Quinolones are important antimicrobials for treatment of leprosy, a chronic infectious disease caused by *Mycobacterium leprae*. Although it is well known that mutations in DNA gyrase are responsible for quinolone resistance, the effect of those mutations on the enzymatic activity is yet to be studied in depth. Hence, we conducted *in vitro* assays to observe supercoiling reactions of wild type and mutated *M. leprae* DNA gyrases. DNA gyrase with amino acid substitution Ala91Val possessed the highest activity among the mutants. DNA gyrase with Gly89Cys showed the lowest level of activity despite being found in clinical strains, but it supercoiled DNA like the wild type does if applied at a sufficient concentration. In addition, patterns of time-dependent conversion from relaxed circular DNA into supercoiled DNA by DNA gyrases with clinically unreported Asp95Gly and Asp95Asn were observed to be distinct from those by the other DNA gyrases.

DNA gyrase supercoiling activity largely changed by acquisition of quinolone resistance.