Contribution of *Lactobacillus iners* to Vaginal Health and Diseases: A Systematic Review

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**INTRODUCTION**

The vaginal microbiome plays an important role in determining human vaginal health. Using high-throughput metagenomic and 16S rRNA sequencing, over 250 bacterial species have been identified in the human vagina (Fredricks et al., 2005; Chen et al., 2020). Among them, *Lactobacillus* is the most frequently detected microorganism in the healthy vagina, and this includes *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus jensenii,* and *Lactobacillus gasseri* (Alonzo Martinez et al., 2021). For decades, *Lactobacillus* species have been regarded as beneficial to the vaginal eoniche by preventing the invasion of pathogens through the production of organic acids, hydrogen peroxide (H$_2$O$_2$), bacteriocin, and other antimicrobial compounds (Petrova et al.,
L. iners has been recognized as the most prevalent Lactobacillus species in the vaginal econiche (Spear et al., 2011; Campisciano et al., 2020). This species has been initially overlooked in past bacteriologically-based studies because of its fastidious requirements and inability to grow on de Man-Rogosa-Sharpe agar (MRS), a selective culture medium that isolates vaginal Lactobacilli (Falsen et al., 1999). Furthermore, L. iners has very unique characteristics compared with other symbiotic Lactobacillus species in the vaginal econiche (Vaneechoutte, 2017). Most vaginal Lactobacillus species exert a protective effect and play a role in the resistance of the vaginal tract to colonization by pathogens. However, the relationship between L. iners and vaginal health is somewhat complicated and ambiguous (Petrova et al., 2017). This review aims to present the overall characteristics, an overview of different arguments, and the dual roles of L. iners in the vaginal econiche.

CHARACTERISTICS OF L. INERS

Culture Characteristics and Gram-Staining Properties

L. iners was first described by Falsen et al. in 1999 in vaginal and urinary tract specimens (Falsen et al., 1999). This species had escaped our attention for a long time due to its inability to grow on MRS agar under the same culture conditions as other Lactobacillus species (De Backer et al., 2007). Nevertheless, L. iners is characterized by small, smooth, circular, translucent, and non-pigmented colonies after 24 h of anaerobic incubation on blood agar (Falsen et al., 1999). Indeed, most L. iners isolates can grow on MRS agar upon the addition of 1–5% sheep and human blood (Vaneechoutte, 2017). In addition, Yoshimura et al. demonstrated that L. iners can grow on MRS agar under anaerobic conditions for a period of at least 7 days, which is evidently longer than that of other Lactobacillus species. In MRS broth with 0.5% cysteine as the reducing agent, which created the anaerobic conditions, L. iners grows slowly to its highest concentration of only $10^7$ CFU/ml and then growth decreases after 12 h (Yoshimura et al., 2020).

L. iners was initially believed to be a Gram-positive, rod-shaped, non-spore-forming, and facultative anaerobic bacterium (Falsen et al., 1999). However, several studies have reported that, unlike other Lactobacillus species, L. iners does not always clearly stain as Gram-positive, and it seems to have a coccobacillary rather than a bacillary morphology (De Backer et al., 2007; Lebeer et al., 2008). Yoshimura et al. reported that L. iners was mostly Gram-negative with a very short rod shape and weak acid resistance, as it was non-viable in pH 3 medium (Yoshimura et al., 2020). This may be the reason why L. iners was initially overlooked by culture and microscopy methods. By transmission electron microscopy, Kim et al. revealed that the peptidoglycan (PG) layer in the cell wall of L. iners was thin enough to give an apparent Gram-negative morphology (Kim et al., 2020). This morphological characteristic and Gram-staining property of L. iners are clinically very important to consider, as Nugent scoring, which is based on the Gram-staining of vaginal smears, remains a common diagnostic tool in the assessment of vaginal health (Wang et al., 2021). The Gram-negative property of L. iners masks the fact that it is a Lactobacillus species and this may lead to the misdiagnosis of BV, which is a condition characterized by the depletion of Lactobacillus species in Gram-stained vaginal smears under microscopy (Vaneechoutte, 2017). This may help explain why as many as 50% of women diagnosed with BV by the Nugent score are asymptomatic (Klebanoff et al., 2004).

Genome Size and Function

L. iners has the smallest genome of ~1.3 Mbp on a single chromosome among the Lactobacillus species identified so far, with its pangenome count of 2300 genes and average GC content of ~33.3% (France et al., 2016). This low genome size is comparable to those of human symbionts and parasites, and is strongly indicative of a more parasitic, host-dependent lifestyle (Petrova et al., 2017). The genome of L. iners seems to have undergone rapid evolutionary events that resulted in large-scale gene loss and genome reduction, as well as the acquisition of genes, such as iron-sulfur genes, for specific adaption to the vaginal econiche (Macklaim et al., 2011).

Comparative genome analysis revealed that L. iners had a severely reduced number of genes related to carbohydrate and amino acid metabolism, whereas it maintained conserved genes for largely core metabolic proteins and membrane transport genes for essential compounds from the host or the community (Macklaim et al., 2011; Kim et al., 2020). Three potential core genes (inerolysin, ZnuA, and hsdR) were identified to be closely related to the specific adaption of L. iners to the vaginal environment (Kwak et al., 2020). Among them, inerolysin is an unusual pore-forming cholesterol-dependent cytolsin that is active in the acidic vaginal environment and creates aqueous pores within the cell membrane. It may be one of the essential L. iners genes required to stably obtain nutrients from the host (Rampersaud et al., 2011; France et al., 2016). High-affinity zinc uptake requires the binding protein ZnuA type I (ZnuA), which is essential for metal ion homeostasis in L. iners. ZnuA may be a key mediator of strong adhesion of L. iners to vaginal epithelial cells (Gabbianelli et al., 2011; McMillan et al., 2013). Type I restriction enzyme R protein (hsdR) was suggested to be involved in the defense mechanism against bacteriophage infection during BV (Miller-Ensminger et al., 2018). In addition, L. iners contains genes that encode all enzymes directly involved in PG synthesis and hydrolysis (Kim et al., 2020). The unique and thin PG layer of L. iners cell membranes may absorb nutrients or secrete proteins more easily than other...
Lactobacillus species, which can provide essential nutrients or respond to rapid changes in the vaginal environment (Kim et al., 2020).

**Ability to Produce Lactic Acid and H$_2$O$_2$**

Lactobacillus species are the main lactic acid-producing bacteria in the vagina, and they reduce the vaginal pH and restrict the growth of potentially harmful bacteria (Jang et al., 2019). Nevertheless, this lactic acid-producing ability is different from the main Lactobacillus species found in the vaginal microbiome (Godovalov et al., 2019). L. crispatus, L. gasseri, and L. jensenii can produce D- and L-lactic acid by fermenting glycogen, whereas L. iners can produce only L-lactic acid because it lacks the gene that codes for D-lactate dehydrogenase in its genome (France et al., 2016). Because of the almost complete absence of L. iners whereas can produce D- and L-lactic acid by fermenting glycogen, L. iners (Beghini et al., 2015). Additionally, D-lactic acid has been transverse the cervix, and initiates upper genital tract infections the breakdown of the extracellular matrix, helps bacteria metallocproteinase inducer (EMMPRIN) and subsequently lactic acid ratio in the vagina may elevate extracellular matrix effects on the host immune system (Witkin et al., 2013). The L/D lactic acid ratio in the vagina is the only Lactobacillus species to host cells is believed to play a role in the exclusion of pathogenic microorganisms through a mechanism that involves the blocking of their binding sites on vaginal epithelial cells (Pino et al., 2019; Mane et al., 2020). Although L. iners lacks most of the main adhesion molecules of Lactobacillus species (Morris et al., 2012), it still shows a strong adhesive ability to vaginal epithelial cells (McMillan et al., 2013). Fibronectin is an insoluble glycoprotein in the extracellular matrix of the vaginal epithelium (Park et al., 2012). The L. iners genome encodes a fibronectin-binding protein that contains a motif (fibronectin-binding protein A) common to pathogenic strains of Staphylococcus aureus, thereby mediating the adhesion and the invasion of S. aureus to host cells (Macklaim et al., 2011; Macklaim et al., 2013). McMillan et al. demonstrated that L. iners bound significantly stronger to human fibronectin than other Lactobacillus species at a more neutral pH, which may contribute to the persistence of L. iners in the vagina despite the presence of pathogens or treatment with antibiotics (McMillan et al., 2013). An in vitro study reported that L. iners may increase the adhesion of BV-causing G. vaginalis (Castro et al., 2013). It was also demonstrated that L. iners produces inerolysin, a pore-forming protein typically found in pathogenic bacteria, which can enhance the adhesive ability (Rampersaud et al., 2011; Ragaliauskas et al., 2019). These findings indicate that the unique adhesive function of L. iners reduces the protection of the healthy vaginal microbiome from pathogenic bacteria.

**Requirement of Nutrients From Exogenous Sources**

L. iners has an unusually small genome with reduced metabolic capabilities, but it contains a broader array of genes that was probably acquired from foreign sources. The nutrient requirements of this species are more complex than those of other vaginal Lactobacillus species, thereby allowing L. iners to adapt to the diverse niche in the vagina (Macklaim et al., 2011). The fluctuation of hormones and other factors may affect the vaginal environment, resulting in changes in mucus and glycogen production, pH, and microbial species, which may provide essential nutrients for L. iners (Kwak et al., 2020). Genome analysis has indicated a higher dependence of L. iners on exogenous sources of amino acids (France et al., 2016). Furthermore, L. iners has the molecular and cellular machinery to ferment glucose, maltose, trehalose, and mannose, among which glucose and maltose are common glycogenolysis products (France et al., 2016). Macklaim et al. reported that L. iners genes for the uptake of mannose and maltose, genes for glycogen decomposition, as well as genes for mucin and glycerol transport, were strongly up-regulated in BV (Macklaim et al., 2013). Although no iron uptake system has been identified in L. iners, ferrochelatase, which is capable of catalyzing ferrous ion and binding protoporphyrin IX to form heme, was detected in L. iners (Macklaim et al., 2011).

The ability of L. iners to produce inerolysin may be one of the most important factors influencing its ability to acquire nutrients from the vaginal environment. L. iners is the only Lactobacillus species known to code for inerolysin, which is related to intermedilysin (69.2% similarity) and vaginolysin (68.4% similarity) produced by Streptococcus intermedius and G. vaginalis, respectively (Rampersaud et al., 2011). Over 10% of genes coding for inerolysin in L. iners are more highly expressed in dysbiosis than in balanced microbial environments (Macklaim et al., 2013). This cytolysin can liberate resources directly from host tissues or cells, which necessitates that L. iners acquire its nutrients from the host in a symbiotic way (Macklaim et al., 2013).
2011). In other words, this characteristic may give *L. iners* a competitive advantage in the vaginal environment when nutrients are scarce, especially under potentially adverse conditions, such as BV, when other *Lactobacillus* species cannot colonize the vagina (Zozaya-Hinchliffe et al., 2010; Li and Ma, 2020).

**L. iners AND WOMEN DISEASES**

**L. iners and Vaginal Dysbiosis**

Compared with intestinal microflora, a typical feature of the vaginal microbial environment in healthy individuals is its extremely low bacterial diversity (Ravel et al., 2011; Collins et al., 2018). There are five major community-state types (CSTs) in healthy premenopausal women, namely, *L. crispatus*-dominated CST I, *L. gasseri*-dominated CST II, *L. iners*-dominated CST III, and *L. jensenii*-dominated CST V, whereas CST IV is characterized by the absence of *Lactobacillus* species (Wells et al., 2020). Vaginal dysbiosis, which is defined by a high bacterial diversity and a mixture of anaerobic bacteria, is frequently associated with a variety of gynaecological diseases (Eastment and McClelland, 2018; Chee et al., 2020; Chen et al., 2021).

*L. iners* can be predominant in the vagina of healthy women, or in those with vaginal dysbiosis, such as BV, or even in those receiving antimicrobial therapy (Ferris et al., 2007; Goodfellow et al., 2021). Many studies have reported that the presence of *L. crispatus* in the vagina is associated with good health, whereas communities dominated by *L. iners* fail to provide sufficient protection against vaginal dysbiosis (Petricevic et al., 2014; France et al., 2016; Tortelli et al., 2020). The existence of *L. iners* is related to higher levels of proinflammatory factors, such as interleukin-1α, interleukin-18, macrophage migration inhibitory factor, and tumor necrosis factor-α, which are responsible for the activation of an inflammatory response in the vagina (De Seta et al., 2019). *L. iners* is even believed to play a role in the onset of vaginal dysbiosis (Petrova et al., 2017), although the precise role of *L. iners* remains debated. However, it seems that the abundance of *L. iners* remains relatively constant, and *L. iners* is not easily displaced by pathogens or infectious conditions. In cases of BV, *L. iners*, rather than *L. crispatus*, usually coexists with other potentially harmful bacteria that colonize the vagina (Ferris et al., 2007; Zozaya-Hinchliffe et al., 2010). The ability of *L. iners* to adapt to dysbiosis, despite its small genome, may be related to changes in genes involved in metabolism and cytolysis, as well as antibacteriophage defense genes, to changing conditions in the vagina (Borgdorff et al., 2016; Leizer et al., 2018). The remarkable ability of *L. iners* to survive under various conditions indicates that this species may be an important member of the host’s defense and may be a persistent symbiotic *Lactobacillus* species that can maintain and restore the vaginal microbiome (Macklaim et al., 2011).

**L. iners and BV**

BV is the most common type of vaginosis in women of childbearing age. It is characterized by a significant reduction or disappearance of *Lactobacillus* species, accompanied by the emergence of more diverse microbiota dominated by anaerobic and facultative bacteria such as *Gardnerella* species, *Prevotella* species, and *A. vaginalis* (Fredricks et al., 2005; Lee et al., 2020; Witkin et al., 2021). However, *L. iners* is usually the only vaginal *Lactobacillus* species coexisting with BV-associated bacteria that can be detected during BV (Macklaim et al., 2011; Santiago et al., 2012). It can persist under the drastically changing vaginal environment of BV due to its ability to respond and regulate its genomic functions (Macklaim et al., 2013). The increased gene expression of *L. iners* may lead to the production of succinate and other short-chain fatty acids and the increase in the pH value in the BV environment (Macklaim et al., 2013). To adapt to the BV environment, *L. iners* can increase the expression of inerolysin and mucin, and promote the production of glycerol and the expression of related metabolic enzymes, which ensures its acquisition of nutrients from foreign sources (Macklaim et al., 2013). In addition, bacteriophages were one of the reasons for the sudden decrease of *Lactobacillus* species during BV, whereas *L. iners* can upregulate defense systems such as the type I RM system and CRISPR, as well as its specific hasR gene, to resist bacteriophage invasion during BV (Kwak et al., 2020). Nevertheless, a recent study discovered three active peptides of bacteriocin produced by a human intestinal strain named *Lactobacillus paragasseri*. These bacteriocins have strong selective inhibitory activity against *L. iners*, whereas *L. crispatus*, *L. jensenii*, and *L. gasseri* were only slightly inhibited, indicating that these *Lactobacillus*-derived effective inhibitors of *L. iners* can be combined with metronidazole to improve the current BV treatments (Nilsen et al., 2020).

As the coexistence of *L. iners* in BV is different from that of other *Lactobacillus* species, the prevalence of *L. iners* can be used as a microbial indicator to predict the onset of BV or the intermediate BV status (Basavaprabhu et al., 2020). Furthermore, *L. iners* is metronidazole-resistant, and it was found to be the predominant *Lactobacillus* species, even after treatment of BV with metronidazole (Ferris et al., 2007; Srinivasan et al., 2010; Mayer et al., 2015; Lehtoranta et al., 2020). Compared with other more protective *Lactobacillus* species, which hardly exist during BV, *L. iners* showed a stronger competitive advantage and coexisted in the disrupted microbiome (Nilsen et al., 2020). It has been proposed that *L. iners* facilitates the transition between BV and non-BV states (Shipitsyna et al., 2013; Petrova et al., 2015). Interestingly, it has been reported that even after BV treatment, the vaginal microbiome does not change from the *L. iners*-dominant state to the *L. crispatus*-dominated state (Lambert et al., 2013). Therefore, the persistence of *L. iners* may lead to long-term vaginal dysbiosis, especially after repeated treatment cycles of BV (Nilsen et al., 2020). Further studies are needed to clarify whether this species is only a biomarker of the vaginal microbiota transition or a contributing factor of BV.

**L. iners and Biofilm Formation**

Biofilms are bacterial structures tightly attached to a surface, and they are known to be more resistant to the host immune response and antibiotic therapy than planktonic cells (Hall-Stoodley et al.,
2012). It has been shown that biofilm formation on vaginal epithelial cells is strongly associated with vaginal infections (Costerton et al., 1999; Srinivasan and Fredricks, 2008). There is sufficient evidence that BV associates with the presence of a dense polymicrobial biofilm, in which G. vaginalis is the dominant bacterial strain on the vaginal epithelium (Machado et al., 2015; Rosca et al., 2020). It has been hypothesized that Gardnerella spp. initiate biofilm formation, which supports the attachment of other BV-associated bacteria (BVAB) to the vaginal epithelium, further enhancing the biofilm thickness (Muzny et al., 2019). Moreover, Gardnerella biofilms serve as barriers to antibiotics and function to protect other BVAB by preventing the penetration of antibiotics (Gustin et al., 2021). It is generally believed that the high rate of BV recurrence is due to the formation of biofilms that protect the bacteria from antibiotic treatment, and even serve as a reservoir for pathogen regrowth (Bradshaw et al., 2006; Gottschick et al., 2017).

Vaginal indigenous Lactobacilli are believed to prevent the colonization of pathogenic bacteria through steric hindrance or receptor masking in the mucosa (Zarate and Nader-Macias, 2006). Previous studies have used a Lactobacillus probiotic approach in an attempt to clear the polymicrobial biofilms, essentially impeding bacterial virulence and suppressing infection in the human vagina (Saunders et al., 2007; Hardy et al., 2017; Chee et al., 2020). Lactobacillus plantarum was reported to significantly reduce the adhesion of Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Pseudomonas aeruginosa in the HT-29 cell line, which made it a potential anti-biofilm agent for BV treatment (Liu et al., 2017). Saunders et al. reported that G. vaginalis biofilms grown in vitro were displaced with Lactobacillus reuteri RC-14, and to a limited extent with L. iners (Saunders et al., 2007). Castro et al. demonstrated that L. crispatus drastically reduced the adhesion of G. vaginalis strains, both from a healthy woman and a woman with BV, to cervical epithelial cells. Interestingly, L. iners significantly reduced the adhesion of G. vaginalis strains from a healthy woman, but markedly enhanced pathogenic G. vaginalis adhesion (Castro et al., 2013), suggesting that L. iners can cohabitute with BV-associated G. vaginalis and may contribute to G. vaginalis-dominated biofilm formation (Gottschick et al., 2017). In addition, it is well known that Candida species, mainly C. albicans, can form thick and tough biofilms, which greatly increases the tolerance to antifungal drugs during the treatment of recurrent vulvovaginal candidiasis (Taff et al., 2013). Mckloud et al. reported the ability of various Lactobacillus species to inhibit C. albicans biofilm formation and biofilm-related gene expression when cocultured (McKloud et al., 2021). Lactobacillus rhamnosus could down-regulate C. albicans biofilm-related gene expression. Conversely, coculture with L. iners resulted in an up-regulation of biofilm-related gene expression (ALS3 and ECE1), suggesting that the presence of L. iners may be indicative of a shift to vaginal dysbiosis; therefore, it should not be used as a probiotic intervention for C. albicans infection (Ponomarova et al., 2017). A further understanding of the interactions between vaginal commensal Lactobacilli and the structure and function of biofilms is of extreme importance to identify novel treatment approaches for biofilm-associated infections (Falconi-McCahill, 2019).

**L. iners and STIs**

Previous studies have reported that L. crispatus-dominated vaginal microbiomes associate with a lower prevalence of STIs, whereas BV associates with an elevated risk of STIs such as infection with *Chlamydia trachomatis*, human immunodeficiency virus (HIV), *Neisseria gonorrhoeae*, cytomegalovirus, and herpes simplex virus-2 (Bayigga et al., 2019; Gondwe et al., 2020; Redelinguys et al., 2020). Van Houdt et al. reported that the vaginal microbiome dominated by *L. iners* at baseline significantly increased the risk of acquiring *C. trachomatis* infection one year later (van Houdt et al., 2018). A lack of D-lactic acid in the *L. iners*-dominated vaginal microbiome may increase the ability of HIV to transverse the cervicovaginal mucus by modulating cervical integrity (Witkin, 2015; Reimers et al., 2016; Hoang et al., 2020). Interestingly, Mehta et al. and Reimers et al. demonstrated that the vaginal microbiome did not differ between HIV-positive and HIV-negative black women in the United States (Mehta et al., 2015; Reimers et al., 2016). However, Spear et al. conversely observed that the percentage of *L. iners* was significantly higher in HIV-negative African Americans than in HIV-positive African Americans (Spear et al., 2011). The reason for these inconsistent results may be differences in the genetic background or complicated social and behavioral factors in black women, as black women without BV were more likely to have vaginal microbiomes dominated by *L. iners* (Fettweis et al., 2014; Wells et al., 2020). The precise role of *L. iners* in HIV infection should be further examined. Many studies reported a higher diversity of vaginal microbes and a lower abundance of *Lactobacillus* species among HPV-positive women (Lee et al., 2013; Oh et al., 2015; Reimers et al., 2016). Norenhag et al. showed that the vaginal microbiome dominated by *L. iners* was associated with high-risk HPV infection compared with *L. crispatus* (Norenhag et al., 2020). It can be speculated that vaginal dysbiosis may affect the host’s innate immunity against HPV infection, resulting in dysplasia/cervical cancer (Kyrgiou et al., 2017). These findings indicate that *L. iners* may exhibit rapid changes in the composition of the vaginal microbiome similar to BV and could be a valuable biomarker of the dynamic vaginal environment under STIs (Ravel et al., 2013; van Houdt et al., 2018).

**L. iners and Preterm Birth (PTB)**

It is especially important to maintain the natural and healthy balance of *Lactobacillus* species in the vaginal microbiome during pregnancy (Zheng et al., 2019; Juliana et al., 2021). Earlier studies confirmed that high estradiol levels and the consequent high glycogen levels in the vagina during pregnancy result in stronger vaginal acidification, thereby promoting the prevalence of *Lactobacillus* species as gestation progresses (Aagaard et al., 2012; Basavarabhu et al., 2020). However, many studies have indicated that the *L. iners*...
dominated vaginal microbiome was more likely to shift towards dysbiosis during pregnancy (Mils et al., 2019; Kumar et al., 2021; Sarmento et al., 2021). In our previous study, we found that the abundance of L. iners decreased significantly in the second and third trimester, whereas that of L. crispatus increased in the second trimester compared with the first trimester in healthy pregnant women (Zheng et al., 2019). In addition, we observed that the increase in the abundance of L. iners, but not that of L. crispatus, was related to the increase in vaginal cleanliness and positive leukocyte esterase activity, which is consistent with the results of a previous study (Vanechouette, 2017).

Increasing evidence indicates that BV is one of the major etiological causes for adverse pregnancy outcomes, especially PTB (Guerra et al., 2006; Basavaprabhu et al., 2020; Redelinghuys et al., 2020). The L. iners-dominated vaginal microbiome, a so-called 'intermediate microflora' and a typical feature of BV, is speculated to be a risk factor for PTB (Petrievic et al., 2014; Kindinger et al., 2017). Petrievic et al. suggested that the prevalence of L. iners detected in vaginal smears of healthy women in early pregnancy can associate with PTB (Petrievic et al., 2014). This was also demonstrated by Kindinger et al., who reported that the vaginal microbiome dominated by L. iners at 16 weeks of gestation is a risk factor for both a short cervix and early PTB (<34 weeks), whereas L. crispatus dominance is protective against PTB in a more ethnically diverse cohort (Kindinger et al., 2017). Recent studies from different countries also showed a significant association between L. iners and an increased prevalence of PTB (Elowitz et al., 2019; Aslam et al., 2020; Goodfellow et al., 2021; Kumar et al., 2021; Payne et al., 2021; Sarmento et al., 2021). However, several studies reported no significant association between L. iners and PTB (Callahan et al., 2017; Bostein et al., 2020; Witkin et al., 2021). L. iners was also demonstrated to be the most abundant Lactobacillus species among pregnant black women (Wells et al., 2020). However, most studies did not identify a significant relationship between the Lactobacillus-dominant vaginal microbiome and PTB in pregnant black women (Hyman et al., 2014; Nelson et al., 2016; Subramaniam et al., 2016; Stout et al., 2017). Conversely, three studies reported that L. iners was associated with a decreased risk of PTB (Fettweis et al., 2019; Tabatabea et al., 2019; Park et al., 2021). Therefore, the association between L. iners and PTB risk is controversial (Table 1). Presently, it is believed that the vaginal microbiome in black women does not play an important role in the pathogenesis of PTB, as it does in Caucasians and Asians (Kindinger et al., 2017). Furthermore, the limited sample size, the time of sample collection, differences in the definition of PTB, ethnic and geographical variations, and differences in the methodology of strain identification, as well as complicated clinical conditions such as genetic abnormalities or a history of PTB, were confounding factors that impacted the results (Ravel et al., 2011; Jespers et al., 2012; Mehta et al., 2020; Wells et al., 2020).

According to the features of L. iners and its lack of protection against pathogens when it is the only Lactobacillus species in the vagina, some researchers have suggested that L. iners cannot be responsible for infections during pregnancy (Petrievic et al., 2014; Peelen et al., 2019). Indeed, because the vaginal microbiome dominated by L. iners is relatively unstable, there is a tendency for transition to BV-associated CST-IV during pregnancy (Verstraeten et al., 2009). In addition, the L. iners-dominated vaginal microbiome may increase the risk of PTB by modulating local tissue inflammation and cervical integrity, thereby disrupting chemical and mechanical mucosal protective barriers against ascending infections (Kindinger et al., 2017). Further studies are needed to clarify the potential mechanisms between the L. iners-dominated vaginal microbiome and PTB.

**L. iners and Infertility**

Previous studies have reported that up to 40% of patients who failed assisted reproduction by in vitro fertilization (IVF) had an abnormal reproductive tract microbiome (Fanchin et al., 1998; Moore et al., 2000; Koedooder et al., 2019). Vaginal dysbiosis, including an elevated pH value, increased flora diversity, BV, vulvovaginal candidiasis, and trichomonal vaginitis, are recognized as risk factors for infertility (Campisciano et al., 2017; Mounne et al., 2021). Campisciano et al. reported that the abundance of L. iners was associated with an increased infertility rate (Campisciano et al., 2020). Chen et al. also recently reported that the L. iners-dominated vaginal microbiome was associated with tubal infertility and C. trachomatis infection (Chen et al., 2021). As a transitional species, L. iners may facilitate the transition between an abnormal and a normal vaginal microbiome under treatment or artificially high estrogen levels that are needed for IVF (Kindinger et al., 2017; Kosti et al., 2020). It is believed that the L. iners-dominated vaginal microbiome is an unfavorable factor for pregnancy.
### TABLE 1 | Main results per study on L. iners and PTB.

| Author (year) | Country | Time of sample collection | Sample size | Tools implied for identification | Main findings | References |
|---------------|---------|---------------------------|-------------|----------------------------------|---------------|------------|
| Petricevic et al. | Austria | At 11-14 weeks of gestation | 111 women (white European, Middle Eastern, Asian) | PCR-DGGE and sequencing | *L. iners* was the predominant vaginal *Lactobacillus* spp. in women who delivered preterm newborns. *L. iners* was predominantly present in 11/13 (85%) of women who delivered preterm newborns and in only 16/98 (16%) of women who delivered at term (p < 0.001). | (Petricevic et al., 2014) |
| Kindinger et al. | United Kingdom | At 16 weeks of gestation | 161 women (30 Black, 104 Caucasian, 27 Asian) | 16S rRNA gene sequencing at V1-V3 region | *L. iners* dominance of the vaginal microbial community at 16 weeks of gestation was significantly associated with both a short cervix <25 mm and early PTB (<34 weeks). By contrast, *L. crispatus* dominance was highly predictive of TB. | (Kindinger et al., 2017) |
| Calihan et al. | United States | Weekly | Low-risk cohort: 39 women; High-risk cohort: 96 women | 16S rRNA gene sequencing at V4 region | *L. crispatus* was associated with the low risk of PTB in low- and high-risk cohorts, whereas no significant association was detected for *L. iners*. A subspecies clade of Gardnerella vaginalis explained the genus association with PTB. | (Calihan et al., 2021) |
| Tabatabaei et al. | Canada | At 8-13 weeks of gestation | 94 spontaneous PTB cases, 356 term controls | 16S rRNA gene sequencing at V4 region | *L. iners* was predominantly present in 11/13 (85%) of women who delivered preterm | (Tabatabaei et al., 2019) |
| Elovitz et al. | United States | At 16-20 weeks, 20-24 weeks and 24-28 weeks of gestation | 539 women (402 African American, 115 white, 22 other) | 16S rRNA gene sequencing at V3-V4 region | *L. crispatus* increased in abundance during pregnancy in women who delivered preterm newborns. Women who delivered at term exhibited significant decreases in the abundance of *A. vaginae* and *G. vaginalis*, and an increase in the abundance of *L. iners*. | (Elovitz et al., 2019) |
| Fettweis et al. | United States | At prenatal visit, at triage | 45 spontaneous PTB cases and 90 term controls (African American predominantly) | 16S rRNA gene sequencing at V1-V3 region | *L. iners* was the dominant species, respectively, but the differences were not statistically significant. | (Fettweis et al., 2020) |
| Aslam et al. | Pakistan | Not available | 8 term vaginal swabs, 8 preterm vaginal swabs, and 8 preterm placental tissues | 16S rRNA gene sequencing at V1-V2 region | *L. iners* and *A. vaginae* were significantly associated with PTB. | (Aslam et al., 2020) |
| Blostein et al. | Peru | Before 16 weeks of gestation (9 weeks on average) | 25 PTB cases and 100 term controls | 16S rRNA gene sequencing at V4 region | Overall, no CST (diverse, *Lactobacillus*-dominated, or *L. iners*-dominated) was associated with PTB. | (Blostein et al., 2020) |
| Sarmento et al. | Brazil | In the second trimester | 146 women | 16S rRNA gene sequencing at V1-V3 region | *L. iners* was the dominant vaginal bacterium in 61.5% of women with spontaneous PTB but only in 31.2% of those who delivered at term (p = 0.035). | (Sarmento et al., 2021) |
| Kumar et al. | India | In each trimester of pregnancy | 18 PTB cases and 20 term controls | 16S rRNA gene sequencing at V3-V4 region | A significantly higher abundance of *L. iners* (all trimesters), *Megasphaera* sp (first trimester), *Gardnerella vaginalis* (second trimester), and *Sneathia sanguinigenes* (second trimester) was identified in preterm samples, whereas a higher abundance of *L. gasseri* (third trimester) was observed in term samples. | (Kumar et al., 2021) |
| Witkin et al. | Brazil | In the second trimester | 613 women | 16S rRNA gene sequencing at V1-V3 region | Spontaneous PTB occurred in 9.6%, 9.3%, and 6.9% of women when *G. vaginalis*, *L. iners*, or *L. crispatus* was the dominant species, respectively, but the differences were not statistically significant. | (Witkin et al., 2021) |
| Goodfellow et al. | United Kingdom | At 15-22 weeks of gestation | 109 high-risk women and 145 low-risk women | 16S rRNA gene sequencing at V3-V4 region | *L. iners* achieved higher bacterial loads compared to the other *Lactobacillus* species and associated with early spontaneous PTB/PPROM recurrence. | (Goodfellow et al., 2021) |
| Park et al. | Korea | At 15-34 weeks of gestation | 38 PTB cases and 56 term controls | Multiplex quantitative real-time PCR | Although most values for single bacteria were not statistically significant, the mean value of the total Bacillus class showed a significant difference between PTB and TB groups, in which the mean value of *L. iners* showed a significant increase in the TB group. | (Park et al., 2021) |
| Payne et al. | Australia | At 12-23 weeks of gestation | 936 women (white race predominantly) | Quantitative PCR | Women who delivered at term had a higher level of *L. crispatus*, *L. gasseri*, or *L. jensenii* DNA in their vaginal swabs. In the remaining women, a specific microbial DNA signature was identified, which was strongly predictive of spontaneous PTB risk, consisting of *G. vaginalis*, *L. iners*, and *Ureaplasma parvum* DNA. | (Payne et al., 2021) |

PTB, preterm birth; TB, term birth; PPROM, preterm prelabor rupture of membranes; DGGE, denaturing gradient gel electrophoresis.
**ANTI-MICROBIAL AND IMMUNE-INDUCING ACTIVITY OF L. iners**

L. iners is the most common and persistent vaginal symbiotic Lactobacillus species with good adaptability to the complex and dynamically changing vaginal environment (Borgdorff et al., 2016; Kwak et al., 2020). Under fluctuating environmental conditions, other Lactobacillus species may not survive, while L. iners persists with relatively constant abundance due to its ability to respond and regulate its genomic functions, such as specific carbohydrate uptake, fibronectin-binding protein activity, bacteriophage defense, and inerolysin synthesis (Rampersaud et al., 2011; Macklaim et al., 2013; McMillan et al., 2013; Kwak et al., 2020). This remarkable ability to survive under a range of conditions contributes to L. iners' being a dominant species when the microbiome is in a transitional stage (Jakobsson and Forsum, 2007), suggesting that L. iners may be an important member of the host defense mechanism as a persistent mutualistic lactobacilli, and even promote the restoration of a healthy vaginal microbiome (Ravel et al., 2011; France et al., 2016).

In fact, several studies have confirmed many probiotic characteristics of L. iners. It has many ecological functions, such as lactic acid production, that are similar to those of other Lactobacillus species (Linhares et al., 2011; O’Hanlon et al., 2011). Although the genome of L. iners lacks most of the adhesion factors of other Lactobacillus species, it can still adhere strongly to vaginal epithelial cells (Morris et al., 2012; McMillan et al., 2013). Thus, L. iners shows the similar phenotypic traits of colonization and host interaction, as well as excluding pathogens, as other vaginal Lactobacillus species (Osset et al., 2001). Shipitsyna et al. reported the loss of L. iners during BV and suggested that it was not the key pathogen causing the disease (Shipitsyna et al., 2013). L. iners can destroy or replace G. vaginalis to form biofilms in vitro (Hummelen et al., 2010; Zhou et al., 2010). Macklaim et al. demonstrated that some specific functions of L. iners, such as the expression of cytokines, absorption of exogenous sources, and bacteriophage defense, facilitated the harsh conditions in the vagina, including BV (Macklaim et al., 2013). L. iners prevents harmful bacteria from obtaining important nutrients, such as iron, and inhibits their sustained growth by triggering the innate immune system in vaginal epithelial cells (Vaneechoutte, 2017). In addition, the anti-inflammatory effects of L. iners were observed through specific molecular interactions between vaginal epithelial cells (Rose et al., 2012).

The L. iners-dominated vaginal microbiome was associated with the induction of a stress response in the vaginal epithelium (Vaneechoutte, 2017; Linhares et al., 2019). Doerflinger et al. discovered that L. iners, but not L. crispatus, significantly upregulated the pattern-recognition receptor signaling pathway in human primary vaginal epithelial cells and increased the mRNA expression of tumor necrosis factor, indicating that the vaginal microbiome regulates the host immune response species-specifically (Doerflinger et al., 2014). It has been suggested that, in response to stress, L. iners can activate the toll-like receptor signaling pathway in vaginal epithelial cells, increase heat shock protein 70 expression, and inhibit autophagy, which would destroy the homeostasis between vaginal epithelial cells and reduce the ability of these cells to recognize and respond to potential pathogens (Doerflinger et al., 2014; Feng et al., 2015). Conversely, many compounds involved in the antimicrobial defense of vaginal epithelial cells, such as neutrophil gelatinase-associated lipocalin, calprotectin, and hyaluronan, were also preferentially induced by L. iners (Leizer et al., 2018). These findings support the fact that L. iners can fight non-physiological threats, and maintain and promote the recovery to a healthier state, as well as exhibit proinflammatory qualities and act less like a commensal microbe under different conditions (Levine et al., 2011).

**CONCLUSIONS**

In conclusion, L. iners is a unique and intriguing Lactobacillus species with extraordinary characteristics. Its small genome and concurrent nutrient dependency are conducive to its high adaptation to both the low and the high pH vaginal environment, as well as both BV-positive and BV-negative conditions. Therefore, L. iners is often classified as a transitional species that colonizes the vagina after an ecological disturbance. However, whether L. iners is beneficial or pathogenic for the host’s microbiome remains controversial. Most researchers are inclined to believe that L. iners offers limited protection against vaginal colonization by pathogens and may contribute to the onset and maintenance of vaginal dysbiosis. L. iners may also be a risk factor for sexually transmitted infections and adverse pregnancy outcomes. A greater understanding of the roles of L. iners in health and diseases in individuals of different races and ethnicities is warranted. In addition, further studies are required to clarify the role of L. iners in vaginal mucosal immune regulation, and to further clarify whether it can be used as a novel biomarker to detect the existence or prognosis of vaginal inflammation and guide subsequent clinical treatment.

**AUTHOR CONTRIBUTIONS**

NZ, RG, JW, WZ, and ZL discussed the contents, wrote, reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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