Staphylococcus aureus is one of the most common pathogens that cause mastitis in dairy cows. Various subtypes, virulence genes and pathogenicity islands have been associated with isolates from bulk tank milk and clinical mastitis. So far, no Danish cattle associated S. aureus isolates have been whole-genome sequenced and further analyzed. Thus, the main objective was to investigate the population structure and genomic content of isolates from bulk tank milk and clinical mastitis, using whole-genome sequencing. This may reveal the origin of strains that cause clinical mastitis. S. aureus isolates from bulk tank milk (n=94) and clinical mastitis (n=63) were collected from 91 and 24 different farms, respectively and whole-genome sequenced. The genomic content was analyzed and a phylogenetic tree based on single nucleotide polymorphisms was constructed. In general, the isolates from both bulk tank milk and clinical mastitis were of similar genetic background. This suggests that dairy cows are natural carriers of the S. aureus subtypes that cause clinical mastitis if the right conditions are present and that a broad range of subtypes cause mastitis. A phylogenetic cluster that mostly consisted of ST151 isolates carried three pathogenicity islands that were primarily found in this group. The prevalence of resistance genes was generally low. However, the first ST398 methicillin resistant S. aureus isolate from a Danish dairy cow with clinical mastitis was detected.