DRUG RESISTANCE IN FOUR IMPORTANT HUMAN VIRUSES: UPDATEDREVIEW

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I. INTRODUCTION

Viruses causes severe infections and associated and linked with outbreaks of some major global diseases such as HIV/AIDS, COVID-19, influenza virus, dengue fever, common cold, and hepatitis. The infective process of viruses varies depending on the viral species; however, they follow certain steps in the infective process. The typical stages in their life cycle include (Fig 1): attachment and entry, viral uncoating, replication and transcription of viral genome, protein synthesis, assembly, and release of progeny virus. Antiviral drugs are used in combating viral infections and the development of these drugs has been major achievement in the global effort to mitigate infectious diseases. Antiviral drugs are available for a number of viruses including severe acute respiratory coronavirus 2 (SARS-CoV-2), hepatitis-A and-B virus, influenza virus, Papillomavirus. Human Immunodeficiency Virus (HIV), respiratory synctial virus, human cytomegalovirus (HCMV). Although most of these agents are not curative, they can efficiently control viral replications. They can be either small or large molecules, synthetic or natural. Based on their mode of action (MOA), antiviral agents are divided into two classes: 1, inhibitors of the viruses or 2. Inhibitors of the target host cells. Viral –targeting antivirals (VTAs) act by either directly or indirectly inhibiting the biological functions of viral protein which results in the inhibition of the ideal viral replication machinery. Host-targeting antivirals (HATs) on the other hand target the hostprotein that are associated with the viral replication cycle, regulating the function of the immune system or other cellular processes in the host (3) The Food & Drug Administration Board has approved more than hundred antiviral agents which are based on either monotherapy and combined therapies (https://www.fda.gov). The approved drugs have different MOAs based on their functions or structures. These includes structural analogues, entry inhibitors, integrase inhibitors, essential enzyme inhibitors such as nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors, protease inhibitors, inhibitors that are specific to certain viruses such as influenza virus and HCV NS5A protein and NS5B polymerase inhibitors, immunomodulators, interferons, antimitotic inhibitors, and oligonucleotides(1).

The global effort to address viral infections is hindered by the ability of the virus to develop resistance. Antimicrobial drug resistance (AMR) is defined as reduction in susceptibility to a drug by a pathogen as a result of genetic alteration. It is expressed as IC50 or IC90 (the

concentration required to inhibit viral growth by 50% or 90%, respectively). AMR is now a global pandemic on its own. In viruses, drug resistance is based on specific mutation in the viral genome which results in changes in the viral target protein or viral drug activator (2). Table 1 outlines factors associated with antiviral drug resistance. Antiviral drug resistance is on the increase which is having an impact of the global effort to manage viral infections especially with the risk of occurrence of pandemics now on the increase. In this review, we discuss selected viral infections of global importance and discuss the mechanism of resistance and types of polymorphisms associated with the resistance. We also discuss the clinical significance, and novel tool for diagnosing antimicrobial resistance.

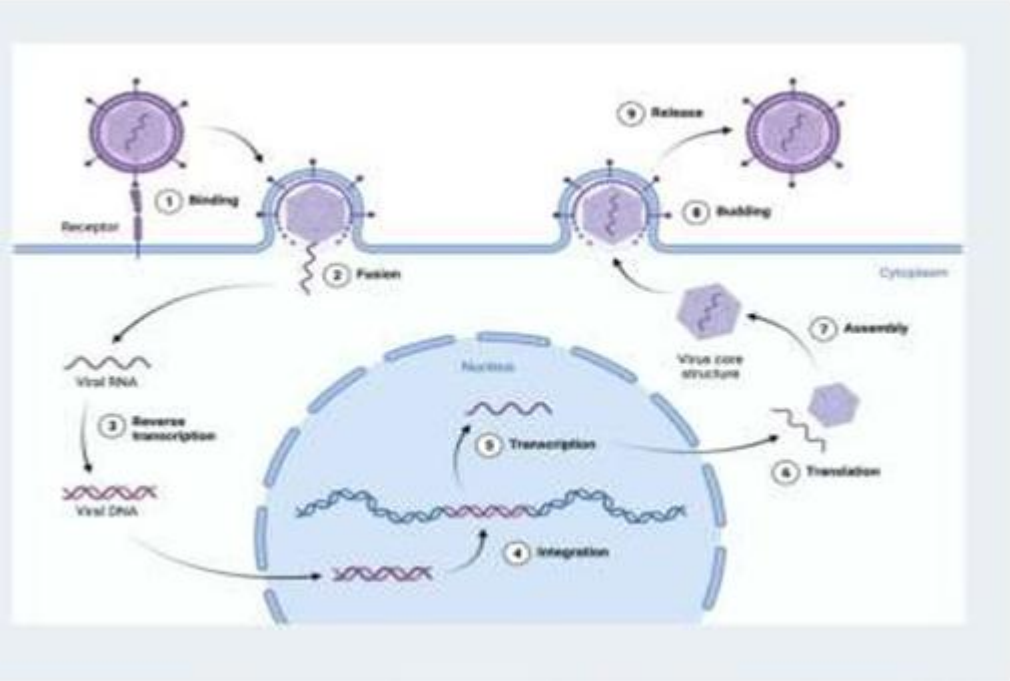

Figure 1: *The general life cycle of viruses (Source: Biorender, 2023)*

Table 1: Factors that Influence the Emergence of Viral Resistance

Mutation rate
Number of mutation sites
Viral replication
Viral load
Fitness, with or without the presence of the antiviral agent

II. CORONAVIRUS DISEASE 2019 (COVID-19)

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the Coronaviruses (CoVs) which are spherical viruses with a positive-sense, single-strained RNA genome (4). The genome also encodes several structural and non-

structural proteins that are important for transcription and replication of the virus. These include the spike protein (S), membrane proteins (M), envelope protein (E), and nucleocapsid protein (N) (7). Other members that have been associated with outbreaks in humans include Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) (4, 6). SARS-CoV-2 was first described in Wuhan, China in December 2019. COVID-19 is associated with series of clinical manifestations ranging from self-limiting respiratory distress to severe pneumonia which can lead to multiple organ failure and death (7). By 03/10/2023, approximately 680,000,000 cases had been reported with an estimated 6,900,000 deaths (https://coronavirus.jhu.edu/map.html).

Like other RNAs viruses, the genome of SARS-CoV-2 is susceptible to random mutations which have an effect on the structural and non-structural genes (7). Due to these genetic mutations, a number of variants of concern (VOC) have been described around the world (7). These genetic mutation changes the phenotypic status of the virus thereby affecting it virulence, transmissibility, and severity of SARS-CoV-2-associated diseases. As of 15 December 2023, five variants of VOCs have been described as Alpha, Beta, Gamma, Delta, and Omicron variants with Omicron DV.7.1 and XBB.1.5 been under monitoring status (https://www.ecdc.europe.eu) . A new variant JN.1 has been reported that has been classified as "Variant of interest" after it spread in the Americas, Europe, and western Pacific. It is related to BA.2.86 but differ due to single mutation in the spike protein (https://www.yalemedicine.org/news/jn1-coronavirus-variant-covid).

At the start of the pandemic , several antibiotics, antivirals, antimalarial agents, and immunomodulators were recommended as novel therapeutic agents for SARS-CoV-2 However, further evaluations showed that these had either limited or ineffective value against the virus. Several drugs have been approved for use in COVID-19 by the Food & Drug Administration includes baricitinib, ritonavir,-boosted nirmatrelvir, remdesivir, and tocilizumab. However, the data on COVID-19 treatment keeps changing rapidly.

Currently, the therapies with published documentation are small molecule drugs, interferon (IFN), and monoclonal antibodies (mAbs). Most of the small molecules used in COVID-19 are repurposed antiviral agents. The antiviral agents are based on two strategies: direct acting antivirals (DAAs) and host-targeting agents (HTA). DAA are used in targeting viral proteins while HTAs are used in inhibiting the human host cells that are required by the virus for replication and transmission (8). Among the DAAs used are Remdesivir, a polymerase inhibitor that was initially developed for hepatitis C virus. It is administered intravenously.

Another agent is molnupiravir, another polymerase inhibitor developed for Venezuelan equine encephalitis and influenza. Nirmatrelvir is a protease inhibitor that is combined with ritonavir, an inhibitor of CYP3A4 as Paxlovid® (9). Azvudine, lopinavir, celgosivir, ritonavir, chloroquine, umifenovir, and favipiravir were all recommended for monotherapy but none were shown to be effective (10, 11, 12)

There are few options in HTA development due to poor investment. For example iminosugarshave activity against SARS-CoV-2. Iminosugars interfere with enzymes that are involved in glycan-mediated endoplasmic reticulum quality control (ERQC) for folding of viral glycoprotein. It was initially developed for treating rare genetic lysosome storage diseases such as Gaucher and Niemann-pick type C (10). Iminosugars have been used for

hepatitis B and C viruses, flaviviruses such as dengue and Ebola virus (10). Others are dexamethasone, tocilizumab, tofacitinib, and baritinib (13).

Virus-targeting MAb binds to the viral S protein thereby resulting in direct neutralization of SARS-CoV-2. These have been used as novel antiviral intervention strategy. Neutralizing mAbs consist of recombinant proteins which are derived from the B cells of convalescent patients or humanized mice. Some mAbs have also been approved for treating non-hospitalised patients with mild-to-moderate COVID-19 (14). These include bamlanivimab, casirivimab, regdanvimab, and imdevimab (13).

The emergence of new SAR-CoV-2 variants led to reduction in the efficacy of therapeutic agents used for SAR-CoV-2 (antiviral agents and mAbs). For e.g., the emergency of Omicronlineages resulted in the VOC mentioned earlier showing reduction in susceptibility to mAbs (17). The risk of SARS-CoV-2 antiviral resistance is based on two factors: prolonged use of antiviral may result in the emergence of variant that are less susceptible to the specific agent and increased use of these agents in immunocompromised patients who are prune to increased period of infection because they are not able to control viral replication.

SARS-CoV-2 like other RNA viruses are associated with high mutation rates which allow them to adapt to environmental changes. Such mutations are linked with the development of drug resistance. Remdesivir is used in COVID-19 and the drug target the non-structural protein 12 (Nsp12). Amino acid substitution in Nsp12 can lead to resistance to remdesivir. Stevens et al reported of mutations in the Nsp12 RNA-dependent RNA polymerase (nsp12-RdRp): V166A, N198S, S759A, V7921, and C700F/R (14). These variants become resistanceto remdesivir through distinct mechanisms (mutations). Previous study showed that single point mutation in RdRp (D484Y) led to the emergence of resistant mutant which conferred resistance against remdesivir (15). In addition mutations were found in ORF1b: nsp5:S144A, nsp5:Q189K, nsp5: H172Y, nsp5: E166A, and nsp5:F140A which were associated with moderate and high resistance to Paxlovid (18). Mutations in main protease (Mpro) had been associated with resistant to nirmatrelvir. It was reported that the effectiveness of nirmatrelvin was reduced due to six mutations: Q189E, Q192T, N142L, Q189I, P132H, and E166M (21). However, nirmatrelvin maintained its efficacy *in vitro* against the following Omicron sub- variants: BQ.1.1, XBB, BA.1, BA.1.1, BA.5, BA.4, BA.2, BA.2.12.1, BA.2, and BA.2.7.5 (21).

Neutralization efficacies of mAbs are also impacted by the emergence of SAR-CoV-2 variants. Ronapreve (casirivimab/imdevimab) was linked with complete reduced efficacy against all the Omicron sub-variants but it was effective against Alpha, Beta, and Delta variants. Regdanvimab was shown to have reduced neutralising effect against BA.1, BA.2 and BA.5 which led to complete resistance by the variants *in vitro* (18). Previous studies on the efficacy of regdanvimab before the emergence of Omicron variants showed that regdanvimab significantly reduced the number of patients that progressed to severe or critical COVID-19-associated symptoms and led to short hospital stays in comparison to their cohort (19). Similarly, sotrovimab had complete neutralising effect against most SARS-CoV-2 variants. However, it showed reduced efficacy against Omicron sub-variants BA.2, BA.4 and BQ.1.1 *in vitro* but not in hamsters. This inconsistence means further research and different techniques to those available maybe needed. Tixagevimab packaged with cilgavimab (Evusheld) had neutralising effect against BQ.1 and BQ.1.1 sub-variants. However, the FDA in a statement warned that there is potential risk of treatment failure as a result of emergence

of SARS-CoV-2 variants that are resistant to Evusheld (20). A simulations analysis showed that combination of L50F with E166M, and E166V alone resulted in reduced binding efficacybetween nirmaltrevir and Mpro.

III. HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV is a lentivirus of the *Retroviridae* family. It is the causative agent of Acquired immunodeficiency syndrome (AIDS). The genome consist of linear single-stranded RNA (ssRNA) encoded by fifteen mature viral proteins. HIV is grouped into two: HIV-1 and HIV-2, which are further divided into extensive groups, subtypes, and recombinant forms. As a result of zoonotic transmission, HIV is transmitted from chimpanzee, gorillas, and sooty mangabeys to humans. HIV is transmitted through contaminated blood or body fluids meaning an individual can become infected through sexual contact, transfusion of blood, sharing contaminated needles, or through maternal transmissions (23). Clinical manifestations associated with HIV infection include lymphoma, cardiovascular disease, lung cancer, gingivitis, neurological diseases, kidney disease, and osteoporosis. In 2021, it was estimated that 38.4 million people were living with HIV infection and 1.5 million new cases were reported (22). The introduction of combined antiretroviral therapy (cART) for HIV was one of the greatest achievements in the fight against infectious diseases. Therapeutic interventions with cART results in HIV viremia suppression, restoration of the immune system, and improvement in the quality of life of HIV-infected individuals (22). By the end of 2021, more than 27.5 million people around the world were receiving cART. In Sub Saharan Africa, the number of people on cART increased from 100 000 infected individuals in 2003 to 6.1 million in 2011 (24). This highlights the important role cART is playing in the management of HIV infection. Currently, the antiviral agents used in HIV infection which target different phase of HIV replication cycle include 1. Nucleoside reverse transcriptase inhibitors (NRTIs) which block the reverse transcriptase enzyme (e.g. abacavir, emtricitabine,Lamivudine, tenofovir disoproxil fumarate, and zidovudine); 2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) which bind and later changes the reverse transcriptase enzyme (e.g. doravine, efavirenz, etravirine, nevirapine, and rilpivirine); 3. Protease inhibitors (PIs) which blocks viral protease enzyme (e.g. atazanavir, darunavir, fosamprenavir, and ritonavir); 4.Fusion inhibitors (FIs) prevents the virus from entering the CD4 T lymphocytes (e.g. enfuvirtide); 5. CCR5 antagonists block the CCR5 coreceptors (e.g. maraviroc); 6. Integrase strand transfer inhibitor (INSTIs) block HIV integrase (cabotegravir, dolutegravir, and raltegravir); 6.Attachment inhibitors (AIs) bind to the gp120 protein (e.g. fostemsavir); 7. Post-attachment inhibitors block CD4 receptors (e.g. ibalizumab-uiyk); 8. Capsid inhibitors interfere with HIV capsid development (e.g. lenacapavir); and 9.

Pharmacokinetic enhancers which are used to improve the effectiveness of drug (s) HIV medicine (e.g. cobicistat) [A]. These achievements are being derailed by the emergence of drug resistance and multidrug resistant strains which are major contributors to treatment failure, risk of HIV-associated diseases progression, and increased mortalities. In addition, cross-resistance between the same classes of drugs has been reported. Three types of drug resistances have been described as acquired resistance, transmitted resistance, and multidrug resistance (24). The World Health Organization (WHO) HIV Drug Resistance Report of 2021 stated that prevalence of acquired and transmitted HIV drug resistance among cART naïve individuals has increased exponentially over the course of years and it would become one of the most essential obstacles to objective of ending HIV pandemic as global health threat by 2030[B] . The report indicated that 10% of adults that commenced HIV treatment

have resistance to NNRTIs while people who were previously exposed to antiretroviral (ARV) agents are three times more likely to develop resistance to NNRTIs. Prevalence of three or four-drug-resistant viruses is approximately 5 to 10% in ARV-experienced individuals in Europe while in North America, it is less than 3% (22).

An important factor in the development of resistance in HIV infection is the virus's high rate of mutation with a study reporting that mutation rate can be 3×10^{-5} mutation per replication cycle while another studies from clinical samples suggested a rapid turnover rate of between 10^8 and 10^9 virions per day. This high mutation rate results in the emergence of genetically diverse set of HIV viruses from a single viral genome leading to the development of heterogeneous population referred to as quasispecies. The main etymology for the high mutation associated with HIV is due to the absence of 3' to 5' exonucleolytic proofreading system of HIV-1 reverse transcriptase. Mutations are therefore initiated during error-prone DNA synthesis when base substitutions, frame shifts, genetic rearrangement, and hypermutations are generated (34). The host RNA polymerase II is also another etymology for emergence of mutation during the synthesis of +-strand viral RNA; although O'Neil argued that majority of changes in the viral genome is due to mistake by HIV-1 RT instead of host cell RNA polymerase (35). Finally, genetic recombination also leads to mutation which is not harmful to the virus and maintains the genomic information under selective pressure like use of ART therapy. Recombination arises during minus-strand or occasionally during +-strand DNA synthesis and linked with single cross-over. The viral ability to recombine provides the virus with an efficient mechanism to enable the redistribution of mutations while increasing the differences in the viral population. Emergence of multidrug resistant variants via recombination impedes ART therapy.

Transmitted drug resistance (TDR) can be transmitted from one individual to another. Because the HIV resistant strain is transmitted to newly infected individual, such individual may carry the drug-resistant strain even though he/she has not started treatment yet. Althoughit is suggested that HIV drug resistant can be transmitted, the numbers remain low. Drug resistance in transmitted resistance is associated with mutation in the gene which confers resistance to either NRTI or NNRT. Drug transmitted resistance to PIs is not common (24), astudy reported that 39.3% harboured NNRTIs resistance-associated mutation, 1.7% harboured PI resistance-associated mutation, 1.0% harboured NNRTI and NRTI resistance- associated mutations, 1.4% harboured NNRTI and PI resistance-associated mutation while 0.3% harboured NNRTIs , NRTIs, and PI resistance-associated mutations with the most common mutation included V179D, E138A, V106I, K103N, V179E, and V179T. K103N was most found in CRF01_AE and CRF07_BC, V179D and E138A were mostly found in CRF08_BC, and V179T was found in CRF01_AE (27). This suggests that mutation can be strain specific.

Acquired drug resistance (ADR) on the other hand is associated with increased drug resistance among patients who are on ART. A systematic review by Stadeli & Richman (61) found that acquired resistance was detected among approximately 7.2% of patients who were on ART for 6-11 months in comparison to 11.1% at 12-23 months. Multi-class drug resistance also increased based with time on ART. This suggests acquired resistance rates depend on duration of ART treatment and types of antiviral agents prescribed. However, a study by von Wyl et al (62) reported that although there was increase in ADR at population level depending on time frame, the population level antiviral drug resistance decreased from approximately 50-57% from 1999 to 35-42% to 2007 which was due to exposure to regimen

that consisted of only nucleosides. This was consistent with the study of Mtambo et al (63) who reported that emergence of ADR was dependent on NNRTI-based regimens vs. PI-based regiment (9.8% vs. 11.0%). Stadeli & Richman (61) also found that for the time periods of 6-11 and 12-23 months, high number of patients on NNRTI developed resistance mutation in comparison to those on NRTI. Higher rate of NNRTI- vs. NRTI- vs. PI-based regimens were also evaluated by Musengimana et al (64) as 90.4%, 75.5%, and 3.5%, respectively. NRTI-based mutations were commonly due to K65R, M184V, and D67N while for NNRTI, K103N and Y181C/I/V/YC were more common. With regards to HIV subtypes, there is the suggestion that some subtypes are more prone to emergence of resistance than others; however this correlation needs to be evaluated.

1. **Resistance to NRTIs:** Resistance to NRTIs is due to three phenotypic mechanisms: 1. Discriminatory mutations thatfavour binding of physiological nucleosides over that of the drug, 2. Nucleoside-associated mutations (NAMSs) facilitates the removal of chain terminators from the cDNA, and 3.

   Insertion at p6 region within the gag gene which favours the virus evading from NRTI via greater accumulation of RT molecules per virion (36). In zidovudine (AZT) therapy, high- level resistance is linked with mutations M41L, D67N, k70R, L210W, T215Y/F, and K219Q, although most AZT-resistant isolates have been found to consist of only a subset of these mutations (37). Furthermore, these mutations were also found in patients who were on stavudine (d4T) therapy and associated with conferring resistant to this drug but at lower extent than AZT. These mutations that result to resistance to AZT and d4T are referred to as thymidine associated mutations (TAMs). It was also been reported in about 10% of individuals who are on didanosine monotherapy (37). Patients on ddl, ddC or 3TC therapies are also associated with rapid emergence of resistance due to a single mutation including L74V, K65R, or M184V. L74V mutation was found in patients on didanosine therapy and also in combination with other mutations in patients who were on abacavir monotherapy.

   This mutation is also associated with 2- to 5-fold resistance to ddc. M184V mutation is rapidly selected in patients who are on Lamivudine monotherapy and associated with conferring high-level resistance to this drug and emtricitabine as well as in abacavir monotherapy but less frequent in didanosine and zalcitabine monotherapy. K65R mutation is linked with intermediate resistance to didanosine, zalcitabine, Lamivudine, abacavir, and tenofovir (37). A study found that this mutation also confer high fold resistance to stavudine when there were no TAMs (38).

2. **Resistance to NNRTIs:** Resistance to NNRTIs in most cases is due to single mutation located at or near the drug binding pocket (36). Several mutations that confer resistance to NNRTIs have been described. As highlighted earlier, NNRTIs bind to the nonconserved sites which were found at some distance from the polymerase active sites. This means high mutation rate in the RT leads to selection pressure that result in the development of resistant mutation in RT against this class of drugs (NNRTIs) (39). The following mutations have been commonly associated with NNRTI resistance: K103N, L100I, K101E, Y188C, G190A, and E128K (Figure 2). Of these, the most common are K103N and Y181C. The protein structure and conformational changes linked with these mutations is yet to be fully elucidated. However, the other mutations that involves residues that link directly with NNRTI is suggested to be due to either loss of essential

interactions with the inhibitor or steric discordance that are introduced by the mutations such as L100I (39).Several studies have therefore suggested that emergence of resistance mutation results in small conformational changes across the RT enzyme with these conformational changes have significant implication on RT dynamics (39)

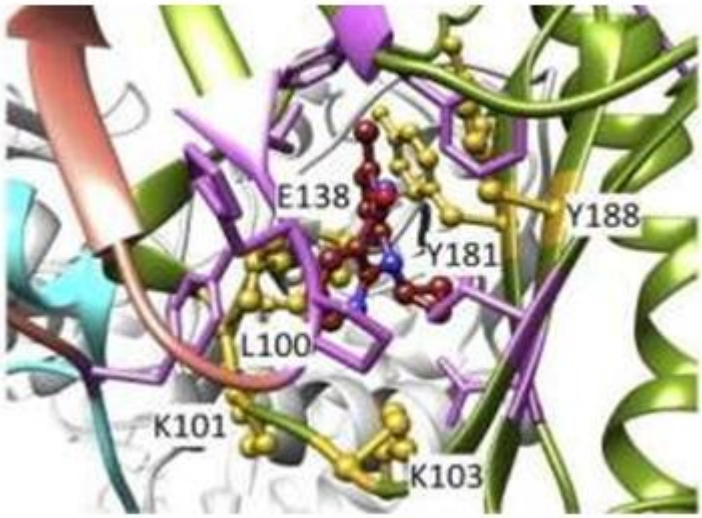

Figure 2: *Some resistance mutations described in NNRTIs (Source: 39)*

3. **Resistance to INSTIs:** The first-generation INSTIs have also been associated with emergence of resistance. Single mutation can lead to resistance to these drugs such as raltegravir (RAL) and elvitegravir (EVG). However, multiple mutations are needed for the emergency of resistance to second- generation integrase inhibitors such DTG. The following mutations were identified with the ability of conferring resistance to INSTIs: G118R, R263L, S153Y, Y143, M501, N155, and Q148. This resistance mutation seems to confer only low level cross-resistance to these secondary-generation drugs, Q148 is involved with significant decreased in susceptibility to all the drugs of INSTIs family (28). Q148 should therefore be monitored closely when using these INSTIs. M501 mutation has been reported in INSTIs-naïve patients who were treated with DTG and also found in combination with R263K in a patient who failed treatment with RAL. This suggests that M501 can compromise the activity of DTG among INSTI-naïve patient and also play a part in the emergence of cross-resistance among treatment- experienced patients (32). However, a study reported that M501 mutation is not associated with restoration of loss in HIV-1 infection linked with R263K and can confer moderate resistance to DTG (33). M501 in combination with R263K increases the risk of DTG resistance than R263K; similar effect was reported with EVG. However M501-R263K resulted in low-level resistance to RAL. M501 alone was not linked with resistance to DTG or RAL but can lead to the emergence of low-level resistance to EVG. Other minor mutationsthat have the potential of contributing to DTG resistance include T97A, S119R, and S147G.

   R263K and E157Q mutations have also been associated with resistance to INSTIs (31). R263K was first identified in vivo following the emergence of DTG resistance mutation. A site-directed mutagenesis study showed that R263K mutation conferred low-level resistance to DTG (32). E157Q can partially restore reduced integrase enzymatic activity

as a result of R263K mutation, suggesting that it might be a secondary compensatory mutation. In addition,double mutation of E157Q and R263K is associated with increased DTG resistance by tenfold in comparison with low-level resistance linked with R263K mutation alone. Another secondary mutation, H51Y is linked with R263K in DTG resistance. Mutation E92Q or N155H have been shown to be factor for the emergence of R263K (31).

MK-2048, a second-generation INSTI have been shown to also select a resistance mutation atG118R. In comparison to the first-generation INSTIs, these second-generation INSTIs possess higher genetic barrier for resistance which can be linked to a moderate fold change (FC) of <2.5. It must be added that accumulation of multiple resistance is needed for FC of <10 (31). Single mutation in the integrase gene is linked to resistance to raltegravir (RAL) with mutations Y143, Q148, and N155 implicated. Similarly, resistance to elvitegravir (EVG) was due to mutations at position Q148 and N155 in the integrase gene. Q148 and N155 also confer cross-resistance to EVG. However, Y143 are specifically associated with RAL.

4. **Resistance in Fusion Inhibitors:** In fusion inhibitors, several mutations have been found in enfuvirtide that reduces the susceptibility of HIV to this drug. With maraviroc, two mechanisms have been reported indrug resistance: either through the virus accumulating mutations that results in it using inhibitor-bound CCR5 or the virus can switch from using CCR5 to utilizing CCR4 as co- receptor for cell entry (24).

   Bouba et al evaluated HIV-1b gp120 sequences in both ART-naïve and ART-experienced individuals and found several mutations which was associated with fostemsavir resistance including L116Q, S375H/M/T, M426L, and M434I which was associated with HIV-1 gp120 polymorphism (25). However, the study by Kozal et al reported no resistance to fostemsavir among adults with multidrug- resistant to HIV-1 infection (26).

5. **Protease Inhibitors Resistance:** Protease inhibitors are known to possess high genetic barrier to resistance. Darunavir (DRV) possess genetic barrier due to its dual mechanism of action; protease enzymatic inhibition activity and protease dimerization inhibitor activity. However, highly DRV-resistant by HIV-1 variant was generated in vitro. These variants were shown to possess eleven mutations in their protease: A71V, V32I, L33F, 154M, L10F, S37N, M461, 147V, 150V, L63P, and 184V. These mutations conferred resistance to DRV (29). Furthermore, DRV resistant has been reported in patients on long-term cART consisting of DRV (30). These DRV-resistance variants are associated with treatment failure. V321 was identified as key substitution in DRV-consisting regimen therefore commencement or continuation of DRV-consisting regimens should be considered and monitored carefully (29). It must be added that a data showed that the presence of A71V is associated with HIV-1 acquiring V321 in the pathway towards the emergence of high-level DRV resistance. This therefore suggests that the presence of A71V facilitates the emergence of V32I. This mutation must be monitored when analysing DRV-associated resistance.

IV. HEPATITIS C VIRUS (HCV)

HCV is a positive, single-stranded RNA virus that belongs to the *Flaviviridae* family of the genus *Hepacivirus*. More than 180 million people are persistently infected with the virus

around the globe, with majority at risk of developing chronic HCV infection which is associated with advanced liver disease and hepatocellular carcinoma (40, 41). Due to increased understanding of the biological process of HCV including its life cycle, there are now increased pools of antiviral agents for treating HCV infection. The utilization of direct-acting antiviral (DAA) agents has led to increased sustained virologic response (SVR) rate which has played a role in managing this epidemic. Currently, HCV DAAs are grouped into 4types: NS3/4A protease inhibitors, NS5A inhibitors, nucleotide analog inhibitors of NS5B RNA-dependent RNA polymerase (RdRp), and non-nucleoside inhibitors of RdRp (40). The approved series of interferon-free regimens for HCV infection includes combination of Boceprevir, Simeprevir (SMV), Dasabuvir (DSV), Sofosbuvir (SOF), Paritaprevir, Declatasvir, Ledipasvir (LDV), Ombitasvir (OMV), Dasabuvir (DSV), Grazoprevir (GZR), Telaprevir,and Elbasvir (43,44). The use of DAA agents has the potential of eradicating HCV. However, HCV drug resistance has become a significant global health burden. Lack of proof-reading activity of RdRp in addition to high replicative ability of the virus is associated with the development of resistance to DAAs. This results in the emergency of large pool of genetically distinct viral variants in an infected individual. These HCV variant are referred to as quasispecies with some quasispecies variant have changes in the drug-targeted gene, with some able to confer resistance to DAAs. These quasispecies are due to amino acid polymorphism that emerges as a result of mutation during the replicative cycle and are selected based on the viral fitness. In addition, these quasispecies enable the virus to evade the immune responses. Emergence of resistance-associated amino acid variants (RAVs) from HCV quasispecies depends on the drug-, host-, and virus-associated factors. This suggests that the potency of the drug is primarily influenced by the susceptibility of the virus, the past exposure to the drug, and the genetic barrier to resistance. The ability of RAVs to persevere and induce treatment failure is associated with its fitness or replication ability in comparison to the wild-type variants (43).

Natural mutation within the viral protein region that are essential for antiviral activities of DAA may lead to reduced susceptibility to DAA or a class of DAA. Such mutation can be found in highly fit viral population. In most cases, mutations in HCV are found in minor viral populations due to their reduced fitness in comparison to wild-type viruses (42). Each of the drugs in DAA is associated with a specific mutation profile which is characterized by differences in the genotype or subtype. In addition, each of the drug type is also associated with difference in the genetic barrier to the induced resistance. Although the induced mutation are specific, there is concern that cross-resistance between these drugs in the same inhibitor class might be possible, especially NS3 protease and NS5A inhibitors (43). Several studies have described resistance-associated mutations in NS3, NS5A, and NS5B in HCV genotypes 1a, 1b, and 3a. Iio et al reported that by exploring the treatment outcome of DSV and ASV among 641 patients enrolled in Japan, the following base-line drug resistant mutations were identified: L31F/I/M/V, Q54H, P58S, A92K, and Y93H in HCV NS5A region while V36A, T54A/S, Q80K/L/R, R155K/T/Q, A156S/V/T, and D168A/E/H/T/V were found in the NS3/4A region (46). These RAVs were associated with treatment failure in patients with chronic hepatitis C genotype 1. The treatment outcome was significantly different between those with and without pre-existing NS3/4A 168 polymorphism after SMV failure. However the treatment outcome was not associated with duration of SMV failure and the commencement of DCV/ASV treatment. A study by Faiz et al (2023) found the following mutations in HCV 3a genotype in Pakistan: Leu36Pro, Gln41His, Gln80Lys/Arg, Ala156Tyr, and Gln168Arg in the NS3 region and Leu159Phe and Cys316Arg in the NS5B region. They reported that the overall prevalence of NS3 resistance-associated mutation was

22.5% for genotype 3a (43) while another study reported that the prevalence of mutation was 30% in the NS3 region. However, the study identified two mutations that involved Val36Leu and Asp168 Gln (45). These mutations can therefore be utilized as predictive biomarkers of HCV genotype 3a resistance. These mutations are associated with resistance to Boceprevir and Telaprevir. Gln80Lys polymorphism is also associated with resistance to Simeprevir in genotype 1a patients while Ala156Thr was associated with resistance to Glecaprevir (43). Furthermore, polymorphism of Glutamine at position 168 to Arginine is linked with resistance to Simeprevir and Gelcaprevir. However, Val170lle substitution was not linked with drug resistance (43). Polymorphism Cys316Asn and Leu159Phe were reported in genotype 1b and genotype 3 who failed Sofosbuvir therapy. The effect of these mutations is yet to be fully elucidated (43).

Another described resistance phenomenon associated with DAA regimens is pre-treatment risk factors in which natural resistance-associated substitutions (RASs) plays significant role. In a proof-of-concept study, Cento et al reported that among 139 patients who were on first-line all-oral DAA regimen, 74% of the patients presented pre-treatment risk factors for which drug failure with natural RASs was detected in 32.1% of the patients consisting of 15.3% NS5A RASs with polymorphism Y93H found in genotype 1b and 3b while polymorphism F28C was found in genotype 2C (47). NS3 RASs has been reported in simeprevir, paritaprevir, and grazoprevir mostly involving genotype 1b due to frequent mutation Y56F grazoprevir RAS while NS5A RASs were also identified in daclatasvir, elbasvir, ledipasvir, Ombitasvir and velpatasvir regimen in HCV genotype 1a and 3a. The most frequent NS5A RASs were due to polymorphism Y93H in genotypes 1b and 3a while in genotype 2c, F28C was more frequent. In some cases, double and triple RASs were detected simultaneously in genotype 1a consisting of polymorphism M28V plus Q30R plus L31M. This risk factors (RASs) should be taking into consideration when initiating treatment for HCV infection with viral resistance profile for first-line DAA regimens evaluated which aid establishing the response among DAA-naïve patients. HCV antiviral resistance can be subtype dependent and loss of susceptibility can be attributed to polymorphisms in the genotypes.

V. INFLUENZA VIRUS

Influenza virus is a member of the *Orthomyxoviridae consisting* of single-stranded RNA (ssRNA) with segmented genome enveloped by viral nucleoprotein (NP) (48). They are grouped into A, B, C, and D types. Types A, B, and C influenza viruses can infect humans. Types A and B are the primary seasonal strains that cause mild to severe respiratory infections as well as other complications among humans. Influenza viruses are further subdivided based on their antigenic characteristics and genomic sequences of their surface glycoprotein hemagglutin (HA) and neuraminidase (NA). There are currently 18 HA and 11NA subtypes which are found in nature for influenza A virus (IAV). Depending on HA, IAV is classified into group 1 and group 2. Influenza B (IBV) on the other hand does not belong to groups or subtypes but it is grouped into two major lineages: B/Yamagata and B/ Victoria (48). Influenza vaccination is one of the best protective methods used against influenza morbidity and mortality with vaccine effectiveness from 10% to 60% in the United States (49). Factors association with this variation includes age, vaccine mismatch, weight, sex, and immune status (48). Improving vaccine efficacy for influenza virus prevention and protection should be focus of global efforts. This effort is being compounded by host and vial factors. Antiviral agents are essential tool for the effort against influenza viruses (Fig 2).

Among the antiviral agents used in treating influenza virus includes Oseltamivir, Zanamivir, Laninamivir, and Peramivir (NA inhibitors that inhibits NA activity and viral egress from the cells). Other agents are Baloxavir which inhibits viral replication through the inhibition of polymerase acidic protein (PA), Amantadine and Rimantadine (M2 ion channel inhibitor), and Favipiravir (RNA-dependent RNA polymerase inhibitor). It should be highlighted that these drugs do not cure influenza infection but are associated with reduction of time to clinical resolution. NA inhibitors and Baloxavir are recommended for treating individuals who have been infected with avian influenza viruses such as A (H5N1), A (H7N9), and A (H5N6) (48). The global effort in effective management of influenza can be impeded by the emergence of antiviral-resistant viruses that can have devastating impact. Emergence and spread of antiviral-drug resistance in influenza virus is driven by high evolutionary rates as a result of combination of rapid mutation which is about 1 error per virus genome, per replication as well as rapid replication (50). This diverse population of influenza virus is generated in an individual. Most of the multitude polymorphisms that are generated in the viral genome play essential roles in the viral phenotype such as ability to confer antiviral resistance. Antiviral resistance has been described for almost all known antiviral agents. The emergence of antiviral resistance among the influenza viruses can vary, with IBV having the lowest resistance rates than IAV (50). The World Health Organization reported that among NA inhibitors, resistance rates is 0% for IBV while for IAV is was 0.80% for A/H1N1 pdm09(50). Although the rationale behind this is yet to be fully elucidated, it may be due to lower antiviral inhibition which is higher for IBV than IAV and the selection pressure that is confer by the drugs on IBV. Amino acid substitutions are the mechanism associated with influenza virus antiviral resistance. These have been described for all the three classes of antiviral agents associated with influenza virus: M2 ion channel inhibitors, NAIs, and polymerase inhibitors. In M2 ion channel inhibitors, S31N substitutions involving a serine to asparagines substitutions at residue 31 in M2 protein is associated with anti-influenza virus resistance.

These substitutions results to the disruption of binding ability of the antiviral agents via alteration of the hydrogen-bonding matrix in the ion channel pore (51). Furthermore, amino acid substitutions at positions 26, 27, 30, 31 or 34 of M2 protein is associated with conferring drug resistance while recombinant A(H1N1) viruses that consist of the following polymorphisms Leu26Phe, Val27Ala, Ala30Thr, Ser31Asn, Gly34Glu, and Val27Ala/Ser31Asn in the M2 gene is also linked with Amantadine resistance (48). M2 mutants preserve their virulence and can be transmitted between humans. While about 95% of resistant viruses had Ser31N, only 1% had Val27Ala polymorphism. This means, Ser31N are the most dominate circulating mutant influenza virus. Data from Vietnam, Cambodia, Malaysia, and Thailand found that M2 mutations consisting of Ser31Asn and Val27Ala were dominant among circulating H5N1 viruses (52).

With NAIs, amino acid substitutions change the shape of NA enzymatic sites which leads to reduction in the ability of antiviral binding to NA thereby conferring resistance. Such polymorphisms are specific based on influenza virus specific and subtype specific which means the same substitution can have differing impact on the binding ability of different NAIs. Several polymorphisms that involve substitutions or deletions are linked with resistance to one or more NAIs. Substitutions such as His275Tyr and Asn294Ser have been described in humans and birds. Mutations R292K, and E119D have been found in oseltamivirand zanamivir, respectively. However, the presence of E119D mutation does not

impact oseltamivir (53). Other mutations reported are Ile117Val, Glu119Ala, and Arg292Lys and found to have impacts on the utilization and efficacy of NAIs. Kode et al reported of I117T polymorphism that conferred resistance to oseltamivir and zanamivir by 18.6 – and 11.8- folds, respectively (56). Further evaluation showed that this substitution conferred cross- resistance to both oseltamivir and zanamivir. Previous study identified E119V polymorphism as able to confer cross-resistance to oseltamivir, zanamivir, and peramivir at 1,727-, 2,144- and 5,-5- folds, respectively (58). Importantly, a study reported that the following mutant influenza viruses were resistant to all NAs: E119D, E119A/D/G-H274Y (57). This means single substitution is capable of conferring resistance to multiple neuraminidase inhibitors.

With regards to viral polymerase, the mechanism of action of based on the presence of RdRp which consist of three subunits, PA, PB1, and PB2. These subunits are associated with transcription and replication activities. This means the viral polymerase is an essential and attractive drug target in anti-influenza drug. Drugs such as Baloxavir acid (BXA) are viral polymerase inhibitors that target the cap-dependent endonuclease activity of PA. A pro-drug Baloxavir marboxil (BXM) is able to inhibit viral polymerase activity of most subtypes of influenza A viruses such as A (H1N2). A study by Mushin et al reported that baloxavir has broad activity against type A, B, C, and D influenza viruses (54). Resistance to viral polymerase has been reported which was described in the presence of these drugs.

Polymorphism through substitutions results in conferring resistance to anti-influenza agents. Substitutions that affect baloxavir activity consist of mutation from Ile at residue 38 to Thr, Phe, or Met. This means for e.g. Ile38Thr leads to reduction in binding affinity of the drug in the binding region thereby resulting in baloxavir becoming less effective. Similarly, *in vitro* study showed that PA/I38X polymorphism led to reduction in susceptibility to baloxavir (55).However, PA/I38X polymorphism does not have an impact on oseltamivir.

Due to selective pressure of antiviral agents, it is recommended that there is the need for regular screening of circulating influenza viruses for susceptibility to recommended antiviral drugs. This is because as a result of lack of proofreading activity, influenza virus is prone to high genetic mutation that leads to about 1 error per replicated genome. This means the global effort should be focused on developing alternative anti-influenza viral infection.

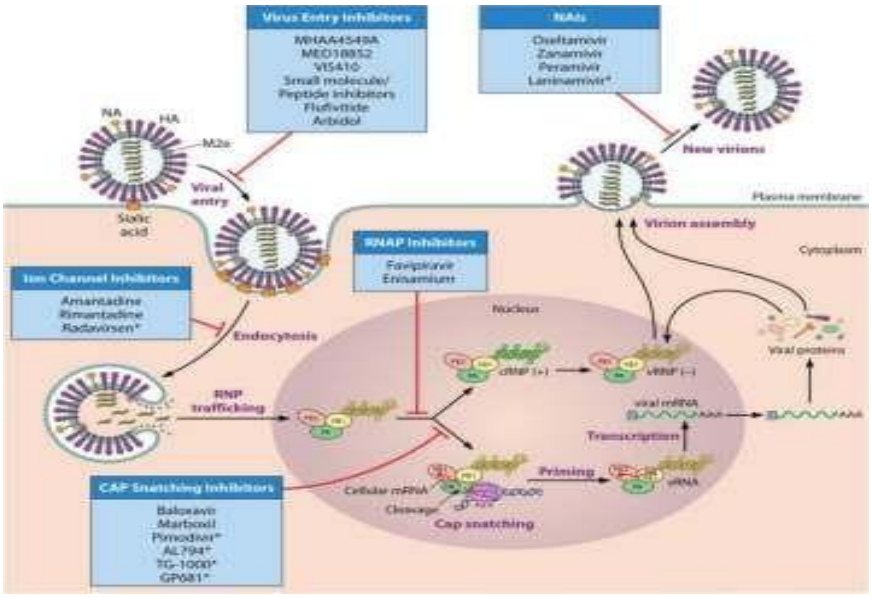

Figure 3: *Life cycle of influenza virus and sites of action of anti-influenza viral targets (Source:Biorender)*

VI. CLINICAL SIGNIFICANCES OF ANTIVIRAL DRUG RESISTANCE

Antiviral drug resistance is associated with several clinical implications including toxicitydue to utilization of second –line antiviral agents, severe diseases and even death not forgetting the high economic burden. For example in HSV infection, antiviral resistance is associated with direct effect of the viral infection and toxic effects of second-line drugs.

When the viral replication is not inhibited by antiviral agents, it leads to progressive and occasionally fatal invasive HSV infection. Chen et al (65) reported that recurrent, chronic, and extensive mucocutaneous HSV ulceration is seen in immunocompromised patients with drug-resistant HSV. During the COVID-19 pandemic, it was observed that frequent use of antibiotics in patients with COVID-19 facilitated the development of antimicrobial resistance (AMR). A study by the Antimicrobial Resistance Collaborators (66) showed that 1.27 millionmortalities were linked to bacterial AMR in 2019. Other evidence suggested that there was increase in COVID-19 associated hospitalization due to increased AMR that included methicillin-resistant *Staphylococcus au*reus and vancomycin-resistant *Enterococcus* (67). Thesuggestion is high consumption of antibiotics during the COVID-19 pandemic may have contributed to the emergence of AMR. However, more research are needed to understand whether there is any molecular mechanism that facilitates the emergency of antibiotic resistance during antiviral infections. Antiviral resistance is also associated with severity of viral diseases. In HCV, the clinical consequences of antiviral drug resistant include worsening of liver histology and hepatic decompensation which in certain cases leads to death. It also leads to increase serum transaminases and other biochemical biomarkers (68). In HIV infection, antiviral drug resistance, leads to limitation of treatment options for individuals with the virus with will result in exhaustion of treatment options with a consequence of HIV disease progression and possible deaths. When drug-resistant HIV strains are transmitted, the new infections means increased number of individuals who will experience drug failure thereby putting considerable strain on the health system. This means transmission of HIV drug resistance mutant can be a threat to the global public health

achievements in reducing morbidity and mortality. Antiviral resistance impacts clinical response. In Influenza, evidences shows that the utilized antiviral agents facilitates clearing of the virus, reduce the duration of disease, transmission and risks of deaths (69). Antiviral resistance in influenza virus infection is therefore a clinical and public health concern. There is strong evidence to suggest that resistance in influenza virus can also affect the susceptibility of vaccines used for B-lineages (70). In addition, influenza resistant leads to the emergence of novel mutant strains thereby raising concern of widespread and sustained transmission of the virus within the communities (69). This means detection of resistance due to previously unknown polymorphism should be an essential component of clinical management scheme of viral infections.

VII. PCR-BASED CRISPR-CAS13A ASSAY FOR ANTIVIRAL RESISTANCE

Rapid detection of nucleic acid using highly sensitive and specific assay would help facilitate the diagnosis of disease, epidemiological studies, monitoring, and other general laboratory activities. Several methods are available that are used for detecting nucleic acid in antiviral assay. However, these assays have some limitations with regards to sensitivity, specificity, cost, and speed (59). Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (CRISPR-Cas) adaptive immune systems that consist of programmable endonucleases can be used as CRISPR-based antiviral diagnostic (CRISPR-avDx). Some Cas enzymes are used in targeting DNA while single effector RNA-guided RNAases such as Cas13a is used in the provision of specific RNA sensing. This is performed by reprogramming with CRISPR RNAs (crRNAs). When the targeted RNA is recognized, the activated Cas13a take part in cleavage of nearby non-target RNAs. This process results in Cas13a detecting the presence of specific RNA via inducing programmed cell death *in vivo* or by nonspecific degradation whereby RNA is labelled *in vitro*. One such *in vitro* nucleic acid amplification which uses Cas13a-mediated cleavage of the reporter RNA is Specific High Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) assay which permits the real- time detection of nucleic acid target. Study by Gootenberg et al (59) suggested that this assay can be utilized as point-of-care with SHERLOCK possessing same level of sensitivity as digital droplet polymerase chain reaction (ddPCR) and quantitative PCR (qPCR), which are sensitive nucleic acid detection systems. Furthermore SHERLOCK has less variation than ddPCR and qPCR. SHERLOCK can also be used in differential detection of different polymorphisms in resistance assay and also readily detect such mutations at low concentrations. This means, platforms such as SHERLOCK can be used for rapid antiviral resistant genotyping. Finally, SHERLOCK can be performed for as low as $0.61 per test.

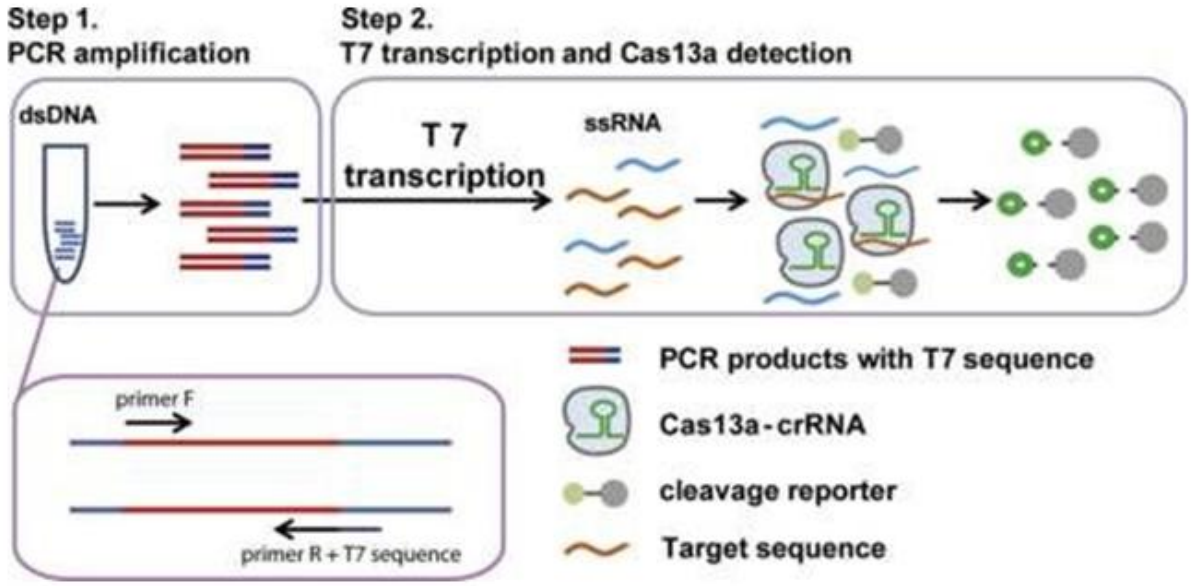

Figure 4: *Schematic representative of CRISPR assay*

PCR-based CRISPR-Cas 13a assay has been used for detecting mutation in infectious diseases. Wang et al (60) used this system in evaluating its ability to detect low level hepatitis B virus DNA and drug mutation in clinical samples. They reported that the system was highly sensitive and specific in the detection of HBV DNA and drug resistance polymorphism. The advantage was drug-resistant mutation in the clinical samples were detected with low concentration of clinical samples. Therefore PCR-CRISPR-Cas13a system can aid in improving the effect of therapeutic interventions which involves drug resistant mutant with low viral loads. PCR-CRISPR-Cas13a is therefore a novel system that can be used for wider utilization for early detection of infectious infections, monitoring treatment efficacy and drugresistance. An international guideline is needed on the potential use of this system in drug resistance diagnosis.

VIII. CONCLUSION

Antiviral agents have been used for effective interventions which have led to reduction in morbidity and mortality of viral diseases. However, this achievement is being derailed by the emergence of drug resistance. Almost all human viruses of international public health interest have developed mechanisms to evade both the antiviral drugs and immune system. Antiviral resistance are associated with certain clinical consequences such as toxicity, severity of diseases, and immense burden on healthcare system. AMR is predicted to be one of the leading risk factors for long hospital stays and possible deaths by 2050. With selective pressure being a risk factor for the emergence of drug resistance, AMR would continue to be a global public health concern. This means more resources should be channel towards identifying molecular mechanisms of resistances for antimicrobial agents and novel management tools including evaluating more treatment options through drug repurposing approaches.

CONFLICT OF INTEREST

Authors do not have any conflict of interest to declare for this work

USE OF ARTIFICIAL INTELLIGENCE (AI)

Authors declare that no aspect of AI was used in preparing this articleFootnote

- https://hivinfo.nih.gov/understanding-hiv/fact-sheets/fda-approved-hiv-medicines
- https://iris.who.int/bitstream/handle/10665/349340/9789240036608-eng.pdf?sequnce=1

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