Introduction: Acanthamoeba is an ubiquitous free-living protozoa which causes serious ocular problems. Acanthamoeba keratitis is becoming more prevalent amongst contact lens wearers. The disease can cause loss of vision and blindness if not treated properly. The objective of this research is to study the sensitivity of six Acanthamoeba spp. isolates, of which three were from the clinical isolates (HKL 95, HTH 40 and HS 6) and the remaining three from environmental isolates (TTT 9, TL 3 and SMAL 8) to antimicrobial agents. Methods: The antimicrobial agents chosen for this purpose were polyhexamethylene biguanide (PHMB) and Chlorhexidine. Serial dilutions were performed for polyhexamethylene biguanide and Chlorhexidine. Cyst suspensions from the chosen isolates were exposed to PHMB and Chlorhexidine respectively. After 48 hours incubation time at 30°C, each mixture was filtered and filtration membrane was put onto non-nutrient agar laid with Escherichia coli. The agar plates were incubated for three days at 30°C and examined daily until day 14 to detect the presence of Acanthamoeba trophozoites under the inverted microscope. The presence of trophozoites indicated ineffectiveness of the antimicrobial agents. Results: Both of the antimicrobial agents tested were found to be effective against Acanthamoeba cysts from all the test strains. Polyhexamethylene biguanide gave a minimum cysticidal concentration (MCC) mean value of 2.848 μg/mL while Chlorhexidine showed MCC mean value at a concentration of 3.988 μg/mL. Conclusion: It can be concluded that Acanthamoeba cysts were sensitive to polyhexamethylene biguanide and Chlorhexidine.