Comparison of Sanger and Next Generation Sequencing for Detection of HIV Protease Inhibitor Mutations

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Introduction

South African national guidelines recommend HIV drug resistance (HIVDR) testing for all HIV-infected patients failing a protease inhibitor (PI) based regimen1. Patients with PI Genotypic Susceptibility scores (GSS) >15 are eligible for 3rd line treatment. Detection of drug resistance mutations (DRMs) using Sanger sequencing remains the gold standard and is currently used for testing purposes. Implementation of next generation sequencing (NGS) considered an alternative approach in order to improve sensitivity, reduce cost and increase sample throughput. The aims of this study were to compare Sanger and Illumina NGS methods for their ability to detect PI drug resistance mutations among patients failing a PI regimen.

Materials and Methods

- Patients failing a PI regimen for at least one year were referred from across South Africa.
- Whole blood specimens were tested for HIV drug resistance at the HIV genotyping Unit at Charlotte Maxeke Johannesburg Academic Hospital.
- 162 specimens with PI mutations by Sanger sequencing were selected for this study.

Results

A consensus sequence was generated for each specimen using 15% cut-off to compare with Sanger sequences.

Validation - Pairwise analysis

Pairwise analysis was done to confirm the sequences similarity between NGS and Sanger. At the nucleotide level sequences pairwise distance were >99%, while at the amino acids level >98%.

Conclusion

- Detection of DRMs using MiSeq using Sanger sequencing showed high concordance (95.7%).
- The difference in the phenotypic interpretation of resistance was due to presence of discrepancies in mutations detected (4.3%).
- The discrepancies had minor impact on clinical interpretation as all GSS >15.
- The use of NGS for HIVDR testing is therefore reliable and allows for large sample numbers to be tested in a more efficient workflow and potentially be more cost-effective.

References


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