Patterns of human immunodeficiency virus drug resistance mutations in people living with human immunodeficiency virus in India: A scoping review

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Abstract

The human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic still exists as a major global public health burden, especially in the middle- and low-income countries. Antiretroviral therapy (ART) remains a sole option to reduce the mortality and morbidity associated with this disease as no approved vaccine candidates are available. About 67% of the people living with HIV (PLHIV) have received the ART in 2019 worldwide. As a consequence of increased ART regimes, the prevalence of drug resistance mutations (DRM) also has been escalating and it would become a significant barrier in achieving the United Nations Programme on HIV/AIDS goal of eliminating HIV by 2030. So far, nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitor-(PI) associated DRM have been reported across the globe with a considerable escalation in the annual prevalence rate of pretreatment NNRTI DRM. Conversely, NRTI-associated DRM is still under 5%, with a few scattered reports of significant increase from few countries such as southern and eastern Africa. Likewise, in India, the propositions of NRTI and NNRTI-associated DRM have increased since the commencement of the nationwide ART program in 2004. In agreement to the global trend, M1841/V, a type of NNRTI, remains as a dominant DRM among PLHIV. In this review, we tried to collate various mechanisms of DRM in PLHIV. In addition, patterns of HIV DRM in India and their future challenges on drug-related mutations have been discussed.

Key words: Drug resistance mutations, nonnucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, people living with human immunodeficiency virus, protease inhibitor

Introduction

The human immunodeficiency virus (HIV) is classified under the genus Lentivirus within the family Retroviridae.[1] Based on its genetic characterization and antigens, it is further subdivided into HIV-1 and HIV-2. However, HIV-1 is relatively more pathogenic and is responsible for the majority of HIV infections around the globe. The first case of HIV infection was reported in 1981 to the Centre for Disease Control, and later in 1983, this was termed as Acquired Immune Deficiency Syndrome (AIDS). As per the United Nations Programme on HIV/AIDS (UNAIDS) data, around 76 million people have been infected since the start of the epidemic and 32.7 million people have died worldwide.[2] In 2019 alone, 1.7 million new infections were recorded and 690,000 died. And as of now, 38 million people are living with HIV (PLHIV). In India, with 2.1 million PLHIV, there are on an average 87,000 new infections and 69,000 deaths, annually.[3] In order to accelerate the process of control and subsequent elimination of HIV/AIDS, the National Strategic Plan has laid strong emphasis on design, development, and implementation of tailored interventions considering context-specific to local evidence. In 1987, zidovudine, a reverse transcriptase inhibitor, was approved by the Food and Drug Administration, USA to treat this disease. Since this first anti-HIV

drug, multiple medicines have been validated for HIV treatment. These include nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). These two major categories of anti-HIV drugs target the reverse transcriptase enzyme thus, blocking the synthesis of proviral DNA. Another category is protease inhibitors (PI), these compounds target HIV protease, which is crucial for the viral maturation process. Subsequently, antiretroviral therapy (ART) was introduced, which is a combination of medicines from NRTI, NNRTI, and PI drug classes, targeting different biological mechanisms in HIV.[4]

At initial stages of the epidemic (during 2004), the WHO guidelines stated that ART should be initiated at CD4 cell count of 200 cells/µl considering the greater chances of acquiring opportunistic infections when the count falls below 200 cells/µl. However, these guidelines have been updated regularly. In 2017, India adopted WHO recommendations of initiating ART "regardless of WHO clinical stage and at any CD4 cell count." Ever since its inception, ART has impacted both mortality and morbidity worldwide in PLHIV.[5] However, success rate of ART relies on susceptibility of virus and adherence to therapy, failure of which leads to unavoidable emergence of drug resistance. The drug resistance mutations (DRM) which impact the effect of all these anti-HIV drugs are observed in individuals undergoing ART. In addition, these reported DRMs are frequently observed in naïve individuals who have not taken any ART regimes. This indicates the spread of HIV DRM at the community level.[6]

Elements like excessive variability and high evolution rate in HIV favors emergence of DRM. Besides hindering the efficacy of ART, the spread of drug-resistant strains is another adverse effect of DRMs.[7] According to WHO Guidelines <5% DRM prevalence is categorized as low, 5%-15% as moderate and >15% as high prevalence, which signifies the grade of surveillance programs required for monitoring primary HIV-DRM. This review discusses the HIV drug-resistant mutations and their molecular mechanisms. In addition, we analyzed the prevalence and patterns of HIV DRMs in India and their future challenges.

Methodology

This review was aimed to provide the pattern of HIV drug-resistant mutations in India. For compiling this review we used a combination of web-accessible data along with manual curation to survey. An initial comparison among many open-access search engines showed that most search results are similar regardless of the search engine used. Therefore, Google Scholar and PubMed were selected as the primary literature search engines. Databases were searched in November 2020 with the keyword "HIV drug resistant mutations" and "India" and retrieved 229 documents for the period of 1988–2020. Search terms were used in combination to systematically search titles, abstracts, and keywords. From this initial set of articles, 195 were excluded from the study, and 34 alone were taken for the analysis. The study includes original articles containing the epidemiological work on HIV DRM
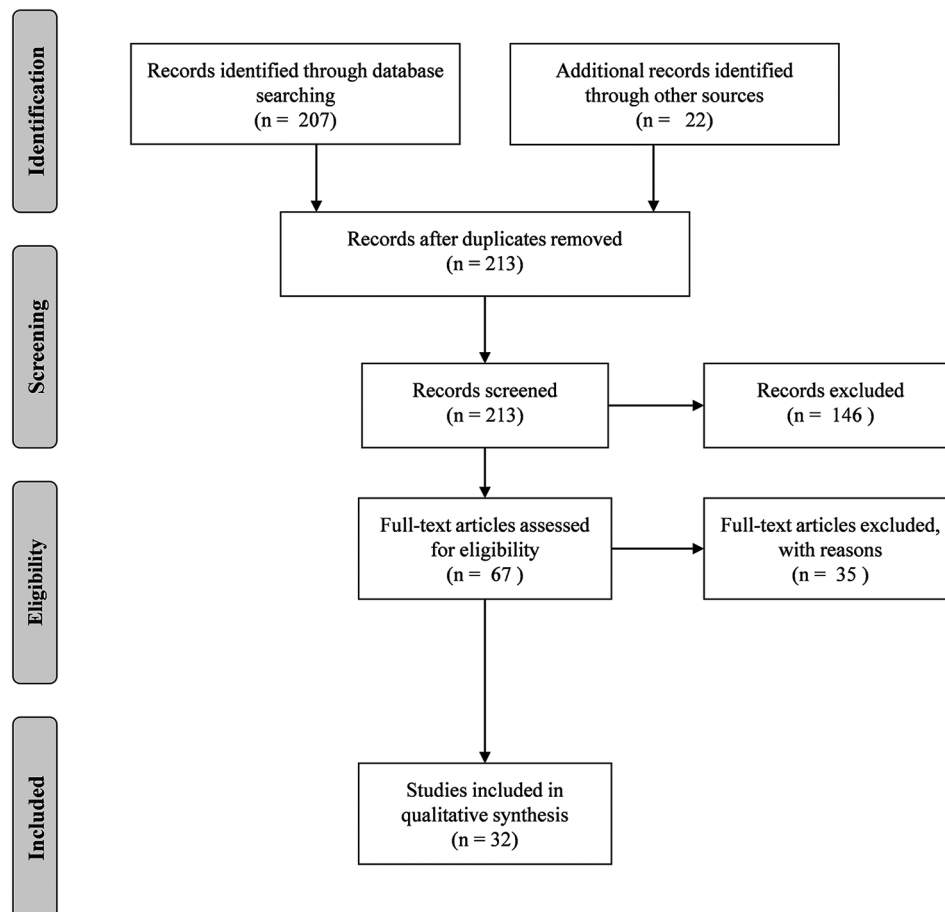

Figure 1: PRISMA flow diagram. Presentation of the procedure of literature searching and selection with number of articles at each stage

Table 1: Common human immunodeficiency virus drug-resistant mutations

Anti HIV drug category	DRM mutation	Location of mutation	Mechanism of action
NRTI	M184V, K65R, Q151, Y115F, K219R, M41L TAMs K70R, D67N, L210W	Active site of RT	Reduced affinity due to competitive inhibition Enhancement of primer unblocking
NNRTI	K103N, Y181C, G190A, A98G, VI79D/E, K101E	Hydrophobic pocket of p66 subunit	Reduced affinity due to allosteric inhibition
Protease Inhibitors	V28S, V82L, N83D, 185V, N88D	HIV protease enzyme	Competitive inhibition-blocks viral maturation

HIV: Human immunodeficiency virus, DRM: Drug resistance mutations, RT: Reverse transcriptase, NRTI: Nucleoside reverse transcriptase inhibitors, NNRTI: Non-nucleoside reverse transcriptase inhibitors
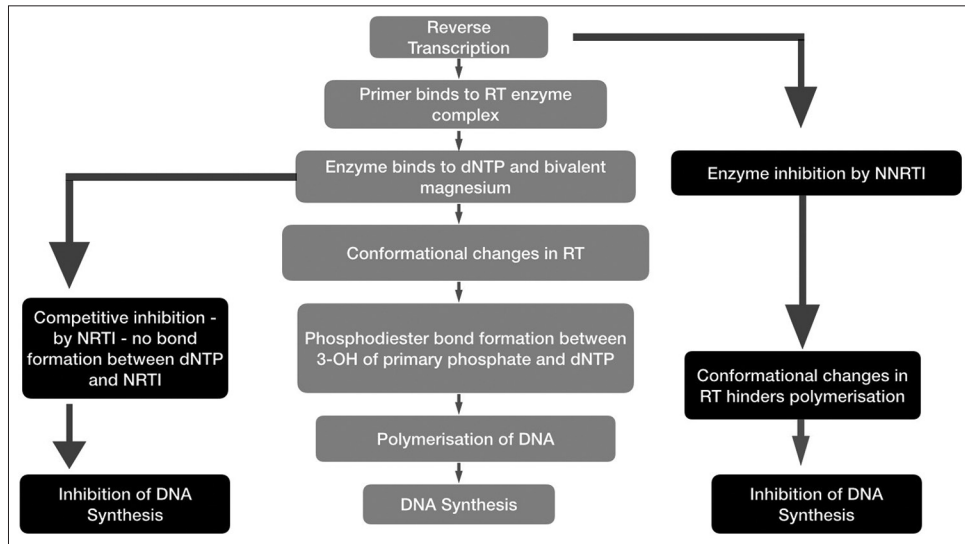

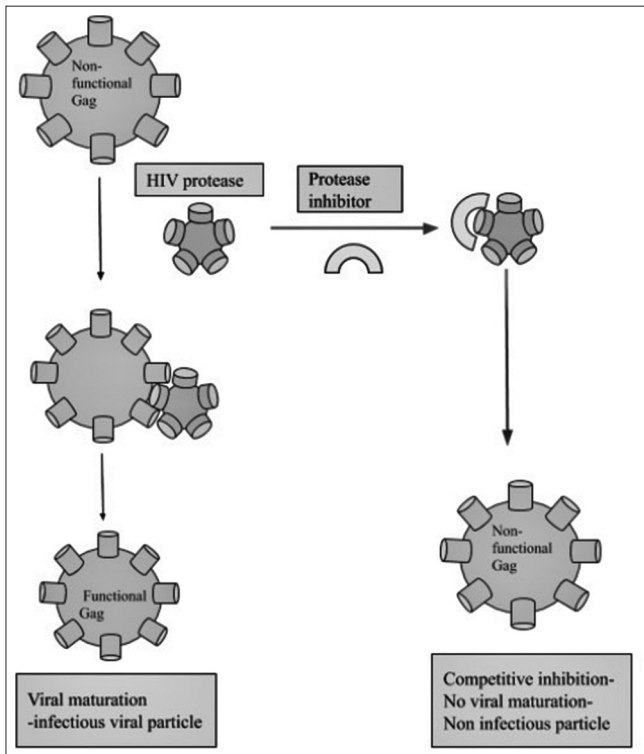Figure 2: Mechanism of anti-HIV drugs NRTIs and NNRTIs

Figure 3: HIV Protease Inhibitor mechanism

from various regions of India. The excluded articles fall under different categories; 24 documents (Books, Reviews, Systemic reviews, etc.,) 18 (Duplications), 143 (Nonrelevant articles). The nonrelevant articles include those from other country, other diseases, computer modeling studies, docking studies, basic research, etc. The literature survey and article selection criteria are detailed in the PRISMA flow diagram [Figure 1].

Human Immunodeficiency Virus Drug Resistance Mechanism

In recent years a great progress in regard to ART for HIV is evinced, but its effects are considerably influenced by DRMs [Table 1]. Most of the anti-HIV drugs target reverse transcriptase enzyme which is a multifunctional enzyme responsible for RNA-dependent DNA synthesis. NRTIs and NNRTIs are anti-HIV drugs which were designed to hinder reverse transcription mechanisms [Figure 2]. NRTIs such as zidovudine, stavudine, abacavir, and lamivudine being dNTP analogs. This competitively inhibits reverse transcriptase enzyme, absence of 3'-OH in NRTI leads to obstruction of phosphodiester bond formation. NNRTIs such as nevirapine, efavirenz, etravirine, on the other hand, follows different mechanism, as they block DNA synthesis by allosterically binding with p66 subunit of reverse transcriptase enzyme leading to conformational changes transcended by restriction of polymerase catalytic triad affecting DNA polymerization.[8]

Nucleoside Reverse Transcriptase Inhibitors Associated Mutations

NRTI-associated mutations give rise to a large number of effects with regard to drug susceptibilities, replication capacity of virus, and biochemistry of reverse transcriptase

enzyme.[9] Point mutations mentioned in Table 1 were observed to be induced after ART, which cause decline in affinity of NRTI to RT enzyme leading to lower incorporation of NRTIs. M184, a mutation located on codon 184 of RT enzyme causes more than 100-fold resistance and develops rapidly in individuals who receive lamivudine monotherapy; this mutation alone is capable of rendering lamivudine therapy ineffective.

The second drug resistance mechanism involves phosphorolytic removal of NRTI from 3 terminus of primer, the process being known as "primer unblocking." Mutations which have an amplifying effect on primer unblocking are Thymidine Analogue Mutations such as K70R and D67N. This can be enhanced by selective drugs like zidovudine and stavudine as they are thymidine analog. While researchers believed that pyrophosphorolysis as a primary mechanism of resistance to zidovudine and stavudine, the process is not drug-specific in the way that discriminatory mutations tend to be. Hence, these pyrophosphorolysis-enhancing mutations can confer reduced susceptibility to all of the NRTIs. Thus, certain drugs are developing resistance quickly and effectively to some NRTI than others.[10]

Nonnucleoside Reverse Transcriptase Inhibitors Associated Mutations

NNRTI-associated mutations are located on a hydrophobic, which usually emerge when patients are administered NNRTI monotherapies, due to incomplete viral suppression. This indicates NNRTI-associated mutations are caused by viruses selected from a mutant preexisting population. Nucleotide substitutions that affect the susceptibility to NNRTIs are usually present on three regions between codons 98–108, 79–190, and 225–236.

Among NNRTI DRM mutations mentioned in Table 1, K103N and Y181C are common which lead to virological failure. These mutations occur in the enzyme pocket targeted by NNRTI, thus reducing the affinity of these compounds toward reverse transcriptase enzyme.[11] K103N present on codon 103 causes 20–50-fold resistance to NNRTIs, enough for virological failure. Another mutation V106A, causes more than 30-fold resistance to NNRTI-Nevirapine. Recent evidences show the differential effects of genetic background on both the type and degree of HIV DRM. Owing to the polymorphic nature of the hydrophobic pocket of HIV reverse transcriptase, it confers a differential degree of resistance among different subtypes and strains.[12]

Protease Inhibitors Associated Mutations

HIV protease, a homodimeric aspartyl protease plays a vital role in viral maturation by cleaving viral Gag and Gag-Pol polyprotein precursors transitioning them into mature, functional viral enzymes and structural proteins.[13] This transition is essential for production of infectious viral particles. The fact that HIV protease is crucial for HIV infection makes it a favorable target for drug design. PIs such as Ritonavir, Saquiunavir, and Indinavir were approved to be used as a part of highly active ART (HAART) by FDA.[14]

PIs competitively inhibit HIV protease significantly, thereby ceasing the viral maturation process, leading to a reduction in the number of infectious viral particles [Figure 3]. However, high mutation rate due to the absence of proof-reading activity and dynamic viral replication along with potential dual infection and treatment interruption contributes to the development of drug-resistant mutation.

A few common HIV drug-resistant PI mutations observed in the Indian population are V28S and V82L.[15] Even though naturally occurring DRMs arise with high frequency in untreated patients they are not significant enough as they rarely cross the threshold of detectable level. Almost all clinically relevant DRMs arise as a consequence of selective drug pressure.[16]

The Current Status of Human Immunodeficiency Virus Drug Resistance Mutations in India

Joint UNAIDS and National AIDS Control Organization (NACO) estimated that the prevalence of HIV/AIDS in India is about 0.3%, which is still above the global average of 0.2%.[2,17] To evade the adverse effects of HIV and AIDS in India, free ART was launched in 2004. This is the second-largest ART program globally and the program achieved 54% decrease in the AIDS-associated mortality after a decade of their initiation. Thus, every newly infected patient receives ART that comprises of 2 NRTIs and 1 NNRTI drug. The PI is reserved for second-line treatment if the first-line treatments like NRTIs and NNRTIs fail due to drug resistance.[17] The emergence and spread of DRM is a major threat to the aim of NACO in combating the HIV epidemic in India. The list of the prevalence of HIV DRMs in India are given in Table 2. In order to identify the frequency of DRM among HIV strains circulating across the states, genotyping techniques are in practice. Karade *et al.*[18] reviewed the DRM status in India by analyzing the 1046 HIV genomic sequences published between 2004 and 2014. The proportion of HIV sequences with any DRM, any NRTI DRM, and any NNRTI DRM was 78.39%, 68.83%, and 73.13%, respectively. Among the NRTI DRM, M184V and K65R were the most predominant mutations found across India. In the case of NNRTI, DRM, K103N, and Y181 C are the prevalent among the reported HIV DRM. Interestingly, the rising trend of K65R mutation was noticed among the Indian population from 2004 to 2014. However, M1841/V continued to be the dominant DRM throughout this study period.

Although ART regimes do not depend upon HIV subtypes, subtype C, which is a predominant subtype in India may develop certain DRM under drug pressure. Thus, systematic sequencing may be practiced to develop a subtype C-specific HIV drug resistance database to overcome the treatment failure. According to recent reports, associated co-infections in HIV patients also can enhance DRM in the Indian population.[7] Among 115 patients analyzed, 13.3% DRM was found in HIV + TB + and 7.5% DRM was found in HIV + TB-. For instance, 10.6%, 4%, and 2.6% of NNRTIs, NRTIs, and PI DRM were respectively found in HIV + TB + patients. On the contrary, only NNRTIs DRM (1<) was found in HIV + TB-. Thus, Tuberculosis (TB) co-infection may enhance the emergence of HIV DRM. However, whether TB alone is responsible for this higher prevalence needs extensive research with scientific methodologies.

Future Challenges for India

In resource-limited countries like India, providing HIV genotyping to all patients with suspected treatment failure is a challenging task. To overcome this, the detection of the most common DRM has been suggested as a cost-effective method. However, this may mislead the evaluation of the emerging DRMs and the changing trends of other uncommon DRM that already exists. Also, different detection techniques used to detect DRM may lead to discrepant

Table 2: List of human immunodeficiency virus drug resistance mutations in India

Study name	Study period	Study region	Significant findings of the study	Type of mutations observed
Chauhan *et al.*[23]	September 2014 to June 2016	North-Western India	Study population: 37 (ART individuals) 22.2% drug resistance observed among FSW and 14.3% was observed among MSM	K219R, L74V, K219N, and Y181C
Thirunavukarasu *et al.*[24]	March 2010 to March 2014	South India (Salem, Tamil Nadu)	Study population: 213 (VF patients: 23) NRTI associated mutations: 87% (20/23) NNRTI associated mutations: 91% (21/23)	I135R/T/V/X, L178 I/M, M184V/I, D67N, K70R, and K103N
Ambike *et al.*[25]	January 2014 to August 2014	Maharashtra	Study population: 213 (treatment naïve individuals) Mutations associated. with RT and PI were observed in 9.37% (3 of 32)	M184V, V111I, K65R and Q151M1
Nesakumar *et al.*[26]	April 2011 to September 2012	Chennai	Study population: 213 (treatment naïve individuals) TDR was found to be 11.3% (6/53) and NNRTI associated mutations in 8.3% (4/53)	K101E, Y188C/Y, K103N,
Azam *et al.*[27]	2010 and 2011	Aligarh	Study population: 54 (treatment naïve individuals). All patients had at least two secondary mutations that are associated with resistance to PIs	H69K, I93L, I15V, L19I, M36I, R41K, L63P, and L89M
Lakhikumar Sharma *et al.*[28]	Not stated	Manipur	Study population: 37 (ART individuals).37%, 29% and 7% individuals harbour DRM at the target sites of NNRTI, NRTI and PI respectively	M184V, T215Y, M41L and V108I, H221Y, M46I and I47V
Kannangai *et al.*[29]	2009 and 2012	South India	Study population: 127 (treatment naïve individuals) 2.4% people have TDRMs, NNRTI PI and NRTI associated mutations were not found	K101E, Y181C and G190A
Dinesha *et al.*[30]	Not stated	South India	Study population: 167 (ART individuals) 90.4% (151/167) people had DRMs. NRTI, NNRTI, and dual mutations (NRTI and NNRTI) were seen in 123 (73.7%), 147 (88%), and 119 individuals (71.3%) respectively	M184IV, K65R, K103NS, V106AM and Y181CIV
Azam *et al.*[31]	2010 to 2012	Aligarh	Study population: 103 (ART individuals) 28% (29/103) people have DRM. Out of this 14 (13.6%) mutations at NRTI and 15 (14.6%) mutations at NNRTI	K219R, E44D, A98G, K103R, V106M, V108I, E138A, Y181C, G190A and T215Y
Azam *et al.*[32]	2010 to 2012	Aligarh	Study population: 102 (59-ART and 43-treatment naïve individuals) DRM was observed in 64% (66/102) of the individuals	D30 N, M46I, G16E, K20R, M36I, D60E, I62V, L63P, I64M, H69K, T74A/S, V77I, V82I, I85V, L89M, I93L, M36I/L/V, H69K, and L89M
Rajesh *et al.*[33]	July 2007 and March 2008	Madurai	Study population: 26 (ART, pregnant women) NNRTI were observed in 33% (12/26) of the individuals	Y181C, K103N and Y188C.
Saravanan *et al.*[34]	2008 and 2009	Chennai	Study population: 107 (ART individuals)	M46I, I54 V/A, V82A, L90M, K103N, Y181C, and G190A
Karade *et al.*[35]	January 2014 and April 2015	Pune	Study population: 52 (treatment naïve individuals) 3.8% (2/52) have NNRTI associated mutations	V106M, K103N
Sharma *et al.*[36]	August 2016 and September 2017	Manipur	Study population: 44 (14-treatment naïve, 30 ART individuals) 35.7% (5/14) had NRTIs, NNRTIs, and PIs associated mutations. 50% (15/30) had NRTIs, NNRTIs and/or PIs	M184V, M184I, M41L, D67N, T69D, K70G/R, Y115F, T215N and K219R/Q, A98G, K103N, V179D/E, Y181C, G190A/R, K101E, V106A/M, V108I, Y181F, H221Y and M230I
Sinha *et al.*[37]	April 2006 to August 2008	New Delhi	Study population: 68 (treatment naïve individuals) 2.9 (2/68) had NRTIs associated mutation observed. No NNRTIs associated mutation were observed. Minor PIs mutation also observed	M184V, K20R, M36I, and H69K
Choudhury *et al.*[38]	Not Stated	New Delhi	Study population: 44 (17-ART and 27-treatment naïve individuals) Naïve individuals: 25.9 and 85.18% of NRTI and NNRTI associated mutations were found ART individuals: NRTI and NNRTI mutations were observed in 78.7 and 80.6% respectively	I135T, I135M, E138A, R211K, M184V
Sen *et al.*[39]	June 2004 to December 2005	Pune	Study population: 44 (50-ART and 25-treatment naïve individuals) No DRM was observed in the Treatment naïve individuals At least one RT mutations were observed in more than 80% (29/44) of ART individuals	T39A, E203D/K, H208Y, D218E, K122E, M184V, K103N/S and Y181C
Gupta *et al.*[40]	January 2005-April 2005	Mumbai	Study population: 51 (ART individuals) 96.1% people had resistance to at least one drug used. In that, 92.2% (47/51) had NRTI resistance, 62.7% (32/51) had NNRTI resistance, and 7.8% (4/51) had PI resistance	M184V, M41L, D67N, K70R, L210W, T215F/Y, V75I, M46I, G48V, I54V, G73S, V82A/F, I84V, L90M, A98G, K101E/P, K103N/S, V106M, V108I, Y181C/I, Y188L, G190A/S/E, F227L, M230L

Contd...

Table 2: Contd...

Study name	Study period	Study region	Significant findings of the study	Type of mutations observed
Karade *et al.*[41]	June and August 2014	Pune	Study population: 80 (ART individuals) 79.75% (63/80) of individuals had at last one type of RT associated mutations The prevalence of DRMs were 58.75%, 78.75%, and 1.25% for NRTIs, NNRTIs, and protease inhibitors (PIs), respectively	M184V/I, K103N, L89M, V77I, L63P, H69K/R/Q, M36I, K20I/M/R/T, G16E, K65R and L10V/I
Ekstrand *et al.*[42]	Not stated	Bangalore	Study population: 92 (ART individuals) 86% individuals had at least one RT mutations. NRTI resistance mutations and NNRTI resistance mutations were observed in 68% and 72% individuals respectively	M184V/MV/I/IM, E44D/DE/A/K, L74V, V75M, V118I, T69D/DN, Y181C/CY/I/V, K103N/KN/R, K101E/EK/Q/KQ, G190A/AG, V108I, V106M/MV
Thorat *et al.*[43]	August 2007 and February 2009	Kakinada	Study population: 47 (ART women individuals) No NRTI and PI associated mutations were found. Only one patient had NNRTI associated mutation	K101E
Neogi *et al.*[44]	November 2009 and February 2010	Bangalore	Study population: 21 (ART individuals) Only 9.56% (2/21) individuals had NNRTI associated mutations	E138A and L210LS
Lall *et al.*[45]	Not stated	Pune	Study population: 40 (treatment naïve individuals) 10% (4/40) of the individuals had RT associated mutations	V82A, M41L, D67N, M184V, and A98G

NRTIs: Nucleoside reverse transcriptase inhibitors; NNRTI: Non-NRTI; PI: Protease inhibitor; TDRM: Transmitted drug resistance-associated mutations; ART: Antiretroviral therapy; DRM: Drug resistance mutations, RT: Reverse transcriptase, FSW: Female Sex Workers, MSM: Man who Sex with Man.

results that ultimately affect the treatment protocols.[19] For instance, the detection rate of K65R DRM will be higher in case of allele-specific PCR, however, the same will be negative for Sanger sequencing. It is worth mentioning that HIV drug-resistant mutation in India depends mostly on conventional Sanger sequencing.[20] So, ensuring the implementation of cutting-edge detection technologies is need of the hour to control and eliminate the HIV epidemics.

It is known that patients in low- and middle-income countries, both acquired and transmitted DRM originate in the first-line ART drugs.[21] However, the predominance of transmitted DRMs can be temporarily impaired by employing new classes of drugs. As per the WHO guidelines in 2016, most of the countries have attained the WHO's 10% transmitted DRM threshold.[22] Thus, switching to a new first-line NNRTI named dolutegravir is in progress, and implementing this strategy may be beneficial for both public health response and appropriate amendments in the policy. Overall, the development of routine HIV genotyping and appropriate database for submission is essential along with the updating in the first line ART as per the WHO to estimate the actual rate and mitigate the HIV DRM in India.

Conclusion

Despite the implementation of effective ART, which declined the mortality rate from 1.9 million in 2006 to 0.95 in 2017, the rapid emergence of HIV DRM would possibly reverse the morality trend across the globe. The first step in the prevention strategy involves the identification of infected populations, for which effective HIV DRM surveillance strategies that include point-of-care testing must be developed worldwide. This would minimize the chance of their dissemination among the high-risk sex networks. Current population genotyping methods for the surveillance of resistance are high cost and low throughputs. Thus, the implementation of cutting-edge technologies like next-generation sequencing combined with simpler sample collection and storage matrices has considerable potential to broaden global surveillance and patient monitoring for HIV DRM. For the long term, WHO has initiated a new global action plan to mitigate and prevent HIV-associated DRM. As factors that influence dissemination of DRM varies between countries, regional data are essential for both public health response and appropriate amendments in the policy. Overall, scale-up of clinical measurements, frequent updating in the ART regimens, and execution of effective strategies like prompt counseling and pretreatment education to improve patient adherence to ART probably would help end the AIDS epidemic by 2030.

Conflicts of interest
There are no conflicts of interest.

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