Human parainfluenza virus type 1 (HPIV-1), a paramyxovirus, is a leading cause of pediatric respiratory hospitalizations globally. Currently, there is no clinically successful vaccine against HPIV-1. Hence, there is a need to characterize circulating strains of this virus to establish the feasibility of developing a vaccine against the virus. The variable HPIV-1 hemagglutin-neuraminidase (HN) protein is found in the envelope of HPIV-1, where it initiates the infection process by binding to cellular receptors. HN is also the major antigen against which the human immune response is directed against. The present study focused on identifying mutations in the HN gene that would be useful in understanding the evolution of HPIV-1. 21 HPIV-1 isolates were obtained after screening nasopharyngeal samples from patients with influenza-like illness. The samples were collected from Mbagathi District Hospital Nairobi from the period July 2007 to December 2010. RT-PCR was carried out on the isolates using HN-specific primers to amplify a 360 nt in the most polymorphic region and the amplicons sequenced. Genomic data were analysed using a suite of bioinformatic software. Forty eight polymorphic sites with a total of 55 mutations were identified at the nucleotide level and 47 mutations at 23 positions at the amino acid level. There was more radical nonsynonymous amino acid changes (seven positions) observed than conservative nonsynonymous changes (one position) on the HN gene fragment. No positively selected sites were found in the HN protein. The result from the analysis of 21 HPIV-1 Mbagathi isolates demonstrated that the HN gene which is the major antigenic target was under purifying (negative) selection displaying evolutionary stasis.