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Use of a Single Step Amplification-Sequencing Method (AmpliSeq) to Analyze Nevirapine Resistance Mutations in HIV-1 Variants from Ugandan Infants.

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Introduction: Several methods have been used to analyze nevirapine (NVP) resistance mutations in HIV from women and infants who received single dose (SD) NVP for prevention of HIV mother-to-child transmission. These include HIV genotyping assays based on population sequencing (e.g. the ViroSeq HIV Genotyping System), sensitive point mutation assays (e.g. ASPCR, LigAmp), single genome sequencing, and analysis of HIV clones (DNA purification and sequencing). In this study, we used a single step amplification-sequencing method (AmpliSeq) to analyze NVP resistance mutations in cloned HIV variants from Ugandan infants who were HIV-infected despite SD NVP prophylaxis. **Methods**: We selected plasma samples from six infants who had two or more NVP resistance mutations detected by ViroSeq. ViroSeq PCR products were ligated into a cloning vector and transformed into bacteria. AmpliSeq was used to analyze HIV-1 variants directly from bacterial colonies. AmpliSeq reactions were performed using commercial DNA sequencing reagents supplemented with additional dNTPs and two oligonucleotides. AmpliSeq reactions were performed using an Applied Biosystems 9700 thermal cycler. Reaction products were ethanol precipitated and analyzed on an ABI PRISM® 3100 Genetic Analyzer. Fifty HIV variants were analyzed for each infant sample. For each HIV variant, a single AmpliSeq reaction was used to analyze mutations at the following codons in HIV reverse transcriptase: 98, 100, 101, 103, 106, 108, 179, 181, 188, and 190. **Results**: In all six infant samples, each of the NVP resistance mutations identified by ViroSeq was also identified in one or more of the HIV-1 clones. Five of the six infants had additional NVP resistance mutations that were not identified by ViroSeq, and five infants had clones with two genetically-linked NVP resistance mutations. Among the 300 clones analyzed, as many as six different amino acids were encoded at a single position associated with NVP resistance. At five of ten positions analyzed, we found two different codons encoding the same amino acid. **Conclusions**: AmpliSeq provides a simple method for analyzing resistance mutations in HIV-1 clones, and provides information about genetic linkage. Analysis of HIV-1 clones from SD NVP-exposed infants revealed numerous NVP resistance mutations not detected by population sequencing, genetically-linked NVP resistance mutations, and a high degree of genetic complexity at codons that influence NVP susceptibility.