Antimicrobial surfaces are important in medical, clinical, and industrial applications, where bacterial infection and biofouling may constitute a serious threat to human health. Conventional approaches against bacteria involve coating the surface with antibiotics, cytotoxic polymers, or metal particles. However, these types of functionalization have a limited life-time and pose concerns in terms of leaching and degradation of the coating. Thus, there is a great interest in developing long-lasting and non-leaching bactericidal surfaces. To obtain a bactericidal surface, we combine μm-scale patterning of borosilicate glass surfaces by ultrashort pulsed laser irradiation and a non-leaching layer-by-layer polyelectrolyte modification of the surface. The combination of surface structure and surface charge results in an enhanced bactericidal effect against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. The laser patterning and the layer-by-layer modification are environmentally friendly processes that are applicable to a wide variety of materials, which makes this method uniquely suited for fundamental studies of bacteria-surface interactions and paves the way for its applications in a variety of fields, such as in hygiene products and medical devices.