This study aimed to compare the antimicrobial efficacy of low temperature atmospheric pressure plasma (LTAPP) design and gaseous ozone delivery system with 2.5% NaOCl on E. faecalis in root canal walls and dentine tubules. The samples were divided into LTAPP (n=12), ozone (n=12), NaOCl (positive control, n=12); and saline (negative control, n=6) groups. Microbial samples were collected using paper points and dentin chips from root canals. Antimicrobial efficacy was assessed by counting the Colony Forming Units of E. faecalis before and after each irrigation protocol. Data were analyzed using Kruskall-Wallis, Wilcoxon signed-rank, Friedman and Bonferroni t (Dunn's test) tests (P=0.05). The microbial sampling with paper points showed antibacterial efficacy of NaOCl, LTAPP, ozone, and saline in descending order, respectively (P<0.05). The microbial sampling with dentin chips demonstrated a superior efficacy of LTAPP compared to NaOCl in the middle third (P<0.05), while both had similar effects in coronal and apical thirds (P>0.05). NaOCl and LTAPP were better than ozone at the coronal and middle parts of the root canals (P<0.05). These findings led us to suggest that LTAPP, which has no thermal and chemical effects, may be of great aid in endodontic treatment. This article is protected by copyright. All rights reserved.