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ORIGINAL ARTICLE

Effect of *Trichomonas vaginalis* lysate on uropathogenic reference strains of *Escherichia coli* and *Candida albicans*.

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Introduction

Antibiotic resistance is a well-known problem in the world of medicine, and drug-resistant pathogens are encountered at an increasing rate in clinical practice. If this trend continues, common infections which are easily treated today may turn into sources of deadly outcomes. This has intensified the search of new drugs and methods to treat infectious diseases.

Trichomonas vaginalis is currently the 4th most common sexually transmitted agent in the world according to WHO, with as many as 145-220 million infected persons. The parasite is easily transmitted during sexual intercourse, however, many cases remain asymptomatic. The presence of virulence factors such as cysteine proteases (CPs), cell-detaching factor (CDF) [1] and the gene NlpC_A1 [2] is well established. Speculation exists whether any of these virulence factors may contribute to a lower occurrence of other urinary tract infections.

The purpose of this research was to investigate the possible antagonistic effect of *Trichomonas vaginalis* lysate on the growth of uropathogenic *Candida albicans* and *Escherichia coli*, respectively. The results of published research so far indicate possible future applications of *T. vaginalis*-derived substances in the treatment and prophylaxis of infections caused by the two pathogens. A concrete example of a possible application is concomitant use of selected protozoan-derived factors with Foley catheters to avoid hospital-acquired UTIs.

Materials and Methods

Strains: The following microorgansims were used in the study:

- *Trichomonas vaginalis* ATCC PRA-92
- *Trichomonas vaginalis* Katedra Mikrobiologii UJCM
- *Candida albicans* DSM 3454
- *Escherichia coli* DSM 103538

Uropathogenic *Escherichia coli* and *Candida albicans* was ordered and originated from the German collection of microorganisms– Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). The *Trichomonas vaginalis* reference strain from ATCC was used in some previous experiments performed at the Chair of Microbiology JUMC and was used by permission from Dr Kochan.

Methodology. Escherichia coli strain obtained from Germany (DSMZ) was cultured initially as per instructions from DSMZ.

Approximately half of the original strain content was then transferred to a test tube with 5 ml of the lysogeny broth media. The remaining half was streaked onto a solid MacConkey agar plate (Figure 1). The test tube and agar media were cultured under aerobic conditions at 37°C for 24 h. The next day, standard paper discs were dipped into freshly prepared *T. vaginalis* lysate (lysate preparation - as per methodology previously described by Rønneberg and Lunde [3]), placed on three bacteria streaked Petri dishes and cultured overnight at 37°C. Petri dishes were then observed for any zone of growth inhibition around the paper disc.

Candida albicans strain obtained from Germany (DSMZ) was cultured initially as per instructions from DSMZ. Approximately half of the content was then transferred to a test tube with 5 ml universal yeast media. The remaining half was streaked onto a solid Sabouraud agar plate (Figure 2). The test tube and agar media were cultured under aerobic conditions at 37°C and room temperature for 24 h. The next day, standard paper discs were dipped into freshly prepared *T. vaginalis* lysate (lysate preparation - as per methodology previously described by Rønneberg and Lunde [3]), placed on three fungi-streaked Petri dishes and cultured overnight at 37°C and room temperature. Petri dishes were then observed for any zone of growth inhibition around the paper disc.

Figure 1. Petri dish with MacConkey agar and the *Escherichia coli* strain DSM 103538.

Figure 2. Petri dish with Sabouraud agar and the *Candida albicans* strain DSM 3454.

Results

We did not observe any antagonistic effect from the lysed *T. vaginalis* on either the *E. coli* DSM 103538 nor the *C. albicans* DSM 3454. No zone of inhibition was visible around the paper discs (Figures 3 and 4).

Figure 3. No effect of *T. vaginalis* lysate (paper disc in the middle) on the *E. coli* growing on MacConkey agar.

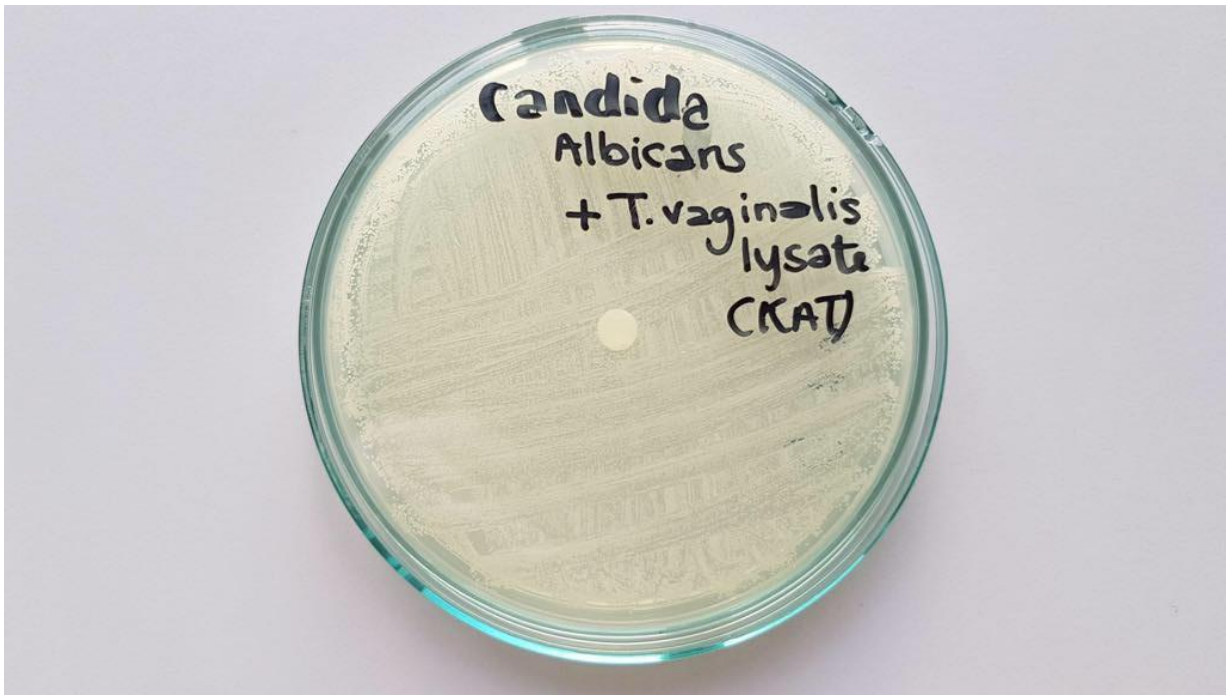

Figure 4. No effect of *T. vaginalis* lysate (paper disc in the middle) on *C. albicans* on Sabouraud medium.

Discussion with conclusion

By creating a thermally intact lysate of *T. vaginalis*, we were hoping to expose some of the important virulence factors of *T. vaginalis*, such as cysteine proteases and cell-detaching factors (CDF). Cysteine proteases (CP) have been shown to induce apoptosis of human vaginal epithelial cells (HVEC) [2], so by exposing these factors, we were hoping to see if they also would be able to induce apoptosis of *C. albicans* and uropathogenic *E. coli*. We did not observe any antagonism of the T. vaginalis lysate to neither C. albicans DSM 3454 or uropathogenic *E. coli* DSM 103538. Sommer *et al.* [1] showed that when the cysteine proteases are isolated from culture, they are dependent on a reducing factor such as cysteine to be cytotoxic. Our lysate did not contain any added cysteine or other reducing factors, which may be a possible factor in why the results showed no antagonism. CP are produced as pro-enzymes and they need to be cleaved before becoming active, so another possible explanation may be that the lysate mostly yielded pro-enzymes and thus not active CP. A fourth possible source of error may be the pH of the lysate. According to Petrin et al. 1998 [4], a pH of <4.5 inactivates CDF. We never measured the pH of the lysate, which could have yielded some important information and should for future reference be done. In the future, it would be interesting to try to isolate the CPs from the *T. vaginalis* medium with an added reducing factor, such as cysteine, to see if that yields some other results.

Another important virulence factor we were interested in was the N1pC_A1 gene. The expression of exogenous NlpC_A1 gene was shown by Pinheiro et.al (2018) [2] to cause a profound reduction in viable vaginal *E. coli* bacteria. The hydrolytic enzymes produced by N1pC_A1 gene causes destruction of peptidoglycans, however, the mechanism is not known. This gene is upregulated in the presence of bacteria and low glucose. Before creating our lysate, the *T. vaginalis* had not been exposed to *E. coli* or *C. albicans*, which might be the reason for why the gene was not upregulated and the enzymes thus not synthesized. Another way of solving this might be to expose *T. vaginalis* to *E. coli* before creating the lysate, so that the gene is upregulated, which might create a solution with high concentration of hydrolytic enzymes. It might then be possible to isolate the proteins and test the antagonism against both *C. albicans* and *E. coli*.

In view of not observing any antagonistic effects of the lysed *T. vaginalis* on the other pathogens, these alterations of the trial mentioned above could be done in another attempt.

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Conflict of interest: none declared

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