

Original Research Article

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# Antagonistic Features of Lactobacilli of the Genital Tract in Torch Infections

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Fertile age, genital tract, TORCH infection, ureaplasmosis, CMV infection, lactobacilli (LB), interspecies antagonism.

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## ABSTRACT

This article presents the results of a study investigating interspecies antagonism between lactobacilli in the genital tract of women with TORCH infections, such as ureaplasmosis and CMV. Data from previous studies have shown that the mechanisms influencing the composition of vaginal lactoflora differ significantly in these infections. For example, while in ureaplasma infection, quantitative changes in lactobacilli were associated with a suppression of their biological properties, in CMV infection, a deficit in both species composition and biological properties was observed. A study of interspecies antagonism between lactobacilli (*L.crispatus*, *L.jensenii*, *L.gasseri*, *L.fermentum*, *L.vaginalis*, *L.salivarius*, *L.iners*) in isolated groups of women with infections showed that lactobacilli isolated from CMV infections have a low level of antagonism, i.e., they were not capable of competition.

## Introduction

The prevalence of TORCH infections such as ureaplasmosis and CMV among women of childbearing age, their frequent asymptomatic course, and their potential for prolonged vaginal persistence determine chronic inflammatory processes, reproductive disorders, and adverse perinatal outcomes. Therefore, they remain controversial issues and objects of research in modern gynecology and microbiology. Two different infections, ureaplasmosis (a bacterium) and CMV (a virus), affect the vagina and trigger the development of a local immune response. *Ureaplasma* spp. occupy a special

position among opportunistic microorganisms of the urogenital tract (15). While they may be present as components of the microbiota, under certain conditions they are associated with pelvic inflammatory disease, habitual miscarriage, and premature birth (2, 5). Cytomegalovirus infection, in turn, is characterized by a pronounced capacity for immune modulation and latent persistence (10). CMV reactivation in the female genital tract often occurs against a background of local immune and microbiome disturbances. However, the role of vaginal microecology in maintaining or limiting the persistence of CMV infection remains poorly understood (12, 13).

Microflora, specifically lactoflora, plays a key role in maintaining the normal functioning of the genital tract. Because they comprise 95-97% of the entire microbial flora in the vagina, they are a dominant and crucial element of reproductive health. The species composition of lactobacilli varies among women. Lactic acid bacteria (*Lactobacillus*) species isolated from vaginal samples of healthy women include: *L.acidophilus*, *L.jensenii*, *L.casei*, *L.gasseri*, *L.crispatus*, *L.plantarum*, *L.fermentum*, *L.cellobiosus*, *L.brevis*, and *L.salivarius* (2). Microaerophilic, hydrogen peroxide-producing lactobacilli are the most common, while anaerobic species are less common (5, 14).

Of particular importance in maintaining normal vaginal microbiocenosis are lactobacilli, which produce hydrogen peroxide; their detection frequency ranges from 46.5 to 100%. Their number in the vagina normally amounts to  $10^5$ – $10^9$  CFU/ml (5). The dominance of lactobacilli ensures the maintenance of an acidic environment, competition for adhesion receptors, and the production of antimicrobial substances (9). At the same time, it has been shown that different species of *Lactobacillus spp.* have different protective potential.

The predominance of *L.crispatus* is associated with stable eubiosis, whereas the dominance of *L.iners* and *L.vaginalis* is often detected in unstable and transitional microbiocenosis states, creating conditions for the persistence of opportunistic and viral pathogens (6, 11). Investigation of interspecies antagonism between isolated lactobacilli species in ureaplasma and CMV infections will provide insight into the mechanisms that maintain the microbiocenosis of the genital tract of women of childbearing age. Furthermore, it will open new approaches for the development of personalized methods for the prevention and treatment of vaginal microflora disorders.

The primary aim of this study is to investigate interspecies antagonistic interactions among *Lactobacillus* species; specifically *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus vaginalis*, *Lactobacillus salivarius*, and *Lactobacillus iners*, within the genital tract of women of reproductive age diagnosed with TORCH infections of diverse etiologies.

## Materials and Methods

In this study used the method of direct co-cultivation of the test and indicator strains on a solid nutrient medium

proposed by N.A.Glushanova (3), as this method appears to be the most informative in our case. A similar method and its modification (on a semi-liquid nutrient medium) are also recommended in regulatory documents for determining the ability of probiotic strains to inhibit representatives of normal intestinal flora and for identifying antagonist strains in the design of complex preparations (7, 8).

A study of interspecies compatibility of lactobacilli was conducted using co-cultivation on a solid MRS-4 nutrient medium. A 24-hour culture, grown in a liquid nutrient medium and standardized for turbidity, was applied to the surface of the solid nutrient medium using a 3-mm diameter bacteriological loop. After the droplet had been absorbed, a droplet of another test culture was applied to the surface of the same medium, 1-2 mm from its edge, in the same volume. The resulting droplet spread until it covered approximately half of the first droplet.

In the superimposed zone, the cultures developed in mutual presence (co-cultivation), competing with each other. After the second drop had dried, the culture dishes were turned upside down and incubated at 37°C using GasPak Anaerobe Gas Generating Pouch System with Indicator (USA). Each experiment was performed in duplicate, with the culture positions varied (to eliminate the influence of the order of layering of the culture drops on the growth pattern in the co-cultivation zone). Drops of the same culture, layered using the method described above, served as controls.

Results were recorded 24 and 48 hours after the start of incubation. If growth of one of the cultures was inhibited, the relationship between them was considered antagonistic, and the cultures themselves were classified as bioincompatible. Cultures were considered biocompatible if complete "fusion" of the spots was detected or if growth of the studied strains in the co-cultivation zone was enhanced (mutualism, synergism, or satellism). If one culture in the co-cultivation zone "rose to the top," suppressing the growth of the other culture, regardless of the order in which they were applied, this was considered weak antagonism.

The presence of a pronounced zone of inhibition (growth retardation) of one culture around the periphery of the spot of another test culture was considered a sign of strong antagonism (Fig. 1). The experiment was repeated three times.

## Results and Discussion

Our previous studies have shown that ureaplasma infection initiates a redistribution of lactobacilli carbohydrate metabolism toward simple sugars with a decrease in the protective potential of the microbiome, which leads to a qualitative transformation of lactobacilli rather than their complete disappearance. Six species were identified among the lactobacilli isolated during ureaplasma infection. Three species occupied the leading positions: *L.fermentum* (36, 6%), *L.salivarius* (27, 6%) and *L.gasseri* (16, 6%). The isolation rate of the remaining three species — *L.crispatus*, *L.jensenii* and *L.iners* — was less than 19, 8%. These results may indicate an unstable, "transitional" state of the microbiota.

Although *L.iners* demonstrated a significant isolation rate, we were unable to include it in the experiment because it could not be cultured in standard nutrient media designed for lactobacilli. It can only be cultured on blood agar and identified using PCR diagnostics.

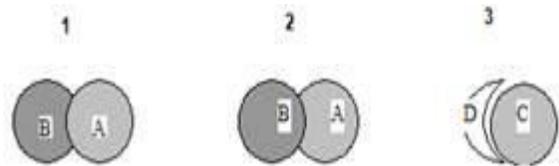
In the presence of CMV infection in the vagina, the local immune response is modulated, leading to changes in epithelial cells, the primary site of lactobacilli colonization. This results in a simultaneous shift in lactoflora species and a functional defect in their dominant status. Thus, the results of identifying isolated lactobacilli in CMV infection revealed low titers of four species: *L.fermentum* (33, 3%), *L.salivarius* (40%), *L.crispatus* (13, 3%) and *L.jensenii* (13, 3%).

A study of interspecies antagonism in vitro between lactobacillus isolates in these infections showed that *L.crispatus* and *L.jensenii*, despite their low isolation frequency, were able to restore all their biological properties as dominant species of vaginal microflora after culture. Our parallel studies examining the production of lysozyme-like enzymes (bacteriocins) and hydrogen peroxide revealed moderate to high levels for these two lactobacillus species. This resulted in the manifestation of complete or partial antagonism to the leading isolates of this group. Although *L.fermentum* and *L.salivarius* are species that quickly adapt to environmental changes, *L.crispatus* and *L.jensenii* remain highly antagonistic.

To fully verify the obtained results, we conducted a study of the antagonism of these isolates to laboratory museum strains of opportunistic and pathogenic bacteria:

*E.coli* (strain 002839, National Collection of Microorganisms of Human Infections, Research Institute of Epidemiology and Microbiology of the Ministry of Health of the Republic of Uzbekistan); *S.aureus* (strain 004134, National Collection of Microorganisms of Human Infections, Research Institute of Epidemiology and Microbiology of the Ministry of Health of the Republic of Uzbekistan); *S.haemolyticus* (strain ATCC 25923, National Collection of Microorganisms of Human Infections, Research Institute of Epidemiology and Microbiology of the Ministry of Health of the Republic of Uzbekistan); *C.albicans* (strain 003848, National Collection of Microorganisms of Human Infections, Research Institute of Epidemiology and Microbiology of the Ministry of Health of the Republic of Uzbekistan). The results showed that strong antagonism to three out of four representatives was demonstrated by *L.crispatus* and *L.jensenii*. This confirms their recovery in vitro. However, this cannot occur in vivo, as the presence of ureaplasma infection causes a negative environmental shift.

**Fig.1** Examples of antagonism between test microorganisms when cultured together on a dense medium: 1 – culture A exhibits antagonism towards culture B; 2 – culture B exhibits antagonism towards culture A; 3 – culture C exhibits strong antagonism towards culture D.



In conclusion, the results of this study demonstrate changes in interspecies and general antagonism of lactobacilli in vitro. Lactobacillus species such as *L.fermentum* and *L.salivarius*, with a high isolation rate and multiple possible mechanisms for adapting to different vaginal environmental conditions, remained less susceptible to interspecies antagonism and resistance to pathogenic and opportunistic vaginal microflora.

Restoration of the biological properties of *L.crispatus* and *L.jensenii*, whose isolation rates were lower in the study groups, was demonstrated in vitro. Due to their high sensitivity, these species reduce all their biological

properties responsible for antagonism (the synthesis of lactic acid, bacteriocins, lysozyme-like enzymes, hydrogen peroxide, and others) when exposed to environmental changes. This further confirms the reason for their prevalence in the vaginas of healthy women. Further study of these species and their use as probiotics to restore vaginal microflora, along with antibacterial and antiviral drugs, is relevant.

### Author Contributions

Guljahon Z. Khaldarbekova: Investigation, formal analysis, writing—original draft.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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