Respiratory syncytial virus (RSV) is classified into subtypes A (RSV-A) and B (RSV-B), which are classified into different genotypes based on genetic variability of the G surface glycoprotein gene. The F surface protein gene is more conserved however variability in signal peptide, transmembrane domain, and antigenic sites have been reported. The study was conducted in the Virology laboratory, Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa. Study participants included patients of all ages from whom respiratory samples were submitted for respiratory viruses diagnoses from 2019 to 2020. The complete RSV F genes were amplified, library prep was preformed using the Nextera DNA prep kits and sequenced using amplicon-based next generation sequencing on the Illumina MiSeq sequencing platform. The Genome Detective Virus tool v2.27 was used to assemble sequencing reads and MEGA X was used for phylogenetic analysis. N-linked glycosylation and amino acid sequence variation was assessed. The overall prevalence of RSV was 5.8% (101/1 734). Seventy (69.3%; 70/101) RSV-positive samples were available for genetic characterisation of the F protein gene and thirty-one (30.7%; 31/101) were excluded due to insufficient sample volume. Only RSV-A strains were identified (91.2%; 31/34). Twenty three of thirty-one (74.2%) of the RSV F gene sequences from 2019 to 2020 clustered together with bootstrap values ranging from 64% to 99% and were NA1-like. A N-glycosylation site at position 120 gained by South African strains from 2018 is retained in strains from this study. This N-glycosylation site is present in approximately 25.8% of RSV strains from this study. The diversity of RSV-A F proteins was low, with amino acid variations observed at 30/571 (5.3%) sites. Ten mutations were detected in 4/6 antigenic sites (I, II, IV and V), with frequencies ranging from 0.3 to 100%. Antigenic changes seen exclusively among South African strains are: Y33H (0.3%) and V384T (7.3%) at site I and S275F (0.3%) at site II. Seven mutations associated with escape of human leukocyte antigen (HLA)-restricted cytotoxic T lymphocyte (CTL) were predicted in seven epitopes. Overall amino acid mutation frequency for 2019 to 2020 RSV-A F genes is similar to that reported for South African strains from 2018 (3.3% to 6.7%). For the first time in South Africa we detected the S275F mutation which causes palivizumab resistance.