ANTIBIOFILM ACTION OF ANTIFUNGALS AND CHLORHEXIDINE AGAINST CO-CULTURES OF *Acanthamoeba* spp. AND *Candida* spp.

DAYARA CORRÊA MATIOLA[1], GABRIELLA DA ROSA MONTE MACHADO[1,2], GABRIELI EDUARDA ISRAEL[1], MARINA ARAÚJO[1,2], ALEXANDRE MENEGHELLO FUENTEFRIA[3], KARIN SILVA CAUMO[1,2]

[1]POSTGRADUATE PROGRAM IN PHARMACY; CENTER OF HEALTH SCIENCES; FEDERAL UNIVERSITY OF SANTA CATARINA; FLORIANÓPOLIS, SC.
[2]LABORATORY OF APPLIED RESEARCH ON EMERGING PROTOZOA; FEDERAL UNIVERSITY OF SANTA CATARINA; FLORIANÓPOLIS, SC
[3]APPLIED MYCOLOGY LABORATORY; SCHOOL OF PHARMACY; FEDERAL UNIVERSITY OF RIO GRANDE DO SUL; PORTO ALEGRE, RS.

Severe infectious keratitis is a major cause of corneal transplantation and vision loss, often caused by the protozoan of the *Acanthamoeba* genus and fungi such as *Candida* spp. These organisms can adhere to contact lenses, lens cases, and the patient's cornea, forming polymicrobial biofilms. Improper use of contact lenses and poor storage and cleaning of the cases are the main factors contributing to biofilm formation. Studies on the adhesion potential of *Acanthamoeba* spp. and the effectiveness of commercial drugs against these biofilms are scarce. Thus, this study evaluated the adhesion potential and biofilm formation of *Acanthamoeba* spp. and *Candida albicans*, both alone and in co-culture. In addition, the efficacy of natamycin, voriconazole, and chlorhexidine against these microorganisms was tested in vitro. The minimum antibiofilm concentration (MAC) and the minimum biofilm-eradicating concentration (MBEC) were determined for $8x10^5$ *Acanthamoeba* trophozoites (ATCC 50492) and a clinical isolate (R40). The same concentration was used for *C. albicans*. The treatments were carried out over 48 hours. Both *A. castellanii* and the clinical isolate demonstrated a high adhesion capacity after 48 hours. At 200 µM, all drugs showed promising antibiofilm activity and were effective in eradicating *C. albicans* and *Acanthamoeba* spp. biofilms, both alone and in co-culture. Natamycin was particularly effective in reducing *C. albicans* biofilms, both alone and in co-culture with *Acanthamoeba* spp. Chlorhexidine was highly effective in eradicating *Acanthamoeba* spp. This study highlights the importance of further research to optimize antibiofilm treatments for managing infections caused by both *Acanthamoeba* spp. and *Candida* spp. A better understanding of the adhesion behavior of *Acanthamoeba* spp. and the evaluation of treatment efficacy could contribute to the development of new treatment and multipurpose solutions for cleaning contact lens cases and preventing infections.

Keywords: biofilms keratitis; *Acanthamoeba*; *Candida.*
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