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Original Article

# *In vitro* antimicrobial activities of metabolites from vaginal *Lactobacillus* strains against *Clostridium perfringens* isolated from a woman's vagina

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**Abstract**

*Background*: More than 50 different species of bacteria may live in a woman's vagina, with lactobacilli being the predominant microorganism found in healthy adult females. Lactobacilli are relevant as a barrier to infection and are important in the impairment of colonization by pathogens, owing to competitive adherence to adhesion sites in the vaginal epithelium and their capacity to produce antimicrobial compounds.
*Methods*: The aim of the present study was to demonstrate the inhibitory capability of *Lactobacillus* metabolites against *Clostridium perfringens*, an anaerobic Gram-positive bacterium. These bacteria were isolated from vaginal swabs by using culture-dependent approaches, and the bacteriostatic effect of *Lactobacillus* metabolites, extracted from different isolates, was assessed using a modified E test.
*Results*: Among the 100 vaginal swabs, 59 (59%) samples showed the presence of *Lactobacillus* strains and only one sample contained *C. perfringens*. *Lactobacillus* metabolites demonstrated the significant potency of *in vitro* activity against *C. perfringens*, with minimal inhibitory concentration values ranging from 15.6 μg/mL to 31.2 μg/mL.
*Conclusion*: This study suggests that women without vaginal *Lactobacillus* strains may be susceptible to nonindigenous and potentially harmful microorganisms.


*Keywords:* antimicrobial activity; *Clostridium perfringens*; *Lactobacillus*; modified E test; vagina

## 1. Introduction

*Lactobacillus* strains are thought to play a major role in protecting the vaginal environment from nonindigenous and potentially harmful microorganisms. This is accomplished through their production of lactic acid, resulting in a low and protective pH (3.5−4.5). Vaginal *Lactobacillus* species are also known to produce other antimicrobial compounds besides lactic acid, including target-specific bacteriocins,[1] and broad-spectrum hydrogen peroxide.[2] *Clostridium perfringens* is an anaerobic Gram-positive bacterium known to be a common pathogen in humans, domestic animals, and wildlife, and is the primary cause of clostridial enteric disease in domestic animals. *C. perfringens* has 10 rRNA operons and 18 polymorphic sites among the 16S rRNA genes.[3−5] A common feature of *C. perfringens* is the production of 17 exotoxins and enterotoxin (CPE). *C. perfringens* is subdivided into five toxinotypes (A−E). Isolates originating from humans with gastrointestinal diseases carrying both cpb2 and cpe have

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recently been described.[6−8] Johansson et al[9] reported that a wide genetic diversity of *C. perfringens* from isolates causing enteric diseases in humans, based on pulsed field gel electrophoresis analysis. *C. perfringens* is part of the normal vaginal flora of 1−27% of healthy women. Therefore, ascending infection from the vagina to the uterus may occur.[6] Under appropriate conditions, the bacteria can cause endometritis that leads to sepsis.[10] Meanwhile, *C. perfringens* infection may occur in the uterus, especially after abortions via the use of contaminated surgical instruments.[10] However, the importance of *Lactobacillus* has been shown in preventing infections such as genital infections, urinary tract infections, and bacterial vaginitis, which are caused by reducing *Lactobacillus* and overgrowth of other microorganisms.[11,12] Accordingly, this study was conducted with the aim of assessing the inhibitory capability of vaginal *Lactobacillus* metabolites against *C. perfringens* isolated from a woman's vagina.

## 2. Methods

### 2.1. Design of study

In this research, 100 reproductive-age women who were referred to the women's department of Imam Khomeini Hospital in Ahvaz, Iran, were considered for this study. The inclusion criteria called for healthy married women who were 25−50 years of age. The exclusion criteria included cervix injuries, previous abortion, and divorced women.

In a 35-year-old nonpregnant woman, the only bacterium isolated from a cervicovaginal smear sample was *C. perfringens*. On physical examination, she had high fever (39°C), a small amount of necrotic tissue, and mild inflammation of the uterus. The patient appeared anemic and icteric, although her blood pressure and pulse rate were normal. On examination, her abdomen was normal and no free air was seen on plain chest X-ray films. Blood tests showed evidence of mild inflammation, with a white blood cell count of 14,150 μL and a C-reactive protein level of 8.0 mg/dL. The cervicovaginal smear samples were collected from vaginal swabs of women, and the swabs were immediately placed in thioglycollate transport medium (Hi Media, Laboratories, Mumbai, India) and transported in ice bags to the university's laboratory. The samples were incubated for 48 hours at 37°C.

### 2.2. Isolation of bacteria

The collected swabs were placed on the selective medium for lactobacilli, De Man−Rogosa−Sharpe (MRS) agar (Conda, Madrid in Spain). The plates were incubated at 37°C for 24−48 hours under anaerobic conditions using a candle jar. The *Lactobacillus* were presumptively identified by their ability to grow well on MRS medium[13] followed by differential biochemical tests including Gram staining, catalase test, fermentation of glucose, maltose, and sucrose in order to confirm the identification of *Lactobacilli*. *Lactobacilli* are rod-shaped, Gram-positive, fermentative, facultative anaerobic or microaerophilic organotrophs.

Isolation of *C. perfringens* from the vagina was accomplished by spreading the cervical swabs on a blood agar medium. Then plates were incubated anaerobically in a candle jar with gas packed for a period of 24−48 hours at 37°C.[14] The colonies that showed double zone hemolysis on blood agar plates were considered *C. perfringens*. Thereafter, other diagnostic tests including stormy clot, Nagler test, gelatin hydrolysis test, and indole test were performed in order to confirm the identification of *C. perfringens*.

### 2.3. Antimicrobial compound extraction from *Lactobacillus spp.*

The isolated colonies of *Lactobacillus* grown on MRS agar were transferred into 100 mL of MRS broth and incubated in an anaerobic chamber at 37°C for 5 days. Then, the antimicrobial compound was extracted using the three following methods.

#### 2.3.1. Method 1

After incubation, a part of the culture medium was directly mixed with ethyl acetate (50:50) and then stirred using a magnetic stirrer for 6 hours. The upper organic layer was separated using a separating funnel and centrifuged at 6000 rpm for 15 minutes. Then the ethyl acetate layer was removed and transferred into a clean flask. The extract was pooled and dried in a rotary evaporator (Heidolph in Schwabach, Germany) at 45°C. The yield from the extract was dissolved in methanol for antimicrobial susceptibility testing.

#### 2.3.2. Method 2

The second part of the medium was shocked by immersion in boiling water for 1 minute, and then placed in cold water for 3 minutes. Then, the extraction was followed by adding ethyl acetate similar to the first method.

#### 2.3.3. Method 3

The third part of the culture medium was stressed using an ultrasonic device for 3 minutes (160 W); then, similar to the first method, extraction of the antimicrobial compound was performed.

### 2.4. Determination of minimum inhibitory concentration of *Lactobacillus spp.* extracts

The minimum inhibitory concentrations (MICs) of *Lactobacillus* extracts against *C perfringens* isolates were determined using an improved E test method (AB Biodisk, Solna, Sweden) in order to show the antimicrobial activity of isolated *Lactobacillus* spp. In the improved E test, several AB Biodisks impregnated with different dilutions of the extracts were used instead of strips. In fact, it was a simulated version of the standard E test.

The *C. perfringens* suspensions of freshly grown cultures were prepared in sterile saline and adjusted to a density of $10^6$ cells/mL, corresponding to 68−82% transmittance at 530 nm. The plate of Mueller−Hinton agar (Hi Media India in