HIV and AIDS remain a major health burden worldwide. Majority of HIV and AIDS impact is borne by women. Among these women, expectant mothers who become infected by HIV and AIDS will transmit the infection to their infants. This can occur during pregnancy, labour and delivery, or breastfeeding. In order to deter HIV-1 virus spread to the unborn infants intervention strategies namely Antiretroviral (ARV) drugs and vaccines must be used. However, ARVs develop resistance due to evolution of new strains of HIV virus which are drug resistant. This suggests that new intervention strategies have to be developed to deter the spread to unborn infants. One of the options is to characterize the HIV virus in order to know the virus strains circulating among the infected population. The unique characteristics in the virus can be targeted in development of new ARVs, diagnostic techniques and vaccines. The aim of this study was to determine HIV-1 gag gene subtypes circulating among antenatal clinic clients in North-Rift Valley, Kenya. A total of 129 patients were sampled and used in this study. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood. Proviral DNA was isolated from PBMCs. Nested polymerase chain reaction (PCR) was carried out to amplify the viral DNA. Presequencing polymerase chain reaction (PCR) was carried out using nested gag primers. Sequencing was carried out in an automated DNA sequencer (ABI Prism 310) using Rhodamine Terminator cycle sequencing ready reaction kit. After sequencing, the generated sequences were analyzed by comparing them with available sequences at the Los Alamos HIV database using the basic Local Alignment Search Tool (BLAST) and then aligned using CLUSTAL W software. Phylogenetic trees were then constructed using the neighbor joining method. Evolutionary trees were used to monitor the extent of HIV-1 subtype variations in infected populations. On phylogenetic analysis of the 117 sequences based on partial gag gene, 81 (70%) sequences clustered with subtype A1, 13 (11%) with subtype D, 8 (7%) with subtype C, 5 (4%) with subtype A1D, 4 (3%) with subtype A1C, 3 (3%) with subtype A2, 1 (1%) with subtype A2C, and 1 (1%) with subtype G. These results suggest that HIV-1 epidemic may be evolving towards more complex subtypes through recombination due to viral mixing. Subtype surveillance of the circulating HIV-1 subtypes is therefore important in designing AIDS vaccines and determining which antigens need to be included in diagnostic testing kits for HIV-1. The HIV-1 antigens in the new strains of HIV-1 virus revealed in this study should be included in the design and development of future anti-HIV-1 vaccine, antiretroviral drugs and diagnostic techniques.