In this study, nano silver particles (NPs) were applied on the surface of cotton fabric and stabilized by padding process using 1,2,3,4-butane-tetracarboxylic acid (BTCA) sodium hypophosphite (SHP). A colloid solution of silver NPs with 50 nm average of particle size was employed as an antimicrobial agent. BTCA and SHP were used as a cross-linking agent and catalyst, respectively. An aqueous solution containing 10 g/L BTCA and 5 g/L sodium hypophosphate was prepared. The cotton fabric samples were impregnated to this aqueous solution through squeezing rolls under 2 bar pressure and 2 rpm using foulard, dried at 85°C for 3 min and then cured at 180°C for 3 min. The treated fabric with BTCA were impregnated in the silver NPs diluted solution prepared with 10 g/L concentration of silver NPs in deionized water for 1 hour. The impregnated fabrics were passed through squeezing rolls under 2 bar pressure and 2 rpm using foulard, dried at 85°C for 3 min and then cured at 180°C for 3 min. In order to evaluate the role of BTCA on antibacterial activity of the fabric, a sample was treated with BTCA without silver NPs. In order to investigate the fixation and durability of silver NPs on cotton fabrics, treated fabrics were washed in an aqueous solution containing 4 g/L standard detergent (non-phosphate ECE standard detergent without optical brightness) according to TS EN 20105-C06: 2001/A2S program at 40 °C for 30 min using a Gyrowash washing machine. Laundering durability was tested up to washing 20 cycles. The antimicrobial property of the samples was evaluated using pathogenic bacteria *Staphylococcus Aureus*. The presence and durability of silver NPs on the surface of treated cotton fabric was proved using EDX spectrum as well as the SEM images. Furthermore, the creation of cross-links between the cellulose and BTCA was confirmed by the means of FTIR spectroscopy. In conclusion, the antibacterial treatment of the textile fabrics was easily achieved by the two-bath padding process of BTCA and silver NPs. Through a synergistic combination of BTCA and silver NPs, antibacterial activity on the cotton fabrics was obtained. It was observed that BTCA plays a prominent role in stabilizing silver NPs on cotton fabric surface. SEM images of treated fabrics indicated silver NPs were well dispersed on the surfaces of specimens. EDX spectrums informed the residual concentration of silver particles on fabrics after 20 times washing.