




Review

Challenges in Elucidating HIV-1 Genetic Diversity in the Middle East and North Africa: A Review Based on a Systematic Search

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Abstract: The extensive genetic diversity of HIV-1 represents a major challenge to public health interventions, treatment, and successful vaccine design. This challenge is particularly pronounced in the Middle East and North Africa (MENA) region, where limited data among other barriers preclude the accurate characterization of HIV-1 genetic diversity. The objective of this review was to analyze studies conducted in the MENA region to delineate possible barriers that would hinder the accurate depiction of HIV-1 genetic diversity in this region. A systematic search of PubMed/MEDLINE and Google Scholar was conducted for published records on HIV-1 genetic diversity in the English language up until 1 October 2024 across 18 MENA countries. The pre-defined themes of challenges/barriers included limited sampling, data gaps, resource and infrastructure constraints, HIV-1-specific factors, and socio-cultural barriers. A total of 38 records were included in the final review, comprising original articles (55.3%), reviews (21.1%), and sequence notes (10.5%). Libya (15.8%), Morocco (13.2%), Saudi Arabia, and MENA as a whole (10.5% for each) were the primary sources of the included records. Of the 23 records with original MENA HIV-1 sequences, the median number of sequences was 46 (range: 6–193). The identified barriers included the following: (1) low sampling density; (2) limited clinical data (21.7% with no data, 60.9% partial data, and 17.4% with full data); (3) reliance solely on population sequencing and insufficient use of advanced sequencing technologies; (4) lack of comprehensive recombination analysis; and (5) socio-cultural barriers, including stigma with subsequent under-reporting among at-risk groups. The barriers identified in this review can hinder the ability to map the genetic diversity of HIV-1 in the MENA. Poor characterization of HIV-1's genetic diversity in the MENA would hinder efforts to optimize prevention strategies, monitor drug resistance, and develop MENA-specific treatment protocols. To overcome

these challenges, investment in public health/research infrastructure, policy reforms to reduce stigma, and strengthened regional collaboration are recommended.

Keywords: molecular epidemiology; phylogenetics; under-reporting; AIDS; HIV infection

1. Introduction

Human immunodeficiency viruses (HIVs) are rapidly evolving viruses characterized by extensive genetic diversity [1–3]. The extensive diversity of these retroviruses is reflected in their classification into two types—HIV-1 and HIV-2—with four major groups for HIV-1: M, N, O, and P [4]. Group M (major) is responsible for the vast majority of HIV-1 infections globally and is further divided into several subtypes (A, B, C, D, F, G, H, J, and K), numerous circulating recombinant forms (CRFs), and a countless number of unique recombinant forms (URFs) [5–9].

The global distribution of HIV-1 group M subtypes/CRFs is shaped by historical factors and contemporary transmission dynamics, including the region-specific distribution of HIV-1 genetic variants [3,4,10,11]. For example, the subtype C of HIV-1, which accounts for over half of global HIV-1 infections, predominates in sub-Saharan Africa (SSA) and India, whereas subtype B is more common in Europe, North America, and certain regions of South America [3,6,12,13]. In Southeast Asia, CRF01_AE is widespread, with evidence indicating the role of sex tourism in its global spread [14,15]. In Russia and former Soviet Union countries, subtype A predominates [3,16], while in West and Central Africa, a mixture of HIV-1 and HIV-2 subtypes circulate, with a predominance of CRF02_AG [3,17,18].

The genetic diversity of HIV-1 is the result of its high evolutionary rate, driven by a high substitution rate and the frequent occurrence of recombination [1,19,20]. The implications of extensive HIV-1 genetic diversity are profound, since it contributes to virus virulence, disease progression, and the development of antiretroviral (ARV) drug resistance [19,21–25]. Specifically, variability in the viral envelope (*env*) gene that encodes the HIV surface glycoproteins gp120 and gp41 is a major factor in humoral immune response evasion [26,27]. Additionally, the swift evolution in the reverse transcriptase, protease, and integrase proteins—the primary targets of ARV drugs—can lead to ARV drug resistance, which would complicate the successful management of HIV/AIDS [25,28].

The Middle East and North Africa (MENA) region has a complex HIV-1 epidemic [29]. The HIV-1 epidemic in the MENA is characterized by diverse transmission patterns and the complex diversity of HIV-1 variants, driven by unique socio-cultural factors in these regions [29–32]. However, the accurate mapping of the MENA HIV-1 epidemic is complicated by limited epidemiological and virus sequence data [29–34]. Historically, the MENA has been considered a region with a relatively low prevalence of HIV/AIDS [34,35]. Current estimates suggest that the overall adult HIV prevalence remains below 0.1% in the MENA region; however, certain countries and sub-populations exhibit much higher HIV prevalence rates [36–39]. Notably, the UNAIDS reported an increase in new HIV infections in the MENA of 116% between 2010 and 2023 [40]. Additionally, recent evidence suggested an accompanying rising trend in HIV-1 prevalence in certain sub-populations [41]. In particular, this trend has been reported among most-at-risk groups, such as men who have sex with men (MSM), injection drug users (IDUs), and female sex workers (FSWs) [42].

Regarding the genetic diversity of HIV-1 in the MENA region, a heterogeneous pattern of distribution of HIV-1 genetic variants was reported based on the limited sequence data from this region [31,32]. This complex pattern of circulation of a diverse range of HIV-1 genetic variants in the MENA likely reflects the region's geographical location at the crossroads of Asia,

Africa, and Europe, with frequent population movement [12,32,43]. Therefore, a comprehensive understanding of the genetic diversity of HIV-1 in the MENA region is an important aspect of the regional response to tailor intervention and management measures [32,44,45].

The genetic characterization of HIV-1 through phylogeny-based studies provides valuable insights into the origins and spread of HIV-1, allowing the identification of networks of HIV-1 transmission and enabling the tracking of cross-border transmissions [32,46–48]. Thus, phylogenetic inference methods can elucidate the role of travel and migration in shaping local HIV-1 epidemics [49–52]. In the MENA region, phylogenetic analysis revealed a considerable proportion of domestic spread of HIV-1, contrary to previous notions [32]. The utility of genetic data analysis results in understanding HIV-1 transmission dynamics extends to involve the informing of targeted interventions [53]. However, the lack of systematic and comprehensive HIV-1 genetic surveillance across MENA countries hinders the ability to map region-specific HIV-1 dynamics comprehensively [54].

In addition to HIV-1 transmission dynamics studies, the genetic characterization of HIV-1 is important to address the challenges of ARV drug resistance [55–58]. The emergence of drug-resistant mutations poses a significant threat to the success of ARV therapy, particularly in regions with limited access to second-line treatment options [59,60]. In the MENA region, preliminary studies showed a range of resistance-associated mutations (RASs), albeit with varying prevalence across countries [61–66]. The variability in ARV drugs' resistance patterns in the MENA highlights the continuous need for region-specific genetic HIV-1 data to guide the selection of first- and second-line ARV drug regimens for the successful management of patients in this region [67].

Regarding the epidemiology of HIV-1 in the MENA, previous studies revealed country-specific patterns: in high-income countries of the Gulf Cooperation Council (GCC), such as Saudi Arabia and the United Arab Emirates, HIV-1 cases have historically been lower, largely due to strict regulations and religious/cultural norms which have traditionally discouraged or legally penalized high-risk behaviors [38,68]. By comparison, North African countries, such as Morocco, Libya, Tunisia, and Egypt, have been characterized by concentrated epidemics among at-risk groups [29,69–72]. In Sudan, perinatal HIV-1 transmission, especially in conflict-affected areas with limited access to healthcare, has been reported [73].

Despite increasing insights through cumulative HIV-1 research in the MENA, the overall picture of HIV-1's genetic diversity in the MENA region remains fragmented. Challenges to the accurate depiction of HIV-1's genetic diversity in the MENA are multifactorial and include the limited number of sequence data, variable clinical and epidemiological data quality, and poor regional coordination in surveillance efforts [29,32,33,68,74].

On a related note, efforts to map the genetic diversity of HIV-1 in the MENA region are hindered by additional challenges, including political instability, limited resources, and social stigma associated with HIV-1 infection [29,75,76]. These factors contribute to the deficiency in robust HIV-1 surveillance, with subsequent gaps in the knowledge regarding the true scale of the HIV-1 epidemic in the MENA region, including its genetic diversity. Additionally, the MENA region is characterized by substantial heterogeneity in terms of health infrastructure and response capacity, ranging from high-income countries with advanced health systems to conflict-affected areas where healthcare access is severely limited [77–80].

The challenges that may negatively impact the elucidation of HIV-1's genetic diversity in the MENA region necessitate deeper understanding through a focused review of the literature. Thus, the current review aimed to identify and describe the specific obstacles that could hinder HIV-1 genetic characterization efforts in the MENA. This description could help people propose actionable strategies to overcome these barriers. By identifying these barriers, we aimed to advance the level of understanding of HIV-1 challenges in

the MENA, enhance regional and global public health responses, and contribute to global efforts to reduce the burden of HIV/AIDS.

2. Materials and Methods

2.1. Review Design

This review was designed to comprehensively analyze the challenges in elucidating HIV-1's genetic diversity within the MENA region. To achieve this, we conducted a systematic search of PubMed/MEDLINE and Google Scholar for records published in English which focused on HIV-1's genetic diversity across 18 MENA countries, including the United Arab Emirates (UAE), Qatar, Bahrain, Oman, Saudi Arabia, Kuwait, Yemen, Iraq, Syria, Lebanon, Palestine, Jordan, Egypt, Sudan, Libya, Tunisia, Algeria, and Morocco.

These 18 countries were selected based on their geographic and cultural inclusion in the MENA region, as well as their shared epidemiological characteristics, which justify a collective assessment. Conversely, we excluded Türkiye, Iran, Israel, Pakistan, Afghanistan, Djibouti, Somalia, South Sudan, and Mauritania, as previous studies have demonstrated distinct epidemic patterns in these countries which differ significantly from the broader MENA region and would therefore fall outside the scope of this review [81–92].

2.2. Search Strategy

The literature search was conducted by the first author (Malik Sallam) on 1 October 2024. The inclusion criteria included the following: (1) published records; (2) the language of publication being English; and (3) any type of publication (original article, review, sequence note, short communication, book, and book chapter).

The exact PubMed/MEDLINE search strategy was ((HIV) AND (“Middle East” OR “North Africa” OR “MENA” OR “Arab” OR “United Arab Emirates” OR “UAE” OR “Saudi Arabia” OR “Qatar” OR “Bahrain” OR “Oman” OR “Yemen” OR “Kuwait” OR “Iraq” OR “Syria” OR “Palestine” OR “Jordan” OR “Egypt” OR “Sudan” OR “Libya” OR “Algeria” OR “Tunisia” OR “Morocco”)) AND (“genetic diversity” OR “molecular epidemiology”).

The Google Scholar search was conducted in Publish or Perish (Version 8) [93]. The exact search strategy was based on the keyword function with 10 hits: HIV molecular epidemiology “Country Name” and HIV genetic diversity “Country Name”.

2.3. Themes to Be Extracted from Included Records

To identify the key challenges associated with HIV-1 genetic diversity research in the MENA region, a structured approach was used to establish a priori themes to be extracted from the included records. These themes were developed through a combination of a literature review followed by consensus between the first and senior authors based on these cited publications [94–114]. The process was further informed by barriers previously identified in the first author's PhD thesis, “Phylogenetic Inference in the Epidemiologic and Evolutionary Investigation of HIV-1, HCV, and HBV,” which included an analysis of MENA HIV-1 sequences from the Los Alamos HIV Sequence Database [115,116].

The first theme identified was limited sampling and gaps in clinical and epidemiology data. This included insufficient sampling, poor representation of HIV-1 sequences, low HIV-1 sequencing density, and reliance on non-representative cross-sectional studies. Additionally, this theme included gaps in clinical and epidemiologic data, such as the sex of the patients, age, risk factors for HIV-1 acquisition, viral load, etc. These issues were deemed deficiencies in the genetic analysis approach to delineate HIV-1's genetic diversity.

Four common themes were identified, which are summarized in Figure 1.

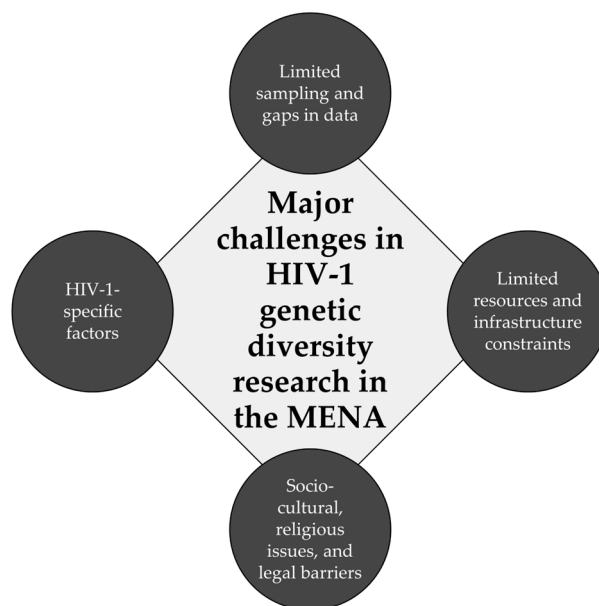


Figure 1. Major challenges in HIV-1 genetic diversity research in the Middle East and North Africa (MENA) region.

The second theme identified was limited resources and infrastructure constraints. This issue highlighted the reliance on older HIV-1 genetic sequencing methods, such as Sanger sequencing, and the limited use of advanced tools like next-generation sequencing (NGS). Restricted research funding and technical capacity were also included in this theme, since these issues were considered as hindrances to progress in HIV-1 genetic diversity research.

The third identified theme was HIV-1-specific factors, which addressed the genetic complexity of HIV-1 due to high rates of mutation and recombination. The lack of full-genome sequencing and robust HIV-1 isolates' recombination analysis can limit accurate subtyping and the characterization of CRFs/URFs and, thus, was deemed as a barrier to HIV-1's genetic characterization.

Finally, the fourth theme identified was the socio-cultural and religious issues and legal barriers. Stigma, cultural taboos, and punitive laws can hinder access to HIV testing and care for key populations, such as MSM and IDUs. These barriers can cause under-reporting and significant data gaps in the investigation of HIV-1 genetic diversity.

2.4. Visualization of HIV-1 Genetic Diversity in the MENA Region

All publicly available HIV-1 sequences annotated as originating from MENA countries were systematically retrieved from the Los Alamos HIV Database (<http://www.hiv.lanl.gov/>; accessed on 10 February 2025) using the advanced search tool [116–118]. Data on HIV-1 subtype/CRF/URF classifications, along with country of origin, were extracted and utilized to construct a comprehensive geographic distribution map of HIV-1 genetic diversity across the MENA region.

3. Results

3.1. Description of the Included Records

A total of 38 records were deemed eligible for inclusion in this review, as shown in Figure 2.

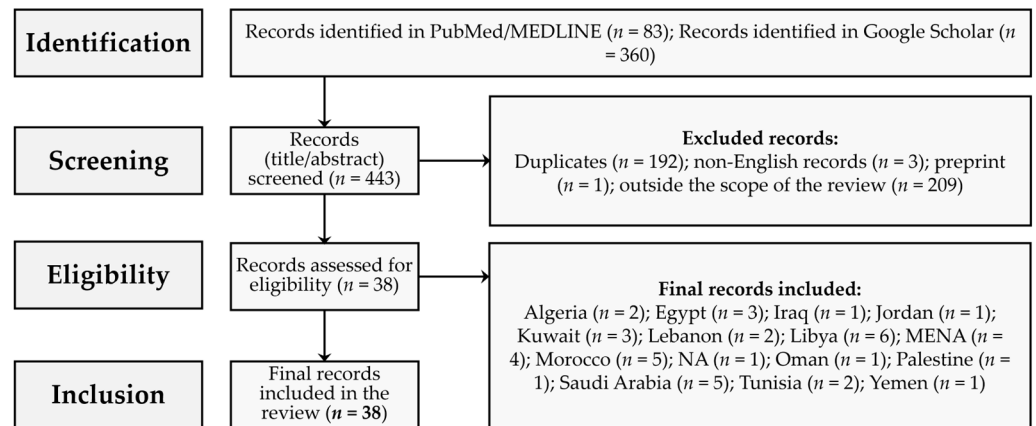


Figure 2. Flow diagram of the record selection process for the review of HIV-1 genetic diversity research challenges in the Middle East and North Africa (MENA) region.

These records were mostly original articles (21/38 (55.3%)), followed by reviews, including systematic reviews (8/38 (21.1%)), and sequence notes/dispatches (4/38 (10.5%)). The most common source location for the included records was Libya (6/38 (15.8%)), followed by Morocco (5/38 (13.2%)), Saudi Arabia and the MENA as a whole (4/38 (10.5%) for both), and Egypt and Kuwait (3/38 (7.9%) for both) (Figure 3 and Table 1).

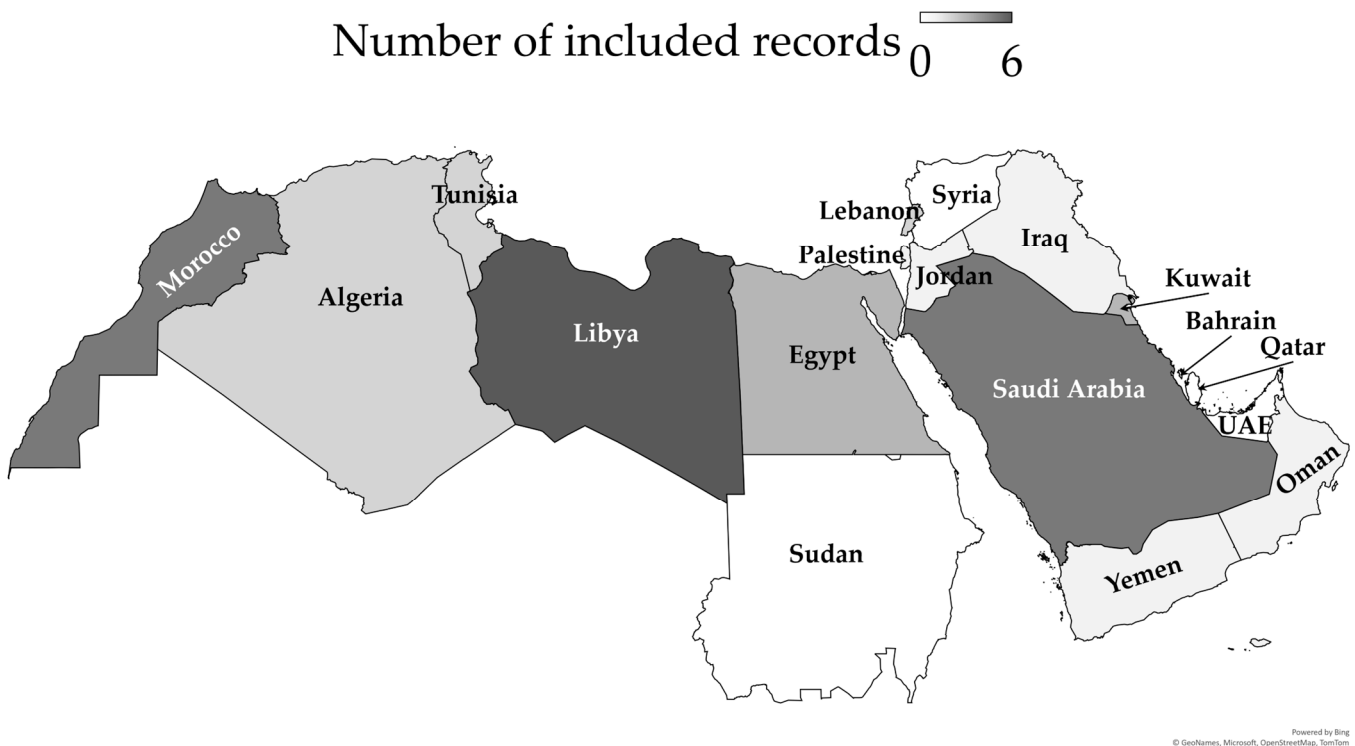


Figure 3. Geographical distribution of the included records on HIV-1 genetic diversity challenges in the MENA region. The map was generated in Microsoft Excel, powered by Bing, © GeoNames, Microsoft, Navinfo, TomTom, and Wikipedia. We are neutral with regard to any jurisdictional claims in this map.

Table 1. Summary of the included records ($n = 38$).

Author(s), Year	Country/Region	Type of Article
Pieniasek et al., 1998 [119]	Lebanon	Dispatch ²
El Sayed et al., 2000 [120]	Egypt	Original article
Ben Halima et al., 2001 [121]	Tunisia	Brief report
Elharti et al., 2002 [122]	Morocco	Review
Saad et al., 2005 [123]	Yemen	Sequence note
Bouzeghoub et al., 2006 [124]	Algeria	Sequence note
de Oliveira et al., 2006 [125]	Libya	Brief communication
Badreddine et al., 2007 [126]	Saudi Arabia	Original article
Bagasra et al., 2007 [127]	Libya	Reply
Yamaguchi et al., 2008 [128]	Saudi Arabia	Other
Mumtaz et al., 2010 [72]	MENA ¹	Systematic review
Mumtaz & Abu-Raddad, 2011 [129]	MENA	Review
Mumtaz et al., 2011 [30]	MENA	Systematic review
Akrim et al., 2012 [70]	Morocco	Original article
Kouyoumjian et al., 2013 [130]	Morocco	Systematic review
Mumtaz et al., 2013 [131]	Morocco	Original article
Mokhbat et al., 2014 [132]	Lebanon	Original article
Cehadeh et al., 2015 [62]	Kuwait	Original article
Abdellaziz et al., 2016 [133]	Algeria	Sequence note
Cehadeh et al., 2017 [63]	Kuwait	Original article
Daw et al., 2017 [134]	Libya	Original article
El Moussi et al., 2017 [135]	Tunisia	Original article
Sallam et al., 2017 [32]	MENA	Original article
Cehadeh et al., 2018 [64]	Kuwait	Original article
Khamis et al., 2018 [136]	Oman	Original article
Alaoui et al., 2019 [137]	Morocco	Original article
Daw et al., 2019 [138]	Libya	Original article
Giovanetti et al., 2020 [17]	North Africa	Review
Zaki et al., 2020 [66]	Jazan, Saudi Arabia	Observational study
Hamarsheh, 2020 [139]	Palestine	Short communication
Amer et al., 2021 [140]	Egypt	Original article
Hamidi et al., 2021 [141]	Libya	Review
Al-Qassab & Utba, 2022 [142]	Iraq	Original article
Gaballah et al., 2022 [65]	Egypt	Original article
Al-Mozaini et al., 2023 [68]	Saudi Arabia	Review
Bakri et al., 2024 [143]	Jordan	Original article
El-Daly et al., 2024 [144]	Saudi Arabia	Original article
Shalaka, 2024 [145]	Libya	Original article

¹ MENA: Middle East and North Africa; ² Dispatch: “updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens.” [146].

3.2. Challenge of Limited Sampling and Limited Data

A summary of the included records with HIV-1 sequence and data availability is included in Table 2.

Table 2. MENA records that analyze HIV-1 molecular sequences, sequencing technique, and clinical data availability.

Author(s)	YEAR	Country/Region	Sample Size of HIV-1 Sequences	Clinical Data	Sequencing Technique
El Moussi et al., 2017 [135]	2017	Tunisia	193	Partial data were available	Sanger dideoxy sequencing
Daw et al., 2017 [134]	2017	Libya	159	Partial data were available	Not mentioned
Abdellaziz et al., 2016 [133]	2016	Algeria	152	Data were absent	Sanger dideoxy sequencing
Bouzeghoub et al., 2006 [124]	2006	Algeria	134	Data were absent	Not mentioned
Alaoui et al., 2019 [137]	2019	Morocco	78	Partial data were available	Sanger dideoxy sequencing
Al-Qassab & Utba, 2022 [142]	2022	Iraq	65	Partial data were available	Sanger dideoxy sequencing
Akrim et al., 2012 [70]	2012	Morocco	60	Full data were available	Sanger dideoxy sequencing
Zaki et al., 2020 [66]	2020	Jazan, Saudi Arabia	57	Partial data were available	Sanger dideoxy sequencing
Badreddine et al., 2007 [126]	2007	Saudi Arabia	56	Full data were available	Sanger dideoxy sequencing
El-Daly et al., 2024 [144]	2024	Saudi Arabia	56	Partial data were available	Sanger dideoxy sequencing
Chehadeh et al., 2017 [63]	2017	Kuwait	53	Data were absent	Sanger dideoxy sequencing
Gaballah et al., 2022 [65]	2022	Alexandria, Egypt	45	Data were absent	Sanger dideoxy sequencing
de Oliveira et al., 2006 [125]	2006	Libya	44	Partial data were available	Sanger dideoxy sequencing
Chehadeh et al., 2015 [62]	2015	Kuwait	43	Partial data were available	Sanger dideoxy sequencing
Bakri et al., 2024 [143]	2024	Jordan	43	Partial data were available	Next-generation sequencing
Chehadeh et al., 2018 [64]	2018	Kuwait	42	Partial data were available	Sanger dideoxy sequencing
Mokhbat et al., 2014 [132]	2014	Lebanon	37	Partial data were available	Sanger dideoxy sequencing
Pieniazek et al., 1998 [119]	1998	Lebanon	25	Full data were available	Sanger dideoxy sequencing
El Sayed et al., 2000 [120]	2000	Egypt	21	Full data were available	Sanger dideoxy sequencing
Ben Halima et al., 2001 [121]	2001	Tunisia	21	Partial data were available	Sanger dideoxy sequencing
Amer et al., 2021 [140]	2021	Egypt	21	Partial data were available	Sanger dideoxy sequencing
Saad et al., 2005 [123]	2005	Yemen	19	Partial data were available	Sanger dideoxy sequencing
Yamaguchi et al., 2008 [128]	2008	Saudi Arabia	6	Data were absent	Sanger dideoxy sequencing

For the studies where original HIV-1 sequences were originated ($n = 23$), the average number of sequences included was 62 ± 50 (median: 46, IQR: 25–65, range: 6–193). The number of sequences per year is shown in Figure 4, which highlights gaps in sequencing for HIV-1 in the MENA over two stretches of years ((2002–2004) and (2009–2011)).

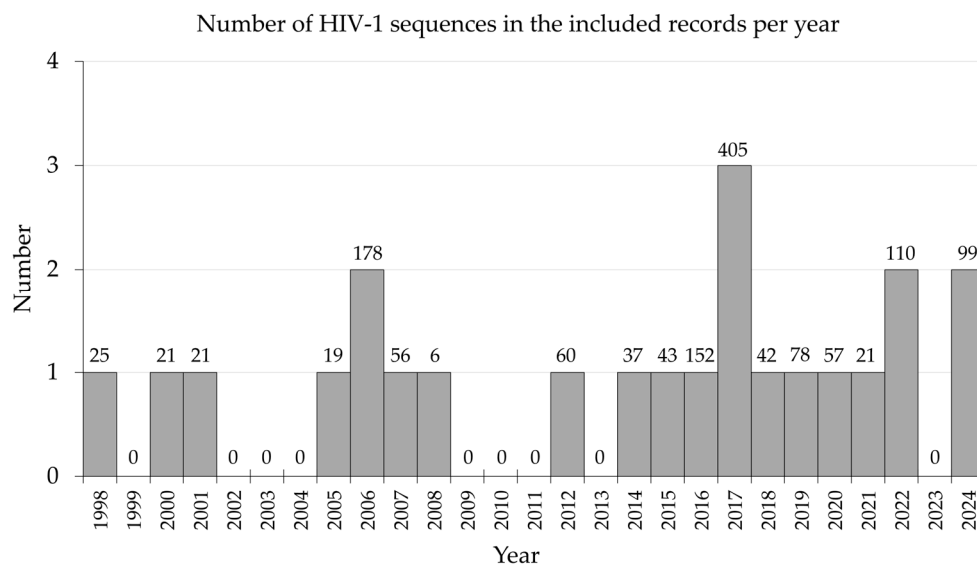


Figure 4. Annual distribution of HIV-1 sequences in the included records in the Middle East and North Africa (MENA) region from 1998 to 2024.

Partial clinical data were available for 14 records (60.9%), and clinical data were totally absent in 5 records (21.7%), while full clinical data were present only in 4 records (17.4%).

In the included records, the following limitations in terms of limited sampling and limited data were identified: In Morocco, a systematic review by Kouyoumjian et al. reported insufficient data on MSM and IDUs, with reliance on convenience sampling and limited behavioral data, often affected by self-report biases [130]. Mathematical model predictions for modes of transmission in Morocco exhibited considerable uncertainty due to inadequate input data [131].

In the sole study which investigated domestic HIV-1 transmission in the MENA region as a single unit, using the phylogenetic maximum likelihood and Bayesian approaches, 8 of the 21 MENA countries lacked HIV-1 sequences, and among the 13 countries with available sequences, only 7 countries had more than 50 unique sequences [32]. Many HIV-1 MENA sequences were derived from non-representative cross-sectional studies with a low sampling density (<1%) [32].

In Libya, discrepancies were noted between government-reported infection rates and independent estimates, as reported by Hamidi et al. [141]. In Saudi Arabia, data quality improved following 2011, but MSM populations remained under-represented, as reported by Al-Mozaini et al. [68]. In Jordan, available data were limited to individuals in HIV care who were willing to participate in research [143].

3.3. Challenge of Limited Resources

The results indicated that HIV-1 surveillance in the MENA region remains limited, with only a small number of cases subtyped, primarily using Sanger sequencing rather than NGS for detecting minor variants, as reported by Mumtaz et al. [30]. Only a single record reported using NGS for HIV-1 typing and resistance testing [143].

Challenges to the accurate depiction of HIV-1's genetic diversity in the MENA linked to limited resources were identified in Libya by Hamidi et al. [141]. In Libya, monitoring relies on mandatory screenings for certificates and hospital-reported cases, resulting in variable accuracy. In Jordan, limited access to HIV testing and care has been identified as a major factor contributing to late diagnoses, with subsequent increases in healthcare costs [143]. Additionally, in Jordan, contributory factors to late HIV diagnosis include limited access to HIV testing and care, with subsequent higher healthcare costs.

On the positive side, Morocco made significant progress in addressing the HIV-1 epidemic and is likely the Arab country with the most advanced HIV-1 surveillance system, research capacity, and response framework, as reported by Mumtaz et al. [131]. In Saudi Arabia, despite the progress made, Al-Mozaini et al. highlighted the need for establishing innovative testing services and improving medicine delivery systems, which could enhance the understanding of HIV-1 genetic diversity [68].

3.4. Challenge Posed by HIV-1-Specific Factors

In the majority of included records, HIV-1 subtyping results varied based on the methods employed, particularly for short sequences, which often lacked the resolution necessary for definitive subtype/CRF/URF assignment [32].

The scarcity of full-genome HIV-1 sequences further complicated these challenges, as short sequences are less reliable for subtyping, particularly to identify recombinant forms. Only a single record reported sequencing full HIV-1 genomes [128]. Several records relied on subtyping tools that may not have accounted for the complexities of recombination, leading to the potential misclassification or incomplete characterization of HIV-1 strains, as highlighted in the study which analyzed HIV-1 sequences in the MENA region as a whole [32]. Based on these challenges, mutation and recombination present significant obstacles to surveillance efforts.

3.5. Socio-Cultural and Legal Issues

Socio-cultural and legal barriers can significantly hinder efforts to characterize HIV-1 genetic diversity in the MENA region, as reported in several included records and in the process shown in Figure 5.

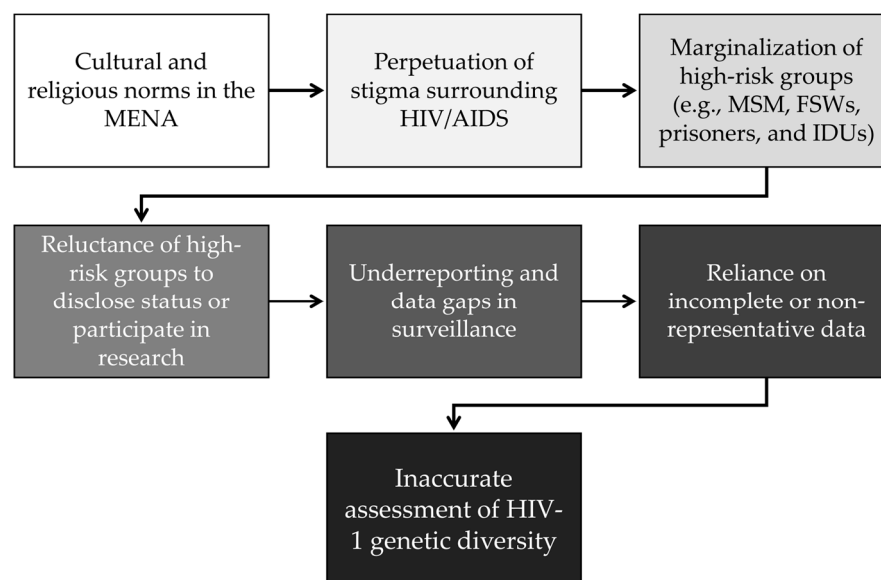


Figure 5. The process of socio-cultural barriers impacting the assessment of HIV-1 genetic diversity in the Middle East and North Africa (MENA) region. MSM: men who have sex with men; FSWs: female sex workers; and IDUs: injection drug users.

Groups of MSM were reported in a few records as stigmatized and hidden HIV risk groups, and these records reported that MSM face substantial obstacles to status disclosure and care [36,72]. Despite increasing epidemiological evidence on HIV-1 and risk behaviors among MSM, stigma and cultural norms in the MENA likely result in the under-reporting of male same-sex transmission in official case notifications [72]. Other high-risk groups, such as truck drivers and prisoners, remain under-represented in research [33,36,42]. In Palestine,

socio-cultural and religious barriers limit the identification of vulnerabilities among high-risk groups, with information gaps persisting in relation to the role of transactional sex in HIV transmission [139]. In Libya, historical policies suppressed accurate HIV data, which were considered as part of “national security”, dismissing scientific evidence and attributing HIV-1 transmission solely to homosexuality [141]. Conspiratorial beliefs, including claims of clandestine HIV vaccine trials by foreign medical staff, have also been reported [127]. High levels of stigma associating HIV/AIDS with immoral behavior lead many individuals to avoid testing to protect personal and family reputations. In the included records, individuals with HIV in the MENA were reported to experience severe social consequences, including family rejection, societal isolation, and restricted access to healthcare, education, and employment [139,141]. Women living with HIV were particularly reported to be vulnerable to violence, humiliation, and invasive practices such as virginity testing [141]. These socio-cultural and legal challenges can impede research efforts to delineate HIV-1 genetic diversity in the MENA through under-reporting and subsequent gaps in data available for analysis.

3.6. HIV-1 Genetic Diversity in the MENA Region

Based on the retrieved HIV-1 sequences available in the Los Alamos HIV Database, a total of 2642 sequences were available, which were assigned to HIV-1 subtype/CRF/URF, from 10 countries: Morocco ($n = 885$), Algeria ($n = 637$), Tunisia ($n = 297$), Saudi Arabia ($n = 226$), Kuwait ($n = 191$), Libya ($n = 148$), Egypt ($n = 97$), Sudan ($n = 81$), Lebanon ($n = 60$), Yemen ($n = 25$), and Iraq ($n = 20$).

The retrieved sequences revealed substantial heterogeneity in HIV-1 genetic diversity across the MENA region. Subtype B was the predominant lineage in Morocco ($n = 607$, 68.6%), Algeria ($n = 304$, 47.7%), Tunisia ($n = 127$, 42.8%), and Yemen ($n = 12$, 48.0%). In Libya, CRF02_AG was exclusively detected, whereas subtype A was exclusively present in Iraq. Subtype C was the most common HIV-1 genetic variant in Saudi Arabia ($n = 100$, 44.2%), while subtype D was the most common variant in Sudan ($n = 37$, 45.7%). CRF01_AE was the most frequently observed HIV-1 genetic variant in Kuwait ($n = 65$, 34.0%). In Lebanon, subtype A accounted for the highest number of sequences ($n = 24$, 40.0%), whereas, in Egypt, CRF02_AG was the dominant form ($n = 42$, 43.3%). Collectively, these results highlight the complex and regionally distinct patterns of HIV-1 genetic diversity across the MENA region, as shown in Figure 6.

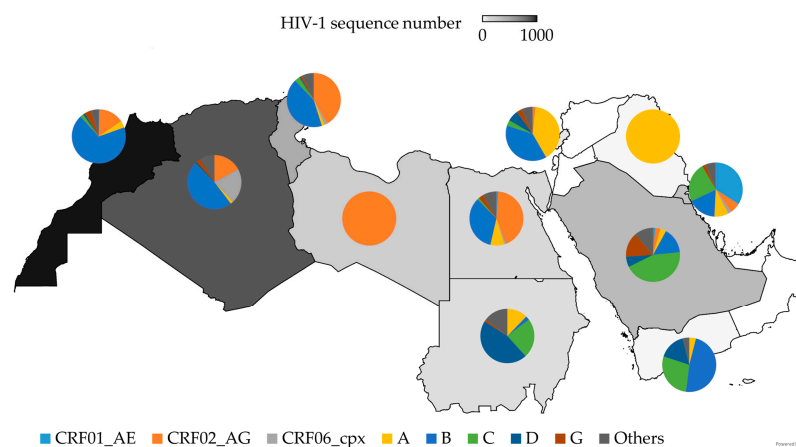


Figure 6. Genetic diversity of HIV-1 in the Middle East and North Africa (MENA) region based on retrieved HIV-1 sequences available in the Los Alamos HIV Database. The map was generated in Microsoft Excel, powered by Bing, © GeoNames, Microsoft, Navinfo, TomTom, and Wikipedia. We are neutral with regard to any jurisdictional claims in this map.

4. Discussion

The identification and investigation of possible challenges that could negatively impact the accurate depiction of HIV-1 genetic diversity in the MENA are highly important. HIV-1 is characterized by a swift evolutionary rate with subsequent extensive genetic diversity [147,148]. This rapid rate of HIV-1 evolution would, in turn, complicate HIV/AIDS management and prevention through the compromised monitoring of ARV drug resistance and negative effects on vaccine design [149,150]. In the MENA region, the HIV-1 epidemic is characterized by distinct features that result from an interplay of unique socio-cultural, legal, and resource-related aspects [30,33,34,36,42]. Thus, it is important to understand the specific barriers and challenges that would compromise the accurate depiction of HIV-1 genetic diversity in the region, which, in turn, would help the development of effective region-specific responses to address the HIV epidemic in the region.

This review identified four key themes that represent barriers to the accurate characterization of HIV-1 genetic diversity in the MENA region. These four themes were limited sampling and data gaps, resource and infrastructure constraints, HIV-specific factors, and socio-cultural and legal issues.

The first challenge of limited sampling and insufficient data can profoundly impact efforts to characterize HIV-1 genetic diversity in the MENA region. The molecular epidemiology of an epidemic, including HIV-1 epidemics, operates at the population level of pathogens [151]. An important issue to be considered upon conducting genetic diversity investigation is the sampling approach, since biased or non-representative sampling will give misleading results [152]. One of the challenging aspects to reconstruct the network of HIV-1 transmission is obtaining a complete sampling density for the population under study given the fraction of undiagnosed HIV-1 infections and ongoing transmissions [107]. In addition, sampling density will affect the subsequent proportion of HIV-1 phylogenetic clustering indicative of the domestic spread of the virus [108,109].

In this review, and across 23 records of HIV-1 sequences, the median number of sequences per record was only 46, with significant gaps in sequencing during two periods, 2002–2004 and 2009–2011. Additionally, as reported in an included record from Morocco, the mathematical models predicting modes of HIV-1 transmission among MSM and IDUs were hampered by insufficient input data and reliance on convenience sampling [131]. Such gaps in data and samples often make it difficult to represent hidden populations, subsequently compromising the reliability of transmission studies [153]. In this review, a record from Libya identified discrepancies between government-reported and independent HIV-1 prevalence rates, which further highlighted the MENA region's data limitation aspects [141]. In Jordan, a recent study reported that data were restricted to individuals in HIV/AIDS care willing to participate in research, creating additional selection bias, which might have been related to stigma as well [143,154].

The second barrier identified in this review was the challenge of limited resources. This barrier continues to impede HIV-1 genetic diversity research in the MENA region. HIV/AIDS surveillance efforts could also be constrained by traditional methods, with most sequence-based records included in this review relying on Sanger sequencing, which lacks the sensitivity to detect minor variants and recombinants [60,155]. Only one study from Jordan reported the use of NGS, an advanced tool used for robust genetic characterization [143]. Unsurprisingly, the study from Jordan, conducted in collaboration with the Center for Infectious Diseases Research at the Walter Reed Army Institute of Research, emphasizes the critical role of international collaboration in addressing resource and capacity constraints faced by MENA countries to address the topic of HIV-1 genetic diversity [143].

The consequences of the health constraints identified in this review can be exemplified by Libya, where monitoring systems relied heavily on mandatory screenings for health

certificates and hospital-reported cases [141]. In Jordan, the included study reported that limited access to HIV-1 testing and care contributes to late diagnoses, further complicating healthcare costs and worsening patient outcomes [143]. These issues resonate with the broader global challenge of healthcare inequity, where underserved populations disproportionately suffer from preventable late-stage complications [156–158]. On the other hand, a few MENA countries, such as Saudi Arabia and Morocco, developed the region's most advanced HIV-1 surveillance system, demonstrating that resource constraints can be overcome with sustained investment and policy prioritization [68,131]. Saudi Arabia, though making progress, still requires innovative testing services and improved medicine delivery systems to address its unique challenges, as reported by the included record in this review by Al-Mozaini et al. [68].

In this review, the third identified challenge was HIV-1-specific factors, particularly the genetic complexity of the virus, which pose notable challenges to the accurate subtyping and characterization of HIV-1 variants in the MENA region. For example, in HIV-1 molecular epidemiology studies, the choice of the genomic regions to be analyzed is of prime importance, with conserved regions unable to resolve the true links compared to phylogenetic noise in hypervariable regions with random links [110–112]. Additionally, recombination in HIV-1 is an evident biological phenomenon and can be viewed as a means for the production of new progeny with higher survival through a sharp reduction in deleterious mutations or the accumulation of advantageous mutations at a higher rate compared to their occurrence through nucleotide substitution by mutation [159,160]. Recombination can have adverse effect on molecular clock analysis, comprising the validity of estimates of the most recent common ancestor for variant introduction into a region or country [161,162]. Furthermore, the construction of phylogenetic trees can be complicated if parts of the nucleotide sequences undergoing analysis are the results of recombination events, which implies that the sequences were generated by two viruses, each of which has a distinct evolutionary history [163]. Analyzing HIV-1 sequences without excluding recombination will result in misleading findings of phylogenetic analysis [162].

The high mutation and recombination rates in HIV-1 demand advanced methodologies to capture its diversity, yet the majority of studies in the MENA region which were included in this study relied on short sequences which lacked the resolution necessary for accurate subtype or recombinant form (CRF/URF) assignment [32,62,64,70,133,135]. Only one study reported sequencing full HIV-1 genomes, underscoring a critical gap in the MENA region's research capacity [128]. Additionally, reliance on subtyping tools other than the gold-standard phylogenetic-based approach with reference full-genome HIV-1 sequences often fails to account for the complexities of recombination, leading to the potential misclassification and incomplete characterization of strains [164].

Finally, socio-cultural and legal barriers remain among the most profound obstacles to understanding HIV-1 genetic diversity in the MENA region. Stigma, deeply rooted in cultural and religious norms in this region, casts a long shadow over public health efforts, particularly for marginalized groups such as MSM and FSWs [45,165–167]. Despite growing epidemiological evidence on HIV-1 and risk behaviors among MSM, the MENA region's societal attitudes may result in the under-reporting of MSM transmission, obscuring the true epidemiological picture of HIV-1 in the MENA [72]. This mirrors the global experience during the early years of the HIV-1 epidemic in the United States, where stigma against MSM delayed effective responses and perpetuated transmission [168]. High-risk populations such as IDUs, FSWs, truck drivers, and prisoners are similarly neglected in HIV research in the MENA, creating significant data gaps [29]. The consequences of these barriers are profound, since individuals with HIV-1 in the MENA region face family rejection, societal isolation, and restricted access to essential services [169]. Women, in particular,

are subjected to violence, humiliation, and invasive practices such as virginity testing [141]. Addressing these challenges requires courageous policy reforms, culturally sensitive public health strategies, and a commitment to addressing stigma to foster transparency and equitable HIV/AIDS research efforts.

In this review, the investigation of HIV-1 genetic diversity in the MENA region based on the sequences available in the Los Alamos HIV Sequence Database revealed the following insights. Subtype B emerged as the predominant HIV-1 genetic variant across the MENA region, which aligns with the results of previous studies and reviews from the region [30,32,33,115,170]. This dominance of subtype B in the MENA region aligns with its historical association with transmission networks linked to Europe and North America, reflecting the global interconnectedness of the epidemic [31,32,115]. The high prevalence of CRF02_AG, particularly in Algeria, Tunisia, and Egypt, reflects patterns seen in SSA, where this variant predominates [171]. This likely indicates historical or ongoing transmission links between North Africa and SSA, facilitated by migration and trade [172].

In the current review, the identification of multiple distinct subtypes and CRFs of HIV-1 emphasizes the complexity of the epidemic across the MENA region. Countries such as Morocco and Tunisia demonstrated particularly high subtype/CRF diversity, which may reflect the long-term establishment of multiple transmission networks, including introduction from various geographic regions into high-risk groups such as FSWs, as illustrated by Mumtaz et al. [131].

4.1. Recommendations

Based on the review findings, several recommendations can be proposed to address the identified barriers that may hinder the investigation of HIV-1 genetic diversity in the MENA. First, investment in infrastructure and research capacity is essential in the MENA region. This includes scaling up the use of advanced sequencing technologies, such as NGS, and increasing funding for HIV-1 molecular epidemiology studies.

Second, targeted policy reforms are needed to address the socio-cultural and legal barriers that impede access to HIV/AIDS testing and care, particularly among marginalized groups such as MSM, IDUs, and FSWs. Public health campaigns that aim to reduce stigma and promote inclusive policies can play a critical role in overcoming these challenges. A major issue is the limited access to HIV testing, treatment, and care across the MENA region, where only 52% of people living with HIV are aware of their status, 38% receive ARV, and fewer than 40% achieve viral suppression, as reported by Karbasi et al. [29,131]. These suboptimal rates reflect substantial barriers to HIV investigation in the MENA, which highlights the challenges of obtaining comprehensive genetic data for phylogenetic analysis.

Third, regional and international collaborations should be strengthened to facilitate data sharing and capacity building. A unified regional database for HIV-1 sequences could improve the availability of comprehensive data and enhance cross-border efforts to monitor HIV-1 genetic diversity.

Fourth, increasing the representation of under-researched populations in HIV-1 studies is crucial. This includes prioritizing studies on high-risk groups, such as MSM, FSWs, IDUs, truck drivers, and prisoners, and addressing the gaps in behavioral data that hinder the development of effective interventions.

Finally, integrating socio-cultural considerations into research design and public health strategies is important. Engaging community leaders and stakeholders can help foster acceptance and support for HIV-1 research and interventions, while culturally sensitive approaches can improve participation and data quality. By addressing these challenges, the MENA region can advance its understanding of HIV-1's genetic diversity, ultimately con-

tributing to more effective public health strategies, improved drug resistance monitoring, and tailored treatment protocols in the region.

4.2. Limitations

This review has several limitations that must be acknowledged. First, the geographic and language constraints enforced in the search strategy may have inadvertently excluded relevant records. The focus on English-language publications inherently excluded studies published in Arabic or French, which are widely used in the MENA region, especially in the Maghreb region. Indeed, this is especially relevant for North African countries, where French is commonly used in academic and governmental reports.

Second, the exclusion of specific countries based on distinct epidemic patterns limits the generalizability of the findings. While these exclusions were justified by the review's scope, this approach may have resulted in omitting valuable insights into regional HIV-1 genetic diversity. Similarly, the reliance on pre-defined country lists might have excluded cross-border insights critical to understanding the genetic diversity of HIV-1 in the MENA region, characterized by significant population movement.

Third, the literature search relied on PubMed and Google Scholar, which may not fully capture gray literature or unpublished datasets of HIV-1 sequences. This may have led to the potential exclusion of valuable data, since relevant findings might exist in non-traditional repositories or institutional reports. Future studies that include multilingual searches and a wider range of databases could provide a more complete picture of HIV-1 genetic diversity in the MENA region and the challenges in characterizing it.

Fourth, the themes extracted for analysis were derived through consensus among authors and informed by the first author's prior research. While this approach provided a structured framework, it introduced potential bias, as the thematic focus may have overlooked emerging challenges not previously documented or emphasized.

5. Conclusions

This review highlighted four key barriers that should be addressed for the thorough investigation of HIV-1 genetic diversity in the MENA region. These challenges were limited sampling and data gaps, resource and infrastructure constraints, HIV-1-specific factors, and socio-cultural issues. Insufficient sampling, reliance on outdated sequencing methods, and a lack of comprehensive recombination analysis hinder the accurate depiction of HIV-1's genetic diversity. Additionally, stigma, cultural taboos, and restrictive policies exacerbate HIV-1 under-reporting, particularly among at-risk groups such as MSM and IDUs, leaving significant gaps in HIV-1 surveillance data. To overcome these challenges, it is essential to invest in health infrastructure, use advanced technologies, such as NSG, introduce policy reforms to reduce stigma and legal barriers, and expand regional and international collaboration and data sharing. Addressing the barriers identified in this review can help in the efforts aiming to accurately map HIV-1's genetic diversity in the MENA region, which is crucial for effective public health strategies. Improved HIV/AIDS surveillance in the MENA would aid the monitoring of ARV drug resistance, the tailoring of treatment protocols, and inform vaccine development efforts.

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Abbreviations

The following abbreviations are used in this manuscript:

ARV	Antiretroviral
CRF	Circulating recombinant form
FSWs	Female sex workers
GCC	Gulf Cooperation Council
HIVs	Human immunodeficiency viruses
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
IDUs	Injection drug users
MENA	The Middle East and North Africa
MSM	Men who have sex with men
NGS	Next-generation sequencing
RAS	Resistance-associated mutation
SSA	Sub-Saharan Africa
UAE	United Arab Emirates
UNAIDS	Joint United Nations Programme on HIV/AIDS
URF	Unique recombinant forms

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