(b) Genotypic Methods —
- Identify specific genetic markers associated with drug resistance, providing a faster and more precise means of determine susceptibility

(i) PCR & Sequencing —
- specific mutations in protozoan genes that confer drug resistance can be identified by PCR & sequencing.

(ii) RTPCR —
- Used to assess the expression levels of gene involved in drug resistance.

(iii) Gene Arrays & Microarrays —
- This technology allows for simultaneous detection of multiple mutations or the analysis of gene expression profiles related to drug resistance.

(iv) NGS —
- NGS is particulary valuable in detecting novel or rare mutations that confer resistance to existing anti-protozoal agents.

• Interpretation of Result —
- The Results determine if the pathogen is susceptible, intermediate or resistant to the drug.
- Results helps doctors choose the best drugs to treat infections.

• Protozoal Species Commonly Tested —
1) Malaria (Plasmodium sp.)
eg. Plasmodium falciparum

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Plasmodium vivax, Plasmodium ovale, Plasmodium malariae.

2) Leishmaniasis (Leishmania sp.)
eg. Leishmania donovani
Leishmania tropica

3) Trypanosomiasis (Trypanosoma sp.)
eg. Trypanosoma brucei
Trypanosoma cruzi

4) Amebiasis (Entamoeba histolytica)
- Treatment involves → Metronidazole, tinidazo and paromomycin.

• Anti-protozoal Drugs Commonly Tested -
1) Chloroquine and Hydroxychloroquine -
- become resistant in Plasmodium falciparum

2) Artemisinin-based combination Therapies -
- first line treatment for malaria.
- against antimisinin-resistant Plasmodium falciparum strains.

3) Sodium stibogluconate -
- for leishmaniasis.

4) Miltefosine
- Leishmaniasis especially in treating Leishmania donovani infections.

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5) Pentamidine & Nifurtimox —
- Treat Trypanosoma infections.

6) Metronidazole and Tinidazole -
- Used for Amebiasis and Giardiasis caused by Entamoeba histolytica and Giardia lamblia.

4) Susceptibility Testing for anti-viral Agents —
- Antiviral susceptibility testing determines how well a virus responds to antiviral drugs.
- This method refers to the process of evaluating the effectiveness of anti-viral agents against viral pathogens.
- As antiviral drugs become essential in managing viral infections such as HIV, Influenza, Hepatitis, and Herpes virus infections.

• Importance —

(i) Emergence of drugs —
- Just as with bacterial & fungal pathogens, viruses can develop resistance to antiviral drugs over time, making them less effective.

(ii) Personalized Treatment —
- Some viruses such as HIV & hepatitis C, exhibit significant genetic variability which can lead to differences in drug efficacy.

(iii) Monitoring Drug Resistance –
- Regular surveillance of drug resistance patterns, especially in high-risk populations, helps identify new resistance mechanisms & improve therapeutic guidelines.

• Methods –
(a) Phenotypic methods –
- Method focus on evaluating the virus's response to antiviral agents in terms of its ability to replicate.
(i) Plaque Reduction Assay (Focus-forming Assay)
- Used for many types of viruses, including herpesviruses & influenza, this method assesses the ability of antiviral agents to reduce the formation of plaques or viral foci in cell cultures.
- This assay provides a clear, quantitative measures of antiviral activity & is widely used for testing herpes simplex virus (HSV) & varicella-zoster virus (VZV).
(ii) RNA or DNA quantification (q-RTPCR)
- q-PCR is often employed to measure the amount of viral RNA or DNA in a sample.
- After treating infected cells with antiviral agents, the amount of viral nucleic acid is quantified.

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(iii) HIV phenotypic Assay —
- Specifically for HIV, the Phenosense assay measures the replication of virus in presence of antiretroviral drugs.
- The results give an idea of whether the virus is resistant to specific antiretroviral drugs & helps clinicians adjust the treatment regimen.

(b) Genotypic methods —
- Detects specific genome mutations that confer resistance to antiviral agents.

(i) PCR & Sequencing — HIV, hepatitis C, Influenza
(ii) RT PCR — RNA viruses → HIV, HCV & Influenza.
(iii) NGS — This method is highly valuable in identifying minor drug-resistant variants that may not be detected using standard sequencing methods.
(iv) Hybridization-Based Techniques -
(Line probe Assay) -
- are used to detects mutations associated with drug resistance.
- These assays involves the use of probes that hybridize to specific mutations in viral genome.

- Common Viruses Tested for Antiviral Resistance -

1) HIV -

- Antiretroviral drugs as - reverse transcriptase inhibitors (NRTIs, NNRTIs).
  - protease inhibitors (PIs)
  - Integrase inhibitors

2) Hepatitis B and C viruses (HBV & HCV) -
- Resistance to nucleotide analogs, for interferons & direct acting antivirals (DAAs) in chronic infections.

3) Influenza virus -
- oseltamivir
- zanamivir (neuraminidase inhibitors)

4) Herpes Simplex virus (HSV) -
- Resistance to acyclovir, valvacyclovir, famciclovir (targets HSV DNA polymerase)

5) Varicella-zoster virus (= VZV) -
- Similar to HSV, VZV can develop resistance to antiviral drugs, particulary in immunocompromised patients.

6) Cytomegalovirus (CMV) -
- Resistance to ganciclovir, valganciclovir & other antiviral drugs is a major concern in transplant patients.

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- Anti-viral Agents Commonly Tested —

1) HIV -
- Nucleoside & Non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs)
- protease inhibitors (PIs)
- Integrase Inhibitors (INSTIs)

2) Hepatitis C -
- Direct acting antivirals (DAAs) targeting NS3 protease, NS5A and NS5B polymerase.

3) Influenza -
- Neuraminidase inhibitors (oseltamivir, zanamivir).
- M2 Ion channel inhibitors (amantadine, rimantadine)

4) Herpes viruses -
- Acyclovir, valacyclovir and famciclovir: (DNA polymerase inhibitors)

5) Cytomegalovirus (CMV) -
- Ganciclovir
- valganciclovir
- foscarnet.