Background and aim: Several of the local anaesthetics used for spinal anaesthesia were shown to possess antibacterial activity. The study was undertaken to evaluate the antibacterial effect of bupivacaine alone and combined with S-ketamine on seven different microorganisms. Material and methods: The microorganisms tested were: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis, methicillin-resistant S. aureus, Enterococcus faecalis ATCC 29212, Escherichia coli 29212, Pseudomonas aeruginosa ATCC 27853 and Acinetobacter baumannii 19091. Bactericidal effect of bupivacaine was tested in concentrations of 0.5, 0.25 and 0.12%. The bactericidal activity was determined by standard microbiological methods. The number of colony-forming unit (CFU) was counted and the number of viable bacteria was plotted against time to obtain a time-kill curve. Anaesthetic was considered to possess antimicrobial activity when a significant difference in CFU/ml (1 log10) was observed between the initial and postexposure values. Results: Bupivacaine with and without S-ketamine significantly reduced (2 to 4 log10) the CFU of S. aureus, S. epidermidis and E. faecalis in all concentrations. The reduction of viable counts was more pronounced with higher concentration of anaesthetic and after addition of S-ketamine. With MRSA significant reduction of viable counts was observed only with 0.5% of bupivacaine. Against A. baumannii bupivacaine produced a small (1 log10) but significant reduction in viable count number in concentration of 0.5 and 0.25% but when combined with S-ketamine the reduction was more pronounced (1 log10 to 4 log10) in all concentrations. Bupivacaine exerted significant bactericidal activity against P. aeruginosa only in the highest concentration. Addition of S-ketamine did not improve antibacterial activity. E. coli exhibited strong and significant decrease in viable counts after exposure to bupivacaine. Conclusions: In this study, the antimicrobial effect of bupivacaine in various concentrations alone and combined with S-ketamine was studied. Significant antibacterial effect of bupivacaine alone and combined with S-ketamine was noticed in all tested bacteria except of P. aeruginosa. Addition of S-ketamine enhanced the antibacterial activity of bupivacaine. Antibacterial activity of bupivacaine was concentration dependent and time-dependent. Bactericidal activity of local anaesthetic could prevent infection in patients with indwelling catheters.