycobacterium bovis* was 0.57%. *M. bovis* BCG also was represented by a single isolate (0.57%). *M.africanum, M.microti*, or *M.caprae* have not been detected so far.

Species identification in the MTBC is an essential

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step in order to limit the transmission from animal to human and to refine the treatment of the affected individuals.

Key words: M. tuberculosis, M.bovis, M.caprae

INTRODUCTION

Tuberculosis (TB) is an infectious disease of public importance, and a leading cause of death among the infectious diseases of all time. According to WHO data, in 2022 10.6 million people were infected with tuberculosis worldwide, and 1.3 million died [1]. Besides the human population, tuberculosis affects domestic and wild animals, forming endemic zoonotic reservoirs

The disease is caused by a group of mycobacteria designated as M.tuberculosis complex (MTBC), which includes different mycobacterial species and subspecies sharing 99.9% of their DNA sequence but differing in their host preferrence [2]. The most common species infecting humans are: M.tuberculosis sensu stricto (Lineage 1-4, Lineage7), M.africanum, M.canettii [2,3,4]. The species adapted primarily to animal hosts are M.bovis, M.caprae, M. pinnipedii, M. microti, M. mungi, M. orygis, M. suricattae, chimpanzee bacillus, dassie bacillus [4]. Although prone to invasion, establishment and spread in a particular animal species, most of the mentioned mycobacteria are capable of infecting other mammalian species, i.e. - human [5, 6, 7, 8, 9]. M.tuberculosis sensu stricto is the leading aetiological agent of human tuberculosis, but has been also reported in sick animals [8]. M.africanum has significance as a pathogen in West Africa, where it causes up to half of the tuberculosis in the human population. [10]. It could be found also in imported cases with migrants from endemic countries [11, 12] Out of the animal-adapted species, M.bovis and M.caprae are the most common causes of TB in humans. Transmission of bovine TB from animal to human occurs primarily through close contact and consumption of unpasteurized milk from infected animal [13]. However, routine surveillance of human and animal populations is insufficient, particularly in the countries where bovine tuberculosis is endemic and laboratory capacity is limited. Cases of zoonotic tuberculosis in humans are uncommon in countries with controlled bovine tuberculosis and high food

safety standards [14].

The clinical manifestation of *M.bovis* infection is identical to *M. tuberculosis* sensu stricto, but the extrapulmonary localization is more frequent. The treatment of *M.bovis* infection in men includes rifampicin, isoniazid, and ethambutol with extended duration 9-12 months. The pyrazinamide is excluded from the treatment regimen due to the innate resistance of *M.bovis*. Even when properly assigned, *M. bovis* infection has often unsatisfactory treatment outcome and high mortality rates [15].

Meta-analysis of the published cases between 2009 and 2019 showed that *M.bovis* causes 9.7% of TB cases in humans worldwide, the rates varying between 0.4% and 76.7% in different countries [16]. The reported cases of human TB caused by *M.bovis* or *M.caprae* in the EU for 2022 were 130, 16 of whom died. Compared with the reported cases from the previous year, there was a 13.2% increase, keeping the trend from the recent years [17].

The estimated incidence of *M.bovis/M.caprae* infection in humans in the EU is 0.03 per 100,000 population for bovine TB free countries and 0. 04/100,000 for the member states with outbreaks of bovine TB in herds. There was a rise in the prevalence of bovine TB among herds, affecting 500 more herds than the previous year [17].

Countries in the EU that have reported outbreaks of bovine TB in herds were Bulgaria, Croatia, Cyprus, Greece, Malta, Romania, Ireland, some parts of Spain, Portugal and Italy. There were 29 reported epidemic outbreaks of bovine TB in herds in 10 different regions in Bulgaria for 2015-2018 [18]. The existence of natural reservoirs of the infection in Bulgaria has also been proven [19, 20, 21]. Although our country is not bovine TB free, the species identification inside the *M.tuberculosis* complex is not routinely performed for human isolates in the TB laboratories and the *M.bovis* or *M.caprae* infections could not be distinguished.

The study aimed to reveal the presence of *M.bovis/M. caprae* as an aetiological agent on the territory of Bulgaria.

MATERIALS AND METHODS

Patients, clinical isolates and study period

The study included only clinical isolates, previously

confirmed as *M.tuberculosis* complex either at the peripheral TB laboratories or at the National Reference Laboratory of Tuberculosis (NRL TB). A total of 175 MTBC strains were examined: n=34 isolates from 2022, n=57 - from 2023, n=74 from 2024, and n=10 from 2025 respectively. Each patient was represented by a single strain. No sensitive personal information was gathered or disposed of in this paper.

Identification

Positive cultures on solid (Löwenstein-Jensen) or liquid media (MGIT) were tested for their affiliation to *M.tuberculosis* complex via rapid immunochromatographic test detecting the MPT 64 antigen [22].

DNA was extracted with GenoLyse[®] VER 1.0 [23] according to manufacturer's instructions.

The species identification inside the *M.tuberculosis* complex was performed by GenoType MTBC VER 1.X (Hain Lifescience GmbH – A Bruker Company): a PCR and subsequent reverse hybridization which provides a reliable discrimination of *M.africanum, Bacillus Calmette-Guérin, M.bovis, M.caprae, M.microti.* whereas *M.tuberculosis* and *M.canettii* cannot be differentiated from one another with the test [24]. GenoType MTBC relies on detection of specific for MTBC 23S ribosomal DNA fragment, the polymorphisms *of gyrB* DNA, and the RD1 deletion of *M. bovis* BCG [25, 26, 27].

RESULTS

The 175 MTBC strains enrolled in this study were retrieved mainly from pulmonary specimens (n=170, 97.1% : sputa, bronchoalveolar lavage, or aspirate). The non-pulmonary specimens (n=5, 2.9%) were presented by pleural fluids (n=2), bone biopsy (n=1), and lymph node biopsies (n=2). The male/female ratio was: 2.4:1, the mean age of the patients was 48.6.

Most of the patients were urban residents (71% vs. 29% living in a rural area (). The number of isolates varied between the districts and is displayed in the table below.

Almost all of the cases were identified as *M.tuberculosis/M.canettii* by GenoType MTBC (n=173; 98.85%). A single isolate was found to be



Figure 1. Age structure of TB patients

Region	n	Region	n	Region	n
Sofia - city	29	Pazardzhik	7	Pleven	2
Vratsa	18	Montana	5	Razgrad	2
Pernik	14	Ruse	5	Silistra	2
Vidin	12	Burgas	4	Haskovo	2
Targovishte	12	Gabrovo	4	Blagoevgrad	2
Dobrich	10	Sofia - district	4	V.Tarnovo	1
Varna	9	Kardzhali	3	S.Zagora	1
Shumen	9	Lovech	3	unknown	4
Kyustendil	8	Plovdiv	3		

Table 1. Number of MTBC isolates by region of TB patients' residence

Mycobacterium bovis BCG (n=1; 0.57%) and one was *Mycobacterium bovis* (n=1; 0.57%). *M.africanum, M.microti* or *M.caprae* were not detected.

DISCUSSION

The MTBC isolates enrolled in this study were sent in NRL TB from peripheral laboratories for drug susceptibility testing or resistance confirmation. Although the sample size was not representative given the small number of tested strains and the disproportionate district distribution, the results revealed the necessity of routine species identification within the *M.tuberculosis* complex at the national level.

For the study period only two isolates were sent in NRL TB with a requirement for species identification. One of them was found to be *Mycobacterium bovis* BCG (n=1; 0.57%), isolated from a lymph node of 8-month old infant with disseminated infection.

The *Mycobacterium bovis strain* (n=1; 0.57%) was isolated from a foreign 70-year-old woman with pulmonary TB, who had settled down in a rural region of Bulgaria. The strain was sensitive to the first line anti-TB drugs (streptomycin, isoniazid, rifampicin and ethambutol).

The discrepancies in the results and the estimated

rate of over 0.4% for *M.bovis* could be related to the profile of the tested TB patients: most of them were urban residents and the location of the infection was mainly pulmonary. The estimated prevalence of *M.bovis* in Bulgarian TB patients in a previous study was 0.46% [28].

Interestingly, *M.caprae* was not detected in humans, though the same is awell known causative agent of bovine TB in Bulgarian cattle herds [18].

Expectedly, *M.africanum* was not found in this study mainly because the number of imported TB cases in the country is low [29] and the immigrants from the endemic region are heading to the western part of Europe [30].

Species identification within the MTBC is important for the early detection of epidemiological links of disease transmission from animal to human and vice versa, for limiting the outbreaks and for refining the treatment of affected individuals.

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INSTITUTIONAL APPROACHES TO HEALTH CRISES - A REVIEW OF GLOBAL PRACTICES

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ABSTRACT

The COVID-19 pandemic demonstrated in practice the need for direct collaboration between governing and scientific institutions. Information exchange proved to be the only tool to control the situation before a vaccine is developed and to determine the dynamics of public trust in institutions. Communication, in these terms, is an instrument for legitimizing imposed measures in society and a decisive factor for their political success.

In the context of a health crisis, the role of politicians as communicators should not be underestimated because of the influence they have over the media and public life on the one hand, and because of the existing intersections between public health goals and political interest, on the other. Political response to pandemic situations in different countries is based on established cultural traditions and political priorities, often focusing on social values that unite the majority within the society. Political orientation could influence factors such as perceived risk and behaviour.

Although politicization of anti-epidemic measures in 2020 determined subsequent attitudes toward prevention in different countries, positive outcomes have been seen when political leaders engaged with the people and called for unity. Empathy and personal example, as well as the use of social media,

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Zhivka Getsova, PhD National Centre of Infectious and Parasitic Diseases 26 Yanko Sakazov Blvd, 1504, Sofia, Bulgaria phone: 359 2 944 6999/255 e-mail: getsova@ncipd.org have been reported as good practices. While initially the induction of fear stimulated compliance, in the long term it has been viewed as harmful and as a source of distrust.

Key words: pandemic communication, emergency, information exchange, political engagement

INTRODUCTION

The COVID-19 pandemic demonstrated in practice the need for direct collaboration between governing and scientific institutions. It also highlighted the barriers to achieving effective synergy between them [1, 2]. Despite the scientific evidence for the possibility of sustained transmission of the new coronavirus and control of incidence through anti-epidemic measures [3], the imposition of such measures was delayed for political reasons and has been costly to society [4]. Given the lack of knowledge in the first weeks of the public health emergency, consensus within the scientific community was difficult to achieve [5] which increased the perceived instability in management approaches to meet the challenge. The pandemic presented the challenge to decision-makers to make decisions "blindly" as there was a lack of hard data on the characteristics of the threat facing the world [6, 7].

The aim of this paper is to review the existing scientific literature for examples of communication practices during health crises occurring within the past 20 years and to compare scientific perceptions on their degree of success.

Potential of communication practices

According to Boin and his colleagues, policymakers faced four aspects of the COVID crisis: understanding the crisis (1), addressing emerging issues (2), communicating risk (3), and developing exit strategies (4) [6]. All of these aspects required prior exchange with technical experts and formal statement of an adopted position and actions to the public. Communicating consistent, coherent and transparent messages is one of the basic principles of communication, and different institutions should coordinate their campaigns [8, 9, 10, 11, 12, 13]. Given the unknowns and uncertainties that characterized the pandemic period, adherence to this standard for success proved difficult [14].

Analysts acknowledge the delicate role of communication as a tool for legitimizing imposed measures in society and as a decisive factor for their political success [6, 15, 16]. Information exchange is the only tool to control the situation before a vaccine is developed [17] and determines the dynamics of public trust in institutions [9]. The design and implementation of communication is based on the management of the acquired knowledge and the reactions of the organizations or individuals involved [18]. Effective information exchange requires structural transformation and recognition of the need for communication by the institutions themselves [19]. Communication units need to have trained staff who understand the principles of social marketing and use social networks not only as a tool for disseminating messages but also as means of tracking social climate (social listening practices) [19]. Conducting pilot tests on the effectiveness and perception of the messages of the information campaign is a must [20].

Inclusion of policymakers: confirming the state of emergency and ensuring funding

According to WHO's expert position, risk communication should be led simultaneously and coordinated by all government sectors with support from partners from external organisations at local and international levels [21]. In the context of a health crisis, the role of politicians as communicators should not be underestimated because of the influence they have over the media and public life on the one hand, and because of the existing intersections between public health goals and political interest, on the other [22, 23, 24].

According to observations from the recent crises, the political response to pandemic situations in different countries is based on established cultural traditions and political priorities, often focusing on social values that unite the majority within the society [7]. Studies have shown that political orientation could influence factors such as perceived risk and behaviour. The authors also note that the politicization of antiepidemic measures determined subsequent attitudes toward prevention among liberals and conservatives in the United States [25]. These observations follow logically from the finding that the behaviour of US politicians from the Republican and the Democratic camps during the pandemic built on the antagonism of political formations found on other issues [26, 27]. It is notable that while persistent attitudes were a leading motive for vaccine hesitancy among Republicans, Democrats were more likely to fall victim to misinformation and, as a consequence, refuse vaccines [28]. Similar to the situation in the United States, Brazil and Mexico also showed a negative correlation between the territorial distribution of the electorate of the two presidents and adherence to anti-epidemic measures [29, 30]. These data suggests that different strategies could be successful with groups of different political orientations given differences in the source of hesitancy, necessitating specific approaches to audience segmentation and targeting.

Sociological studies on risk communication during the COVID pandemic have shown that, in parallel with the presence of governance figures, the inclusion of experts as individuals in communication with the public is crucial for the acceptance of social isolation, but remains irrelevant with regard to socially less severe counter-epidemic measures [31]. Involvement of experts brings significant benefits for timely prevention especially in the initial stage of spread of infectious pathogens when political mobilization is delayed [32]. Collaboration between public health and policy makers is largely shaped by the policy positions of the government. During the COVID pandemic, government support for health promotion was determined by the attitudes of those in power [22]. Political will and the ability to provide social and economic support to the population, as resorted to by European leaders [33], as well as the provision of resources for health care and research [7], have a direct role in dealing with the emergency. Political willingness to support the community is part of the communication signals to the public with different support tools having different weight given cultural understandings in different countries. The effectiveness of an overall communication strategy in the context of an epidemic emergency is seen as the result of two elements: political advocacy for the need for funding and measures, and evidence-based risk communication [22].

Observations and analyses on the conduct

of vaccination campaigns on the occasion of COVID-19 have found that, in an emergency setting, communication on the topic of COVID vaccines has undergone some transformation over time [14]. Given the aims of their study on the implications of the change in vaccination policy, Vernon-Wilson and her colleagues found three key moments in the vaccination campaign in terms of justifying the need for vaccine uptake: to protect vulnerable groups (1), to achieve collective immunity (2), and following the emergence of scientific evidence on the perishable nature of immunity (3) [14]. In the pandemic setting, the COVID-19 vaccination campaign emphasized the need for collective vaccine uptake to achieve collective immunity and presented immunoprophylaxis as a condition for a return to "normal" [34, 35].

Practices in action - in search of the unifying element

Voluntary prevention is essential to avoid simulation of compliance, which only masks the risk of virus spread. In this sense, tools other than fear of punishment must be sought. A retrospective study conducted in Japan demonstrates that a large proportion of people behaved responsibly by cancelling trips and gatherings at the onset of the pandemic, even before the state of emergency was declared in the country [36]. Their behaviour was most likely influenced by the WHO statements and the Japanese authorities' decision to close schools and was not a product of obligation. The data collected by the researchers show that recommendations from the government are most highly valued in shaping behavior, followed by each individual's own perceptions [36]. Of course, one must keep in mind that subjective worldviews are a product of variables such as age, residence, gender, political persuasion, and others.

Government-supported public services are a reliable communication channel to reach the population. According to Betsch and her colleagues, countries need communication tools that meet 5 basic criteria - be evidence-based (1), offer rapid (2) and periodic relevance (3), be simple and with the option to adapt to changing situations (4), and come at a low cost (5) [37]. An analysis of information flow regarding the COVID pandemic in the United States indicates that despite the few reports of the benefits of protective mask use, the public sector covered the essential components of disease prevention [38]. The study team noted the benefits of engaging individuals from the administration who enjoy high levels of trust among the population. Despite implementing the good practice, an opportunity for scientific justification of the recommendations made was still missed [38].

Similarly, institutions could also directly influence external information content, as the use of press releases is a common journalistic practice especially in the health sector. Press releases provide controlled access to information delivered in the way the communicator wants [39]. Press releases allow government entities to maintain their role as the main primary source of information in the media. According to various studies, representatives of state institutions are used as sources in newsrooms in between 75 and 89.1% of the examples included in the studies [14, 18, 19, 40, 41, 42, 43, 44, 45]. Another study published data from the United States concluding that, at least in state-specific social science, government representatives are among the most trusted sources of information [46].

Another example of success in conducting risk communication by crisis managers to the public is the use of social media as a means to reach audiences and in particular to rebuild lost trust through the possibility of two-way information exchange [47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64]. Research in this direction has shown that maintaining institutional accounts has mainly 3 objectives: increasing the transparency of activities of public importance, encouraging citizen participation, and developing collaboration at different levels [65, 66, 67]. The possibility of using hashtags facilitates interaction with the audience and stimulates participation in the communication process by the audience [68]. One of the studies reviewed highlights as a recommendation that institutional interaction with the public via social media should be done through text messages, which during the pandemic reported greater user engagement as opposed to those accompanied by photos or videos [53]. These observations reported by different research teams [49, 53, 69] are surprising given the multimedia communication environment and the principles of easier content uptake in this type of information

channels, which according to other research remain valid in non-crisis periods [52, 70, 71, 72, 73].

Despite the reported benefits of text messages, practice shows that in some cases, such as in Canada, for example, social media content replicated messages from official institutional websites by making institutional accounts formal [74, 75, 76, 77, 78, 79, 80]. Other negative practices are the delay in information submission due to the continuous activity of other communicators, and the failure of institutions to contact citizens directly [51, 81].

During the pandemic, however, some leaders used social media not just as a channel to reach citizens, but also as a tool to reinforce a sense of belonging and cohesion among individuals. Jacinda Ardern, who was the acting Prime Minister of New Zealand during the pandemic, engaged the public in a dialogue about the crisis through podcasts and livestreaming positions via the Facebook platform [82]. Seeking contact with the public from her position as a leader is an approach with proven benefit given the build-up of nervous anticipation in the public space [83]. In this case, Prime Minister Ardern's presence had a therapeutic effect in the context of a turbulent situation and helped her maintain her credibility [84]. A similar strategy was generally pursued by political leaders present in social media, with a gradual softening in the tone of infection alerts (with a peak of 62% of all government Tweeter posts by early March 2020). In early May, the reassuring tone permanently prevailed [57]. While Rao's team found that government entities were much more careful with alarmist messages in contrast to nongovernment Tweeter accounts, which even as of May 2020 had a lower percentage of reassuring messages [57], Xie and his colleagues concluded that positive messages from institutions and citizens on another social platform, Weibo, predominated [58]. In economics, there was no statistically significant difference between stressful messages and reassuring messages [57]. However, one of the studies reviewed provides evidence that negative attitudes towards the pandemic in messages shared by the US President at that time increased interest in the content he shared [26].

Indeed, creating emotional connection with the audience is a factor in communication and determines

the receptivity and subsequent life of a message online. However, neutrality in the style of messages addressed by political leaders to the community was prevalent compared to emotionally charged messages during the pandemic [56]. Research has shown that positive attitudes of the communicator influence his or her social image in a good way and are positively associated with the recipient's willingness to share the information [85, 86, 87]. Studies have reported that in the context of an epidemic situation, with their online behavior (comments, shares, and likes), users value health advice, new information, and accountability from institutions to address the problem as a personal priority [53, 68, 85, 88].

Research of communication conducted by state leaders have shown that the inclusion of a call to concrete action is most often present in public speeches -86.81% of Australian head of state appearances include such a component [89, 90]. Demonstrating empathy, solidarity and personal commitment are other common elements in communicating with the public. The demonstration of personal commitment to contribute to a positive outcome of the crisis by leaders is seen as a crucial element in gaining public trust. An example is Obama's decision in 2010 to immunize his daughters against swine flu [46]. A wellestablished approach to creating positive attitudes toward vaccines recommends emphasizing benefits and focusing dialogue with the public on the priority of "being protected" [91].

Various sources point to Ardren's overall management of the health crisis as a success due to her ability to lead people. Her first statement did not discuss specific measures, but the personal responsibility of each individual [89]. That approach materializes the principle of agency in risk communication messages of the CERC (Crisis and Emergency Risk Communication) model [8, 92]. Placing the community at the centre (the 'team of 5 million' concept [93]), her approach was to connect with people's experiences, calm anxiety and urge citizens not to leave their homes to disrupt transmission [89, 93]. Observations have shown that the call to specific action in the face of crisis is the most frequently used instrument by political leaders, with the best results in influencing public behavior reported when combined with intimacy in communication style and sharing details

of the leader's personal life [56]. Ardern used empathy as a tool to increase acceptance of health recommendations and interventions among the public. Her behaviour united on the basis of shared values, an important condition for cooperation from the public [12, 13, 94, 95, 96], and inspired confidence without being conflated with feelings of pity and sympathy, emotions associated with helplessness and inaction [97]. Analysts have also appreciated the benefits of this approach in Poland, where the leitmotif of local government messaging was that the community would face the challenge together [49, 98].

Achieving unity is considered with no alternative for social recovery during crises. Dividing the community even on objective grounds such as health status (risk groups) in the context of a reported risk of infection in public statements is deemed troublesome due to negative connotations of difference and inferiority of the disabled and elderly population [99, 100].

Another useful practice reported in the scientific literature to amplify social trust and social support for measures points to the references to European instruments to deal with the crisis, inserted as an integral part of institutional communication [101]. Lerouge et al. explain the negative correlation between levels of fear in society and information saturation about the activities of European structures with the Union's financial mechanisms to deal with the consequences of the crisis.

Fear as an alternative measure, sanctions and coercion - a radical move of the authorities

"The masses have never thirsted after truth. They turn aside from evidence that is not to their taste, preferring to deify error, if error seduce them. Whoever can supply them with illusions is easily their master; whoever attempts to destroy their illusions is always their victim." - Gustave Le Bon, [102].

The constant maintenance of a certain amount of fear in the masses is an instrument which guarantees the maintenance of order [103]. An analysis of the content and communication discourse of political leaders' Tweeter posts reveals that a significant percentage of their posts have a negative emotional charge - over 21% of all posts made by leaders of the United States, Australia, and Canada [56]. However,

according to the same study, positive messages regarding the state of emergency caused by COVID-19 dominated the content of their accounts, with the highest proportions reported in Singapore and Ireland [56]. Positive communication enhances social cohesion between individuals by mitigating the fear of uncertainty [103]. Despite the benefits of positively charged communication cited in the previous section, and its use during the pandemic, the induction of fear by political leaders is a quick way to induce change in human behavior [104]. The reason for success when using fear as a driver to achieve a specific goal has been linked to perceptions of the magnitude of danger under the influence of emotion [104, 105]. Because of this feature, during epidemic situations, pessimistic forecasts awaken people's instinct for self-preservation and are used more frequently at the onset of the emergency [106], when the unknown is of the greatest magnitude for both the public and political leaders.

However, according to Bavel and his colleagues, when coping mechanisms are not available in human capital, such psychological manipulations trigger a defensive response in the individual who feels helpless [107]. Results from an older study on the theory of behavioral change through fear induction indicated that message persuasiveness increases when stronger fear is induced in the recipient, but the motivational effect depends on the accompanying signal of an effective success mechanism [108, 109]. Despite theories about the inevitability of using fear instrumentation in the service of the public interest, more modern authors associate this approach with immaturity and an inability to effectively lead the public [110]. According to researchers in the field of crisis management, the main role of institutions and state authorities is to balance between warnings of danger and assurance of a successful outcome of an adverse situation [111], while controlling the level of fear in society [112].

A study found that Médecins Sans Frontières' behaviour on Instagram during the Ebola outbreak in Africa was an example of good practice as it combined information about the severity of the situation but also offered an alternative to reduce the impact on the individual [113]. During the COVID-19 pandemic, theorists and practitioners from the fields

Vol.53, 2025, 1

of medicine, social sciences, and communications came to a consensus that counteracting to the overwhelming panic in society was no alternative to establishing trust and a two-way exchange of information between governmental structures and society to manage the crisis in the long term [7, 12, 13, 114, 115, 116]. Data on the spike in the number of mental health problems triggered at the onset of the pandemic also confirmed the counterproductive role of fear [117]. However, Sandman argues that in a crisis, fear is a necessary element without which society would neglect the problem. Nevertheless, he elaborates that the presence of fear necessitates a communication style that indicates a direction for coping and eliminates doubt about recovery [118]. Without such support from governing structures, the result of fear-mongering is negatively perceived, as it fuels a sense of panic or leads to denial. Experience shows that uncontrolled fear is unconstructive and threatens established order and social norms [119, 120].

The announcement of a pandemic caused by a previously unknown virus is itself a stressor with researchersfindingthatanunmetneedforinformation may exacerbate feelings of fear and weightlessness [121, 122, 123, 124]. In this sense, inducing additional fear to stimulate responsible behavior among citizens seems unwarranted and unhealthy. According to Hugelius et al. communication through the media has the task of helping the community to overcome the crisis by substantiated influence in the direction of stress reduction [125].

CONCLUSION

The COVID pandemic reminded policy makers that public health is an interdisciplinary field and requires cross-sectorial cooperation. At the face of a health crisis political leaders, technical experts and scientists, all have their role in responding to the adverse situation. Communication should be evidence-based and a product of careful planning. Prior exchange between all stakeholders is required for coherence and to meet the needs depending on the social climate.

The mission of policy-makers is to provide reassurance and feeling of unity among the citizens they represent. Although fear-mongering might have a short-term beneficial effect, in the long run, it creates distrust. Therefore, political leaders should not resort to such instruments but use their image to motivate individuals to contribute to public health.

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