Planktonic bacterial cells were exhaustively evaluated for growth inhibition by various antimicrobials. Some bacteria, however, have the ability to form communities intra and inter species - biofilms. The formation of these biofilms on hospital surfaces and instruments may be related to the difficulty in controlling their transmission by the use of disinfectants and antiseptics proven effective for the elimination of the bacteria by testing in planktonic culture. *Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus* are important opportunistic pathogens associated with nosocomial infections, with resistance to most antimicrobials used in therapy. The objective of this study was to compare the antimicrobial activity of different Brazilian propolis and chlorhexidine against *S. aureus, K. pneumoniae* and *P. aeruginosa*. The methodology used was the quantification of bacterial mass by colorimetry and the amount of colony forming units (CFU) after growing planktonic and biofilm in microplate treated by antimicrobial chlorhexidine and two types of Brazilian propolis - green and red. The results show that chlorhexidine and two Brazilian propolis from different regions did not show the same antibiofilm activity observed for the planktonic cells. Chlorhexidine, however, presented antibiofilm activity for *K. pneumoniae*. This study proves that biofilms hamper the antimicrobial activity of antiseptics and disinfectants, and highlight the need to determine the minimum antibiofilm concentration for these products.

**Palavras-chaves:** Biofilm, antimicrobial, chlorhexidine, propolis.