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IN VITRO CO-CULTURE MODEL OF *Trichomonas* vaginalis, *Candida* albicans AND *Lactobacillus crispatus*

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Trichomoniasis, caused by the protozoan *Trichomonas vaginalis,* is the most common non-viral sexually transmitted infection globally and increases the risk of HIV/AIDS transmission and acquisition. It often coexists with a dysbiotic vaginal environment characterized by reduced *Lactobacillus spp.,* particularly *L. crispatus. S*imilarly, C*andida albicans*, the most predominant vaginal yeast, disrupts the microbiota, causing vulvovaginal candidiasis, which frequently recurs. Both infections are increasingly challenged by treatment-resistant isolates, highlighting the urgent need for novel therapies. In this study, we aimed to standardize the co-culture of *T. vaginalis*, *C. albicans*, and *L. crispatus* to simulate the vaginal microenvironment, providing a platform for testing active compounds and developing new drugs. MRS liquid medium was used, with initial densities of ATCC or fresh clinical isolates of *T. vaginalis* (1.00 x 10^6 trophozoites/mL), *C. albicans* (3.33 x 10^4 CFU/mL), and *L. crispatus* (5.53 x 10^6 CFU/mL, LC2, co-culture with ATCC isolate; or 5.53 x 10^7 CFU/mL, LC1, co-culture with fresh isolate). After 24 hours, densities reached 2.00 x 10^6 trophozoites/mL (*T. vaginalis*), 2.6 x 10^6 CFU/mL (*C. albicans*), and 7.9 x 10^6 CFU/mL (LC2), and 8.3x10^6 CFU/mL (LC1) for *L. crispatus*. Higher *L. crispatus* densities reduced *T. vaginalis* and *C. albicans* viability. MIC for metronidazole against *T. vaginalis* isolates and MFC for fluconazole against *C. albicans* decreased in co-culture (twofold for ATCC isolate and fourfold for clinical isolate). Co-culture increased cytotoxicity of ATCC isolate, inhibited *C. albicans* biofilm formation (up to 92%), reduced its metabolic viability (up to 90%), and suppressed the yeast-to-hyphal transition (up to 70%). These findings suggest that this *in vitro* system is an effective tool for evaluating antimicrobial efficacy against pathogens causing vaginitis and for studying microorganism interactions.

Keywords: Antimicrobial activity; Co-culture; Vaginal microenvironment.