J Physiol 595.2 (2017) pp 451–463

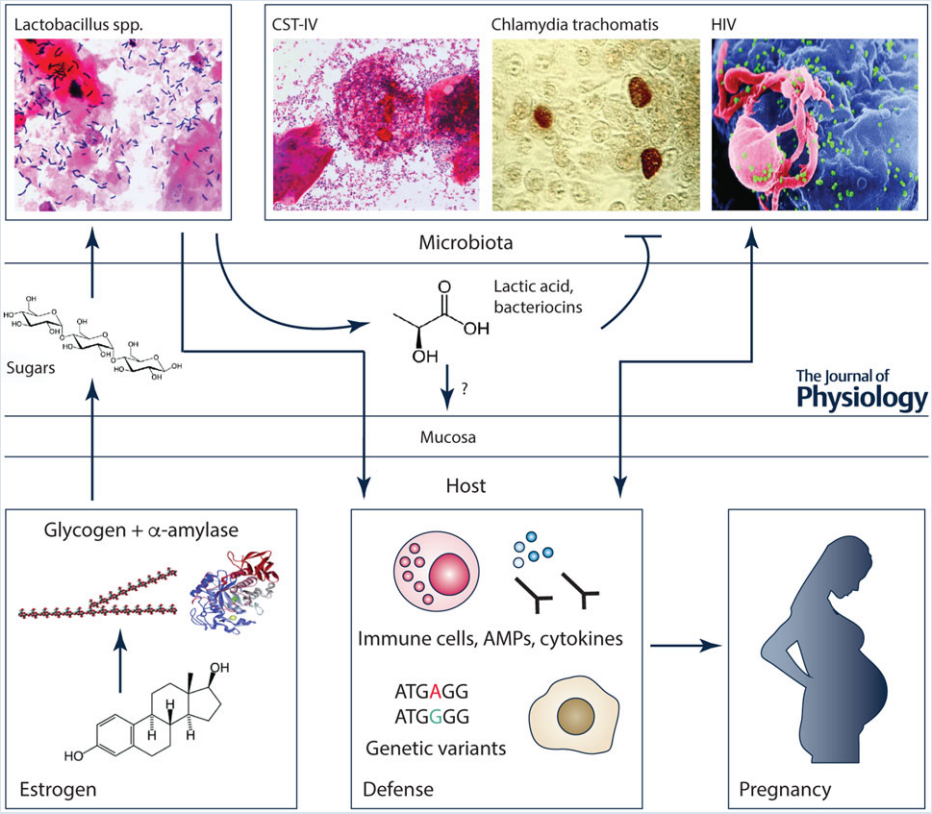
The Journal of Physiology

TOPICAL REVIEW

The vaginal microbiota, host defence and reproductive physiology

Steven B Smith[1,2] and Jacques Ravel[1,3]

[1] *Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[2] *Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA*
[3] *Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA*



Abstract The interaction between the human host and the vaginal microbiota is highly dynamic. Major changes in the vaginal physiology and microbiota over a woman's lifetime are largely shaped by transitional periods such as puberty, menopause and pregnancy, while daily fluctuations in microbial composition observed through culture-independent studies are more likely to be the

Steven Smith is a PhD candidate at the University of Maryland with a focus on bioinformatics and computational biology. His past and current experiences include integration of experimental and high-throughput sequencing datasets for use in systems biology approaches. He is currently studying how changes in vaginal bacterial community composition and abundance and associated BV status affect human vaginal transcriptomic responses. **Jacques Ravel** is a Professor of Microbiology and Immunology and the Associate Director for Genomics at the Institute for Genome Sciences (IGS) at the University of Maryland School of Medicine in Baltimore, Maryland. His research focuses on applying combinations of modern genomics technologies, systems biology approaches and ecological principles to understand the relationship between the host and the genital microbiota as it relates to women's health.

DOI: 10.1113/JP271694

results of daily life activities and behaviours. The vaginal microbiota of reproductive-aged women is largely made up of at least five different community state types. Four of these community state types are dominated by lactic-acid producing *Lactobacillus* spp. while the fifth is commonly composed of anaerobes and strict anaerobes and is sometimes associated with vaginal symptoms. The production of lactic acid has been associated with contributing to the overall health of the vagina due to its direct and indirect effects on pathogens and host defence. Some species associated with non-*Lactobacillus* vaginal microbiota may trigger immune responses as well as degrade the host mucosa, processes that ultimately increase susceptibility to infections and contribute to negative reproductive outcomes such as infertility and preterm birth. Further studies are needed to better understand the functional underpinnings of how the vaginal microbiota affect host physiology but also how host physiology affects the vaginal microbiota. Understanding this fine-tuned interaction is key to maintaining women's reproductive health.

(Received 19 October 2015; accepted after revision 28 January 2016; first published online 8 March 2016)
Corresponding author J. Ravel: Institute for Genome Sciences, University of Maryland School of Medicine, BioPark II, 801 W. Baltimore Street, Baltimore, MD 21201, USA. Email: jravel@som.umaryland.edu

Abstract figure legend Interaction of the human vaginal microbiota with physiology, host defence and reproduction. The host supplies glycogen and glycogen breakdown products via stimulation by oestrogen production as a carbon source to the vaginal microbiota, therefore favouring *Lactobacillus* growth. In turn, lactic acid and bacteriocins produced mainly by *Lactobacillus* spp. contribute to the host defence while species associated with community state type (CST)-IV, aerobic vaginitis or bacterial vaginosis (such as *Gardnerella vaginalis* or *Lactobacillus iners*) contribute to mucosa degradation and susceptibility to other infections. Specific host defences including the innate immune response/inflammation, cell recruitment (such as NK cells or macrophages, pink and yellow circles, respectively), cytokines (pink 'Y' shapes) and antimicrobial peptides (AMPs; blue circles) are dependent on host genetic polymorphisms, CST and pathogen presence (such as *Chlamydia trachomatis* and HIV) and may also have a negative effect on reproduction and pregnancy. Gram stain images of *Lactobacillus* spp. and CST-IV are unpublished data. All other images are in the public domain (Creative Commons Licence).

Abbreviations AMP, antimicrobial peptide; AV, aerobic vaginitis; BV, bacterial vaginosis; CCL, chemokine (C-C motif) ligand; CDC, cholesterol-dependent cytolysin; CST, community state type; HBD, human β-defensin; HIV, human immunodeficiency virus; HNP, human neutrophil peptide; HSV, herpes simplex virus; HPV, human papillomavirus; IgA, immunoglobulin A; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; PRR, pattern recognition receptor; TLR, toll-like receptor; SCFA, short chain fatty acid; SLPI, secretory leukocyte peptidase inhibitor; SP, surfactant protein; NF-κB, nuclear factor-κB; TNF, tumor necrosis factor.

Introduction

The assemblages of microbes (microbiota) associated with the human body have been shown to affect human physiology, immunity and nutrition (Kau *et al.* 2011; Chen *et al.* 2015; Conlon & Bird, 2015). In the vagina, microbes exist in a finely tuned mutualistic relationship with the host and provide the first line of defence against the colonization by opportunistic pathogens. Throughout a woman's lifespan, the vaginal microbiota undergoes major changes associated with transitional reproductive periods such as puberty and menopause, as summarized in Farage & Maibach (2005). During these periods, the vaginal microbiota can affect host reproductive physiology but can also be affected by host physiology.

Recent high-throughput 16S rRNA gene sequencing studies examining vaginal bacterial species composition and abundance in reproductive-aged women have shown that there are at least five major types of vaginal microbiota called community state types (CSTs) (Zhou *et al.* 2007; Ravel *et al.* 2011; Gajer *et al.* 2012). Four of these CSTs are dominated by *Lactobacillus crispatus* (CST-I), *L. iners* (CST-III), *L. gasseri* (CST-II) or *L. jensenii* (CST-V) and one, CST-IV, does not contain a significant number of *Lactobacillus* but is composed of a polymicrobial mixture of strict and facultative anaerobes including species of the genera *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella* and other taxa in the order *Clostridiales* (Fig. 1; Fredricks *et al.* 2005; Campos *et al.* 2008; Ravel *et al.* 2011; Gajer *et al.* 2012). The frequency of these CSTs has been shown to differ in different ethnic backgrounds, with CST-IV more common (~40%) in black and Hispanic women (Ravel *et al.* 2011). The polymicrobial condition known as bacterial vaginosis (BV) is compositionally similar to CST-IV since it is defined by a loss of *Lactobacillus* spp., the presence of anaerobes and strict anaerobes, and sometimes associated clinical symptoms including discharge, odour and irritation. In research settings, a Gram-staining scoring procedure that relies on the identification of bacterial morphotypes known as the

Nugent test (appropriately renamed Nugent-BV by Martin (2012)) is used to establish a BV diagnosis (Nugent *et al.* 1991). Clinically, the diagnosis of BV is accompanied by an evaluation of the following signs and symptoms: discharge, malodour, the presence of clue cells and vaginal pH > 4.5 as defined by the Amsel criteria (Amsel *et al.* 1983).

Daily fluctuations in the composition of the vaginal microbiota have been previously documented by microscopy and cultivation studies (Hay *et al.* 1997; Keane *et al.* 1997; Schwebke *et al.* 1999). These findings were confirmed and extended in longitudinal culture-independent analyses such as those of women who self-collected vaginal swabs twice weekly for 16 weeks (Brotman *et al.* 2008, 2010; Gajer *et al.* 2012), or daily for 10 weeks (Ravel *et al.* 2013) or 4 weeks (Srinivasan *et al.* 2010). It was observed that some vaginal microbial communities transitioned in and out of CST-IV. The amount of time spent in a particular CST could vary individually as some women experienced consistent and stable CST longitudinal patterns (defined as community class), while others frequently transitioned between CSTs and most frequently to CST-IV (Gajer *et al.* 2012;

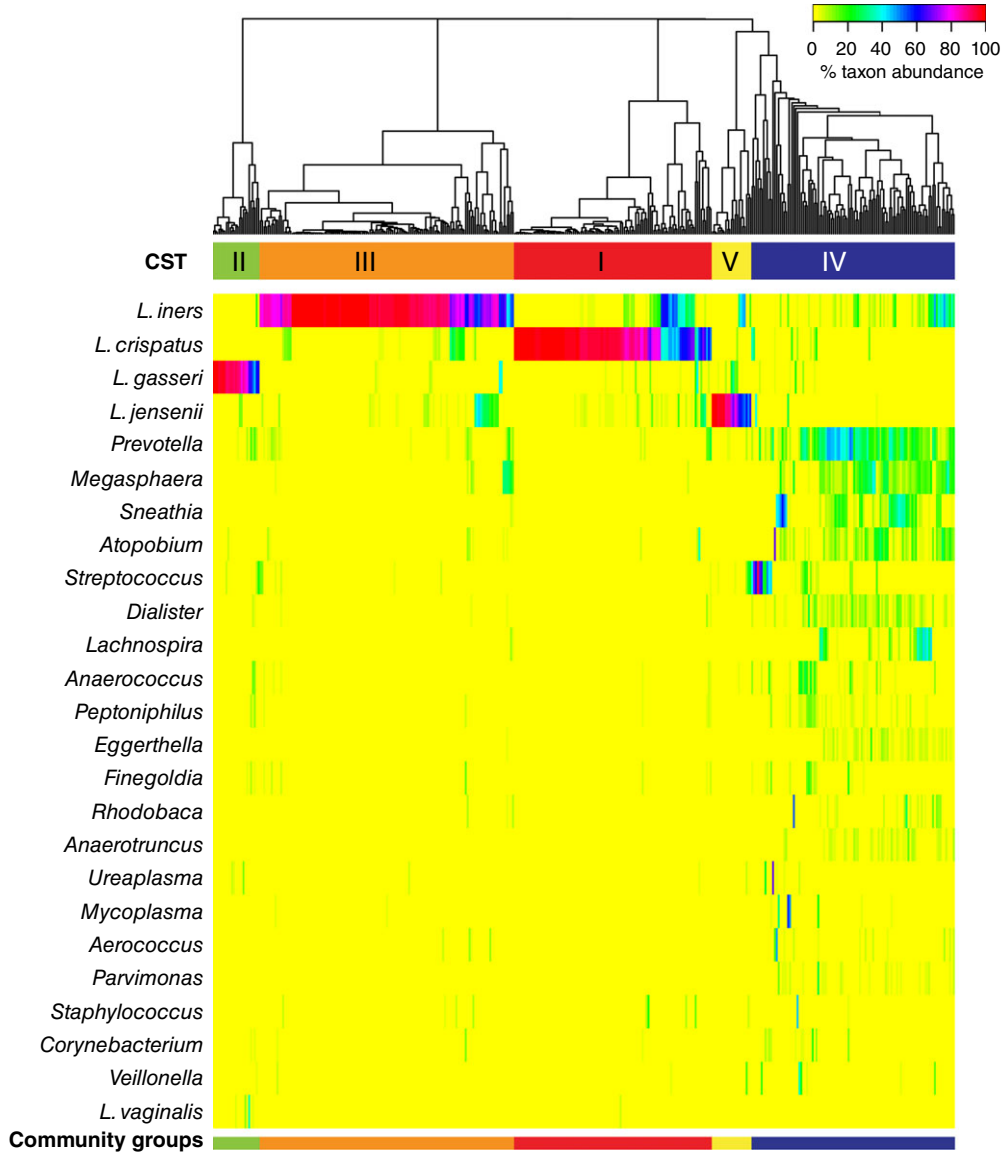


Figure 1. Heat map of vaginal microbiota community state types
Hierarchical clustering shows that the vaginal microbiota of reproductive-aged women clusters into five distinct community state types, four of which are dominated by *Lactobacillus* spp. (*Lactobacillus crispatus* (CST-I), *L. iners* (CST-III), *L. gasseri* (CST-II) or *L. jensenii* (CST-V)) and the fifth (CST-IV) is composed of a polymicrobial mixture of strict and facultative anaerobes including species of the genera *Atopobium*, *Megasphera*, *Mobiluncus*, *Prevotella* and other taxa in the order *Clostridiales*. Figure reproduced from Ravel *et al.* (2011).

Ravel *et al.* 2013). In some cases, CST transitions were triggered by menstruation or sexual behaviours, but in other cases they seem to be driven by uncharacterized factors (Gajer *et al.* 2012). These longitudinal studies highlight the highly dynamic nature of vaginal microbial communities and emphasize the need to better understand the underlying biological factors modulating fluctuations in composition and functions that affect host physiology.

Historically, *Lactobacillus*-dominated vaginal microbial communities have been associated with healthy reproductive-aged women and are characterized by the production of copious amounts of lactic acid and thus a pH < 4.5 (reviewed in Linhares *et al.* 2011; Ma *et al.* 2012; Petrova *et al.* 2015). This acidic environment is thought to be highly protective against infections or colonization of the vagina by pathogens and non-indigenous microbes. An additional benefit of *Lactobacillus* spp. (i.e. *L. crispatus* and *L. gasseri*) is the supply of bacteriocins or bacteriocin genes (i.e. gassericin T, acidocin lF221A, type-A lantibiotic, and Bacteriocins IIa, IIc and J46) to inhibit growth of undesirable species (i.e. *Klebsiella* spp., *Staphylococcus aureus*, *Escherichia coli* or *Enterococus faecalis*) (Stoyancheva *et al.* 2014; Zheng *et al.* 2015). However, the notion that a *Lactobacillus*-dominated vaginal microbiota is necessarily the norm has been called into question since mounting evidence suggests that about 25% of asymptomatic women do not possess a *Lactobacillus*-dominated microbiota at any given time, a staggering proportion that does not support a diseased state (Zhou *et al.* 2004; Ravel *et al.* 2011; Anahtar *et al.* 2015). These differences between women appear to be driven by a combination of cultural, behavioural, genetic and other unknown underlying factors (Ravel *et al.* 2011; Gajer *et al.* 2012; Anahtar *et al.* 2015). However, a strong association between CST-IV (as established by Nugent-BV) and increased risk to sexually transmitted infections (Martin *et al.* 1999; Peters *et al.* 2000; Cherpes *et al.* 2003), including human immunodeficiency virus (HIV) (Cohen *et al.* 1995; Taha *et al.* 1998; Cu-Uvin *et al.* 2001; Coleman *et al.* 2007), and reproductive tract and obstetric sequelae has been established through thorough epidemiological studies (Gravett *et al.* 1986; McDonald *et al.* 1992; Hillier *et al.* 1995; Meis *et al.* 1995; Goldenberg *et al.* 1997). Hence, while CST-IV might be normal (asymptomatic) in some women, it is still associated with significantly increased risk of adverse outcomes. An illustration of how CST-IV can help further foster infections is the case of chlamydial infection, where interferon (IFN)-γ production is thought to be critical for chlamydia clearance. IFN-γ activates the human enzyme indoleamine-2,3-dioxygenase, which catabolizes tryptophan, eventually leading to tryptophan starvation and chlamydia clearance since genital chlamydia cannot synthesize tryptophan. However, production of indole compounds by anaerobes and strict anaerobes contained in CST-IV enables chlamydia to shunt its deficiency and produce tryptophan, thus bypassing this host defence mechanism and establishing a long-term infection (discussed in Aiyar *et al.* 2014). Similarity, relative to other *Lactobacillus*-dominated community states, CST-IV-like communities increase the risk of HIV infection (Pyles *et al.* 2014; Anahtar *et al.* 2015). However, not all *Lactobacillus* spp. are necessarily beneficial and protective since, for example, some strains of *L. iners* might carry pathogenicity factors, such as inerolysin, a cholesterol-dependent cytolysin (CDC) and a host epithelial cell pore-forming enzyme, which was found to be up-regulated at least sixfold in women with BV (Macklaim *et al.* 2011; 2013). Therefore, when considering the impact of the microbiota on host defence and reproductive physiology, it is important to place it in the context of these dynamic and individualized relationships.

The effect of the microbiota on host defence

The vagina contains a number of immune-related cells and receptors to help sense the microbial environment (Wira *et al.* 2005). Surveillance for microbes within the female genital tract of both commensal and pathogenic microbes is generally achieved by microbial motif pattern recognition by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) or the dectin-1 receptor (which helps recognize the fungal pathogen *Candida albicans*; Carvalho *et al.* 2012; Usluogullari *et al.* 2014), and nucleotide-binding oligomerization domain (NOD) receptors present in and on both squamous epithelial cells lining the vagina and the columnar cells lining the upper female genital tract (as reviewed in Wira *et al.* 2005; Witkin *et al.* 2007*a*; Horne *et al.* 2008; Mitchell & Marrazzo, 2014). Microbial stimulation of PRRs initiates cytokine/chemokine signalling cascades, for example secretion of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor-α (TNF-α), in order to recruit or activate specialized cells, such as NK cells, macrophages, $CD4^+$ helper T-cells, and $CD8^+$ cytotoxic T-cell lymphocytes and B lymphocytes (as reviewed in Wira *et al.* 2005; Brotman *et al.* 2013*a*; Nguyen *et al.* 2014). Genetic variants of PRRs such as the IL-1R antagonist gene, TLR4, TLR9, IL-1R2 and TNF-α may play a role in how a woman responds to a particular microbial challenge or pregnancy outcome, as evidenced by several genetic-disease association studies (Genç *et al.* 2004*a*,*b*, 2007; Giraldo *et al.* 2007; Ryckman *et al.* 2011; Royse *et al.* 2012; Mackelprang *et al.* 2015). Women with CST-IV-like states show significant increases in IL-1α, IL-1β, TNF-α, IFN-γ, IL-4, IL-8, IL-10, IL-12p70, and fms-like tyrosine kinase 3 ligand relative to CST-I as well as significantly higher IFN-γ in CST-III relative to CST-I. Specifically, in one study, *Prevotella amnii*, *Mobiluncus mulieris*, *Sneathia amnii* and *Sneathia sanguinegens* (all commonly found in CST-IV) were found

to induce higher levels of IL-1α, IL-1β, and IL-8 relative to *L. crispatus* dominated communities (CST-I), whereas *L. iners* dominated communities (CST-III) induced moderate IL-8 levels relative to CST-I. The authors also showed how there were significant increases in IL-1α, IL-1β and TNF-α longitudinally in subjects that transition from a CST-I, to CST-III and to a CST-IV (Anahtar *et al.* 2015). Conversely, mock communities dominated by *L. crispatus* (CST-I) and *L. jensenii* (CST-V) on reconstructed three-dimensional vaginal epithelial models do not strongly elicit cytokine IL-1β or IL-8 secretion relative to medium control, and also inhibit some pro-inflammatory responses after TLR 2/6 and 3 agonist induction (Rose *et al.* 2012). These studies continue to support the notion that the innate immune response is largely driven by vaginal bacterial community states, with CST-IV potentially having a larger pro-inflammatory response than CST-I or CST-II, and with CST-III triggering an intermediate response.

Additional factors contributing to vaginal defence include mannose binding lectin (MBL), vaginal antimicrobial peptides (AMPs) and immunoglobulin A and G (IgA, IgG). As its name suggests, MBL binds mannose, *N*-acetylglucosamine and fucose carbohydrate moieties present on microbial cell surfaces. Eventually, this interaction leads to cell lysis or targeting for the immune system (Neth *et al.* 2000; Turner, 2003). IgA and IgG may help to prevent vaginal epithelial cell adherence and uptake, as well as contribute to the neutralization and clearance of infectious microbes from the vagina (Tramont, 1977; Wang *et al.* 2014). Vaginal AMPs exist in various classes and may recruit immune cells via chemotaxis or possess anti-endotoxin activity. Mechanisms for each AMP have been thoroughly reviewed elsewhere (Ding *et al.* 2009; Wilson *et al.* 2013; Yarbrough *et al.* 2015), and while the specific association between AMPs and vaginal microbiota has not been extensively investigated, key findings are emphasized here. Defensins are a class of cationic and amphipathic AMPs with diverse mechanisms of action against common vaginal bacteria, pathogens and viruses including HIV, herpes simplex virus (HSV) and human papillomavirus (HPV). In organotypic models of the vaginal epithelium, human β-defensin (HBD)-2 expression, but not that of HBD-1, was associated with colonization by *L. iners*, *Atopobium vaginae* and *Prevotella bivia* (Doerflinger *et al.* 2014), while in another study using similar experimental *in vitro* conditions, *L. jensenii* but not *Gardnerella vaginalis* was shown to induce HBD-2 transcription (Valore *et al.* 2006). As expected, many human defensins bind to viral-specific proteins to prevent viral attachment to a human cell surface, as for example, with retrocyclin-1, retrocyclin-2, human neutrophil peptide (HNP)-1, HNP-2, HNP-3 and to a much lesser degree HNP-4 (Maddon *et al.* 1986; Münk *et al.* 2003; Wang *et al.* 2003, 2004; Wu *et al.* 2005; Furci *et al.* 2007); however, the topic is outside the scope of this review. In addition to defensins, other AMPs are found in the human vagina and include the secretory leukocyte protease inhibitor (SLPI), human epididymis protein 4 (HE4), LL-37, and surfactant protein (SP)-A and SP-D. SLPI expression is associated with BV organisms (Nasioudis *et al.* 2015) but not with *L. crispatus*, *L. iners*, *A. vaginae* or *P. bivia* (Doerflinger *et al.* 2014; Orfanelli *et al.* 2014). HE4 is associated with *G. vaginalis* (Orfanelli *et al.* 2014) and LL-37 inactivates the sexually transmitted pathogen *Neisseria gonorrhoeae* while having no effect on *L. crispatus* and *L. jensenii* and comparatively little effect on *L. iners* (Moncla *et al.* 2012). The lack of AMP stimulation in response to some *Lactobacillus* spp. is associated with their needed maintenance in the vagina (Valore *et al.* 2002). Similar to defensins, SP-A and SP-D contribute to viral inhibition, including HIV where they act via binding to the viral protein gp120 and human CD4, but with SP-A simultaneously enhancing gp120 binding to dendritic cells and therefore also facilitating HIV uptake. (Gaiha *et al.* 2008; Pandit *et al.* 2014). Thus, overall, microbes, environments, immune regulatory actions and genes tightly interact to govern homeostasis of the vaginal environment.

Bacterial vaginosis and aerobic vaginitis. As mentioned, the vaginal microbiota can be characterized by one of five CSTs, with CST-IV lacking a relatively high abundance of *Lactobacillus* spp. Generally, CST-IV can clinically manifest as aerobic vaginitis (AV) or BV, so the immune response to CST-IV outlined above overlaps considerably with BV or AV. AV is mainly differentiated from BV by the presence of an inflammatory response predominately associated with aerobes, such as group B *Streptococcus*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus* (Donders *et al.* 2002; Donders, 2007). The AV inflammatory response manifests symptomatically as itching or burning, molecularly as increased IL-6 and IL-1β, and cellularly as the presence of leukocytes or primary blood cells in a microscopic wet mount (Han *et al.* 2014). In contrast, the clinical definition of BV does not involve any overt inflammatory responses such as recruitment of neutrophils, redness, itching or burning (reviewed in Cauci, 2004). A number of immune factors including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-γ, chemokine C-C motif ligand 5 (CCL5) and SLPI have been variably and inconsistently associated with BV (summarized in Mitchell & Marrazzo, 2014). These conflicting findings may be due to different study designs (longitudinal versus cross-sectional, or *in vitro* versus *in vivo*), different definitions of BV (symptomatic versus asymptomatic BV or Nugent-BV *versus* BV diagnosed according to the Amsel criteria) or that additional features actively suppress the inflammatory response in BV, such as IgA degradation, TLR expression

inhibition, or immune-related genetic variants (Cauci *et al.* 2003; Witkin *et al.* 2007*b*). As an example of BV's effect on host defence, cytokine analysis from a vaginal epithelial cell model co-colonized with mock communities representing CST-I to -IV as well as Nugent scores corresponding to respective BV diagnosis showed significant increases in IL-1β, IL-8, TNF-α, CCL5 and IL-1RA in CST-III or CST-IV, but not CST-I or CST-II (Pyles *et al.* 2014). The ability of individual BV-associated bacterial species to elicit an *in vitro* immune response has also been studied, as in the cases of *A. vaginae*, which induces expression of chemokine C-C motif ligand 20 (CCL20), HBD-2, IL-1β, IL-6, IL-8 and TNF-α via nuclear factor-κB (NF-κB), TLR2 and MyD88 signalling pathways; *G. vaginalis*, which induces IL-6 and IL-8 transcripts; and *L. iners*, which stimulates PRR signalling but not downstream inflammatory response cytokines IL-6, IL-8 or mucins (Libby *et al.* 2008; Doerflinger *et al.* 2014). Bacteria-derived short chain fatty acids (SCFAs), namely acetate, butyrate, propionate and succinate, some of which exist at relatively higher proportions during BV, can induce a pro-inflammatory response under the hypothesis that SCFAs may act to ultimately inhibit chemotaxis and inflammation in BV (Al-Mushrif *et al.* 2000; Chaudry *et al.* 2004; Mirmonsef *et al.* 2011; Gajer *et al.* 2012; O'Hanlon *et al.* 2013). Relatively high concentrations (2–20 mM) of acetate and butyrate, but not propionate, induce cytokine IL-6, IL-8 and IL-1β secretions and also induce IL-8 and TNF-α with TLR2 and TLR7 ligand stimulation in a dose- and time-dependent manner *in vitro* (Mirmonsef *et al.* 2011). However, whether or not the host is actively downplaying the sensing of BV-associated microbes or a specific attribute of BV is evading inflammation remains to be demonstrated since the aetiology of BV is still unknown and the necessary longitudinal studies are lacking (Schwebke, 2009).

Lactic acid and host defence. Lactic acid is produced mainly by vaginal microbes (Boskey *et al.* 2001) and helps maintain healthy host physiological functions since it has been shown to directly inhibit *Chlamydia trachomatis* infection (Gong *et al.* 2014), and potentially both HSV-2 and HIV *in vitro* and *in vivo* if there is sufficient lactic acid to acidify the vagina to pH < 4 (Conti *et al.* 2009; Aldunate *et al.* 2013; Isaacs & Xu, 2013, 2014). Lactic acid also inactivates a broad range of BV-associated microbes at pH < 4.5 (O'Hanlon *et al.* 2011). When *Lactobacillus* spp. dominate the vaginal microbiota, they acidify the vagina to a strongly acidic mean pH of 3.5 ± 0.2 that is likely to help protect against a broad range of infections (O'Hanlon *et al.* 2013). Recent studies aimed to uncover the mechanism by which lactic acid can directly affect host immune functions, as for example by directly inhibiting pro-inflammatory responses IL-6, IL-8 and IL-1RA, (Hearps *et al.* 2014), inducing the Th17 lymphocyte pathway via IL-23 in a dose-dependent manner upon lipopolysaccharide co-stimulation (Witkin *et al.* 2011), and helping to release mediators from vaginal epithelial cells and stimulating antiviral response by release of transforming growth factor-β (Mossop *et al.* 2011). In the gut, lactate and acetate from *L. casei* and *Bifidobacterium breve* inhibit cell proliferation, but whether these molecules play a similar role in the vagina has not been studied (Matsuki *et al.* 2013). Interestingly, lactic acid isomers may also play a role in determining host response and the subsequent host–microbiota relationship. Lactic acid exists in the vagina in both D-(−)- and L-(+)-isomers, with the host contributing only about 4–30% of the total lactate (Boskey *et al.* 2001), suggesting a large reliance on microbes to supply the majority of lactic acid for protection. In one study, only D-(−)-lactic acid was correlated with α-amylase, SLPI, hyaluronidase-1, neutrophil gelatinase-associated lipocalin and matrix metalloproteinase 8 (MMP-8) expression *in vitro* (Nasioudis *et al.* 2015). The authors suggest that epithelial cell exfoliation and subsequent breakdown of glycogen helps favour *Lactobacillus* spp. growth, and thus helps sustain D-(−)-lactic acid production (see discussion on α-amylase and glycogen below). Moreover, women with BV were found to be deficient in both isomers, while those with vulvovaginal candidiasis have elevated L-(+)-lactic acid as well as CD147 and MMP-8 genes (Beghini *et al.* 2014). *Lactobacillus iners* does not produce D-(−)-lactic acid and fails to produce the L-(+)-lactic acid in abundance as high as *L. crispatus* or *L. gasseri* while *L. jensenii* produces only D-(−)-lactic acid (Witkin *et al.* 2013), suggesting potential *Lactobacillus* species-specific effects on the host. Consequently, the composition of the vaginal microbiota, and specifically the ability of vaginal microbes to produce D-(−)-lactic acid, may help to inhibit pathogens and inflammatory responses while also favouring *Lactobacillus* spp. survival by using host cells resources for carbon sources.

The effect of the microbiota on the vaginal mucosa

The vaginal mucosa plays an important role as a physical barrier to separate host epithelial environment from harmful pathogens, including HIV (Nunn *et al.* 2014, 2015), whereas vaginal microbes can affect the integrity of the mucosa (Arnold *et al.* 2014). The BV-associated species *G. vaginalis* secretes sialidases, which have been shown to deglycosylate secretory immunoglobulin A (sIgA) and other sialoglycan substrates via the cleavage of sialic acid from gylocoprotein at the α2–3 and α2–6 linkage Neu5Ac present on both *N*- and *O*-glycans, thereby hydrolysing protective mucosal sialoglycoproteins (Lewis *et al.* 2012). *Gardnerella vaginalis* can consume and neutralize liberated sialic acid residues to further evade the host response (Lewis *et al.* 2013). In addition, *G.*

vaginalis produces vaginolysin, a cholesterol-dependent cytolysin that could contribute to BV symptomology by forming pores in the vaginal epithelium with or without CD59 (Gelber *et al.* 2008; Zilnyte *et al.* 2015). BV-associated bacteria quantified by Nugent score also significantly associate with mucins-1, -4, -5AC and -7 (Moncla *et al.* 2014). Conversely, certain types of vaginal communities could enhance the integrity of the mucosal barrier. A recent study showed that *L. crispatus*-dominated vaginal microbiota were able to reinforce the diffusional barrier properties of cervicovaginal mucus against HIV, hence hindering HIV penetration, while communities dominated by *L. iners* facilitated the penetration of HIV through the cervicovaginal mucus barrier (Nunn *et al.* 2015). Thus, a change in vaginal community composition and function is strongly associated with the integrity of the protective mucus layer. Therefore, vaginal bacteria, including species of *Lactobacillus*, can reduce or increase susceptibility to infectious agents such as HIV.

The effect of the microbiota on reproductive functions

The vaginal microbiota in combination with other factors is associated with adverse reproductive and obstetric outcomes. For example, a meta-analysis revealed that BV-like vaginal microbiota are significantly more prevalent in women with tubal infertility when compared with women with other causes of infertility, but is not associated with decreased conception rates (van Oostrum *et al.* 2013). Preterm labour and delivery has been thought to be in part associated with changes in the vaginal microbiota composition, namely bacteria found in CST-IV (i.e. *G. vaginalis* and *Ureaplasma*), AV or BV (preterm labour and delivery defined as occurring before 37 weeks in Caucasian women (Donders *et al.* 2009), mostly African American women (Nelson *et al.* 2014), and mostly Caucasian women (DiGiulio *et al.* 2015)). Jakovljević *et al.* assessed differences of gestational time to delivery in BV *versus* non-BV women, with a statistically significant difference of 37.72 $\pm$ 3.9 *versus* 39.59 $\pm$ 1.1 weeks (Jakovljević *et al.* 2014). However, another study did not find any association between CST-IV and preterm births (defined as 28–33.1 weeks *versus* term births of 38.8–40.7 weeks in mostly African American women) even though the study was well-powered and preterm births were phenotypically well controlled (Romero *et al.* 2014). It should be noted that differences in ethnicity, definition of preterm birth and analytical methods of microbiota data could explain these different observations. The possibility remains that functional differences exist and that hypotheses should be further explored using metagenomics- or metatranscriptomics-based approaches.

The interplay between host polymorphism and the vaginal microbiota could play an important role in the mechanisms by which microbes affect reproductive health. Polymorphisms in genes that control inflammatory response (protein kinase Cα, fms-like tyrosine kinase 1, IL-6 and TNF-α) are associated with preterm delivery in combination with CST-IV vaginal microbiota, although the direct functional impact of these polymorphisms is unknown (Gómez *et al.* 2010; Jones *et al.* 2010). These studies and others have suggested that the inflammatory and antimicrobial peptide response, associated with certain vaginal microbiota, exhibit a role in rupturing and invading cervical plug or amniotic membranes, eventually triggering pro-inflammatory cascades that could lead to premature labour and delivery (reviewed in Goldenberg *et al.* 2000; Witkin, 2014; Yarbrough *et al.* 2015). In a rhesus monkey model infected with group B *Streptococcus*, there was an observed increase in amniotic fluid cytokines TNF-α, IL-1β and IL-6 occurring before uterine contractility or any clinical signs of infection, suggesting a direct role of infection in preterm labour (Gravett *et al.* 1994). Specific AMPs are expressed upon exposure *in vitro* and *in vivo* to the BV-associated bacteria *A. vaginae* (CCL20, HBD-2) or pathogens, such as *C. trachomatis* (elafin) and *N. gonorrhoeae* (SLPI), but not *L. crispatus* or *L. jensenii* (Libby *et al.* 2008; King *et al.* 2009; Cooper *et al.* 2012; Eade *et al.* 2012; Doerflinger *et al.* 2014). The immune response to pathogens can trigger signalling cascades, which could ultimately lead to miscarriage, intrauterine infection, preterm labour, and tubal and ectopic pregnancy. For example, if *C. trachomatis* ascends past the cervix, such an infection in the upper genital track could lead to tubal scarring and potentially tubal infertility, ectopic pregnancy and chronic pelvic pain (Hafner, 2015, and discussed in Barlow *et al.* 2001). *C. albicans* triggers IL-6 secretion in the placenta ultimately leading to an increase in NF-κB and an inflammatory response (Tyutyunnik *et al.* 2014). A study measuring the effects of immunomodulation in pregnant women with BV found that *C. albicans* and *Trichomonas vaginalis*, but not *C. trachomatis* or *N. gonorrhoeae*, had an effect on vaginal cytokines and none of these pathogens had any effect on anti-*G. vaginalis* haemolysin IgA, sialidase or prolidase activity (Cauci & Culhane, 2007). Clearly there is still much to learn about the dynamics, function and mechanisms driving the role of the vaginal microbiome in reproduction health.

Physiology affects vaginal microbiota composition

The composition of the vaginal microbiota changes throughout a woman's lifetime from birth, through puberty, reproductive age and menopause. The early childhood vaginal microbiota comprise a variety of anaerobes, diphtheroids, coagulase-negative staphylococci, and *E. coli*, whereas postmenopausal women often experience a loss of *Lactobacillus* spp. associated with

the decrease in oestrogen controlling vaginal epithelial proliferation, maturation, and accumulation of glycogen, which is directly or indirectly nutritionally necessary for the maintenance of *Lactobacillus* spp. (Hammerschlag *et al.* 1978*a*,*b*; Hillier & Lau, 1997; Galhardo *et al.* 2006; Brotman *et al.* 2013*b*). Indeed, oestrogen levels peak during reproductive age and contribute to shaping the composition of the vaginal microbiota. In menopause, vaginal application of oestrogen cream is associated with vaginal epithelial maturation, the accumulation of glycogen and acidic pH (<4.0), the latter indicative of the presence of high number of *Lactobacillus* spp. (Nilsson *et al.* 1995). Interestingly, *Lactobacillus* spp. was originally thought to directly ferment glycogen in the vagina. However, this idea was gradually refuted and recent evidence suggests that human α-amylase catabolizes glycogen into smaller polymers, namely maltose and maltotriose, which can then be used by *Lactobacillus* spp. for metabolism, even in newborns who have residual circulating maternal oestrogen (Cruickshank, 1934; Cruickshank & Sharman, 1934; Stewart-Tull, 1964; Bernbaum *et al.* 2007; Spear *et al.* 2014). This model puts forward that the influence of oestrogen, glycogen and especially α-amylase provides a positive selection pressure for a *Lactobacillus*-dominated microbiota (Spear *et al.* 2014). These findings highlight the tight interplay between host physiology and the vaginal microbiota.

Conclusion

The human vaginal ecosystem is a dynamic environment in which microbes can affect host physiology but also where host physiology can affect the composition and function of the vaginal microbiota. Species of *Lactobacillus* have been historically associated with vaginal health in reproductive-age women due to the direct and indirect protective nature afforded by *Lactobacillus* products, such as lactic acid and bacteriocin among others, against mucus degradation and inhibition of pathogens. The reported inconsistent innate immune response observed with non-*Lactobacillus*- or *L. iners*-dominated microbiota (CST-IV, BV, AV and CST-III, respectively), coupled with recent findings that question the definitions of normality, highlight the need for more in-depth functional understandings of the interaction between the vaginal microbiota and host physiology, reproduction and defence.

References

Aiyar A, Quayle AJ, Buckner LR, Sherchand SP, Chang TL, Zea AH, Martin DH & Belland RJ (2014). Influence of the tryptophan-indole-IFNγ axis on human genital *Chlamydia trachomatis* infection: role of vaginal co-infections. *Front Cell Infect Microbiol* **4**, 72.

Aldunate M, Tyssen D, Johnson A, Zakir T, Sonza S, Moench T, Cone R & Tachedjian G (2013). Vaginal concentrations of lactic acid potently inactivate HIV. *J Antimicrob Chemother* **68**, 2015–2025.

Aldunate M, Tyssen D, Latham C, Ramsland P, Perlmutter P, Moench T, Cone R & Tachedjian G (2014). Vaginal concentrations of lactic acid potently inactivate HIV-1 compared to short chain fatty acids present during bacterial vaginosis. *AIDS Res Hum Retroviruses* **30** Suppl 1, A228.

Al-Mushrif S, Eley A & Jones BM (2000). Inhibition of chemotaxis by organic acids from anaerobes may prevent a purulent response in bacterial vaginosis. *J Med Microbiol* **49**, 1023–1030.

Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D & Holmes KK (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* **74**, 14–22.

Anahtar MN, Byrne EH, Doherty KE, Bowman BA, Yamamoto HS, Soumillon M, Padavattan N, Ismail N, Moodley A, Sabatini ME, Ghebremichael MS, Nusbaum C, Huttenhower C, Virgin HW, Ndung'u T, Dong KL, Walker BD, Fichorova RN & Kwon DS (2015). Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity* **42**, 965–976.

Arnold K, Birse K, Mckinnon L, Lingappa J, Novak R, Westmacott G, Ball TB, Lauffenburger D & Burgener A (2014). Mucosal integrity factors are perturbed during bacterial vaginosis: a proteomic analysis. *AIDS Res Hum Retroviruses* **30** Suppl 1, A30.

Barlow RE, Cooke ID, Odukoya O, Heatley MK, Jenkins J, Narayansingh G, Ramsewak SS & Eley A (2001). The prevalence of *Chlamydia trachomatis* in fresh tissue specimens from patients with ectopic pregnancy or tubal factor infertility as determined by PCR and in-situ hybridisation. *J Med Microbiol* **50**, 902–908.

Beghini J, Linhares I, Giraldo P, Ledger W & Witkin S (2014). Differential expression of lactic acid isomers, extracellular matrix metalloproteinase inducer, and matrix metalloproteinase-8 in vaginal fluid from women with vaginal disorders. *BJOG* **122**, 1580–1585.

Bernbaum JC, Umbach DM, Ragan NB, Ballard JL, Archer JI, Schmidt-Davis H & Rogan WJ (2007). Pilot studies of estrogen-related physical findings in infants. *Environ Health Perspect* **116**, 416–420.

Boskey ER, Cone RA, Whaley KJ & Moench TR (2001). Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum Reprod* **16**, 1809–1813.

Brotman RM, Ghanem KG, Klebanoff MA, Taha TE, Scharfstein DO & Zenilman JM (2008). The effect of vaginal douching cessation on bacterial vaginosis: a pilot study. *Am J Obstet Gynecol* **198**, 628.e1–628.e7.

Brotman RM, Ravel J, Bavoil PM, Gravitt PE & Ghanem KG (2013*a*). Microbiome, sex hormones, and immune responses in the reproductive tract: Challenges for vaccine development against sexually transmitted infections. *Vaccine* **32**, 1543–1552.

Brotman RM, Ravel J, Cone RA & Zenilman JM (2010). Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. *Sex Transm Infect* **86**, 297–302.

Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, Viscidi RP, Burke AE, Ravel J & Gravitt PE (2013*b*). Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause* **21**, 450–458.

Campos ACC, Freitas-Junior R, Ribeiro LFJ, Paulinelli RR & Reis C (2008). Prevalence of vulvovaginitis and bacterial vaginosis in patients with koilocytosis. *Sao Paulo Med J* **126**, 333–336.

Carvalho A, Giovannini G, De Luca A, D'Angelo C, Casagrande A, Iannitti RG, Ricci G, Cunha C & Romani L (2012). Dectin-1 isoforms contribute to distinct Th1/Th17 cell activation in mucosal candidiasis. *Cell Mol Immunol* **9**, 276–286.

Cauci S (2004). Vaginal immunity in bacterial vaginosis. *Curr Infect Dis Rep* **6**, 450–456.

Cauci S & Culhane JF (2007). Modulation of vaginal immune response among pregnant women with bacterial vaginosis by *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and yeast. *Am J Obstet Gynecol* **196**, 133.e1–133.e7.

Cauci S, Guaschino S, de Aloysio D, Driussi S, De Santo D, Penacchioni P & Quadrifoglio F (2003). Interrelationships of interleukin-8 with interleukin-1β and neutrophils in vaginal fluid of healthy and bacterial vaginosis positive women. *Mol Hum Reprod* **9**, 53–58.

Chaudry AN, Travers PJ, Yuenger J, Colletta L, Evans P, Zenilman JM & Tummon A (2004). Analysis of vaginal acetic acid in patients undergoing treatment for bacterial vaginosis. *J Clin Microbiol* **42**, 5170–5175.

Chen J, Li Y, Tian Y, Huang C, Li D, Zhong Q & Ma X (2015). Interaction between microbes and host intestinal health: modulation by dietary nutrients and gut-brain-endocrine-immune axis. *Curr Protein Pept Sci* **16**, 592–603.

Cherpes TL, Meyn LA, Krohn MA, Lurie JG & Hillier SL (2003). Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis* **37**, 319–325.

Cohen CR, Duerr A, Pruithithada N, Rugpao S, Hillier S, Garcia P & Nelson K (1995). Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. *AIDS* **9**, 1093–1097.

Coleman JS, Hitti J, Bukusi EA, Mwachari C, Muliro A, Nguti R, Gausman R, Jensen S, Patton D, Lockhart D, Coombs R & Cohen CR (2007). Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. *AIDS* **21**, 755–759.

Conlon M & Bird A (2015). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* **7**, 17–44.

Conti C, Malacrino C & Mastromarino P (2009). Inhibition of herpes simplex virus type 2 by vaginal lactobacilli. *J Physiol Pharmacol* **60** Suppl 6, 19–26.

Cooper MD, Roberts MH, Barauskas OL & Jarvis GA (2012). Secretory leukocyte protease inhibitor binds to *Neisseria gonorrhoeae* outer membrane opacity protein and is bactericidal. *Am J Reprod Immunol* **68**, 116–127.

Cruickshank R (1934). The conversion of the glycogen of the vagina into lactic acid. *J Pathol Bacteriol* **39**, 213–219.

Cruickshank R & Sharman A (1934). The biology of the vagina in the human subject. *BJOG* **41**, 208–226.

Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CC; HIV Epidemiology Research Study (2001). Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. *Clin Infect Dis* **33**, 894–896.

DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DSA, Wong RJ, Shaw G, Stevenson DK, Holmes SP & Relman DA (2015). Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci USA* **112**, 11060–11065.

Ding J, Chou Y-Y & Chang TL (2009). Defensins in viral infections. *J Innate Immun* **1**, 413–420.

Doerflinger SY, Throop AL & Herbst-Kralovetz MM (2014). Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. *J Infect Dis* **209**, 1989–1999.

Donders GGG (2007). Definition and classification of abnormal vaginal flora. *Best Pract Res Clin Obstet Gynaecol* **21**, 355–373.

Donders GG, Van Calsteren K, Bellen G, Reybrouck R, Van den Bosch T, Riphagen I & Van Lierde S (2009). Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG* **116**, 1315–1324.

Donders GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G & Spitz B (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *BJOG* **109**, 34–43.

Eade CR, Diaz C, Wood MP, Anastos K, Patterson BK, Gupta P, Cole AL & Cole AM (2012). Identification and characterization of bacterial vaginosis-associated pathogens using a comprehensive cervical-vaginal epithelial coculture assay. *PLoS One* **7**, e50106.

Farage M & Maibach H (2005). Lifetime changes in the vulva and vagina. *Arch Gynecol Obstet* **273**, 195–202.

Fredricks DN, Fiedler TL & Marrazzo JM (2005). Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med* **353**, 1899–1911.

Furci L, Sironi F, Tolazzi M, Vassena L & Lusso P (2007). Alpha-defensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4. *Blood* **109**, 2928–2935.

Gaiha GD, Dong T, Palaniyar N, Mitchell DA, Reid KBM & Clark HW (2008). Surfactant protein A binds to HIV and inhibits direct infection of CD4+ cells, but enhances dendritic cell-mediated viral transfer. *J Immunol* **181**, 601–609.

Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UME, Zhong X, Koenig SSK, Fu L, Ma ZS, Zhou X, Abdo Z, Forney LJ & Ravel J (2012). Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**, 132ra52.

Galhardo CL, Soares JM, Simões RS, Haidar MA, Rodrigues de Lima G & Baracat EC (2006). Estrogen effects on the vaginal pH, flora and cytology in late postmenopause after a long period without hormone therapy. *Clin Exp Obstet Gynecol* **33**, 85–89.

Gelber SE, Aguilar JL, Lewis KLT & Ratner AJ (2008). Functional and phylogenetic characterization of Vaginolysin, the human-specific cytolysin from *Gardnerella vaginalis*. *J Bacteriol* **190**, 3896–3903.

Genç MR, Onderdonk AB, Vardhana S, Delaney ML, Norwitz ER, Tuomala RE, Paraskevas L-R, Witkin SSMAP Study Group (2004*a*). Polymorphism in intron 2 of the interleukin-1 receptor antagonist gene, local midtrimester cytokine response to vaginal flora, and subsequent preterm birth. *Am J Obstet Gynecol* **191**, 1324–1330.

Genç MR, Vardhana S, Delaney ML, Onderdonk A, Tuomala R, Norwitz E, Witkin SSMAP Study Group (2004*b*). Relationship between a toll-like receptor-4 gene polymorphism, bacterial vaginosis-related flora and vaginal cytokine responses in pregnant women. *Eur J Obstet Gynecol* **116**, 152–156.

Genç MR, Vardhana S, Delaney ML, Witkin SS, Onderdonk ABMAP Study Group (2007). TNFA-308G>A polymorphism influences the TNF-alpha response to altered vaginal flora. *Eur J Obstet Gynecol* **134**, 188–191.

Giraldo PC, Babula O, Gonçalves AKS, Linhares IM, Amaral RL, Ledger WJ & Witkin SS (2007). Mannose-binding lectin gene polymorphism, vulvovaginal candidiasis, and bacterial vaginosis. *Obstet Gynecol* **109**, 1123–1128.

Goldenberg RL, Andrews WW, Yuan AC, MacKay HT & St Louis ME (1997). Sexually transmitted diseases and adverse outcomes of pregnancy. *Clin Perinatol* **24**, 23–41.

Goldenberg RL, Hauth JC & Andrews WW (2000). Intrauterine infection and preterm delivery. *N Engl J Med* **342**, 1500–1507.

Gong Z, Luna Y, Yu P & Fan H (2014). Lactobacilli inactivate *Chlamydia trachomatis* through lactic acid but not H_2O_2. *PLoS One* **9**, e107758.

Gómez LM, Sammel MD, Appleby DH, Elovitz MA, Baldwin DA, Jeffcoat MK, Macones GA & Parry S (2010). Evidence of a gene-environment interaction that predisposes to spontaneous preterm birth: a role for asymptomatic bacterial vaginosis and DNA variants in genes that control the inflammatory response. *Am J Obstet Gynecol* **202**, 386.e1–386.e6.

Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA & Holmes KK (1986). Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. *JAMA* **256**, 1899–1903.

Gravett MG, Witkin SS, Haluska GJ, Edwards JL, Cook MJ & Novy MJ (1994). An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am J Obstet Gynecol* **171**, 1660–1667.

Hafner LM (2015). Pathogenesis of fallopian tube damage caused by *Chlamydia trachomatis* infections. *Contraception* **92**, 108–115.

Hammerschlag MR, Alpert S, Onderdonk AB, Thurston P, Drude E, McCormack WM & Bartlett JG (1978*a*). Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol* **131**, 853–856.

Hammerschlag MR, Alpert S, Rosner I, Thurston P, Semine D, McComb D & McCormack WM (1978*b*). Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics* **62**, 57–62.

Han C, Wu W, Fan A, Wang Y, Zhang H, Chu Z, Wang C & Xue F (2014). Diagnostic and therapeutic advancements for aerobic vaginitis. *Arch Gynecol Obstet* **291**, 251–257.

Hay PE, Ugwumadu A & Chowns J (1997). Sex, thrush and bacterial vaginosis. *Int J STD AIDS* **8**, 603–608.

Hearps A, Gugasyan R, Srbinovski D, Tyssen D, Aldunate M, Anderson DJ, Cone R & Tachedjian G (2014). Lactic acid, a vaginal microbiota metabolite, elicits an anti-inflammatory response from vaginal and cervical epithelial cells. *AIDS Res Hum Retroviruses* **30** Suppl 1, A238–A239.

Hillier SL & Lau RJ (1997). Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. *Clin Infect Dis* **25**, S123–S126.

Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG & Rao AV; the Vaginal Infections and Prematurity Study Group (1995). Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med* **333**, 1737–1742.

Horne AW, Stock SJ & King AE (2008). Innate immunity and disorders of the female reproductive tract. *Reproduction* **135**, 739–749.

Isaacs CE & Xu W (2013). Theaflavin-3,3'-digallate and lactic acid combinations reduce herpes simplex virus infectivity. *Antimicrob Agents Chemother* **57**, 3806–3814.

Jakovljević A, Bogavac M, Nikolić A, Tošić MM, Novaković Z & Stajić Z (2014). The influence of bacterial vaginosis on gestational week of the completion of delivery and biochemical markers of inflammation in the serum. *Vojnosanit Pregl* **71**, 931–935.

Jones NM, Holzman C, Friderici KH, Jernigan K, Chung H, Wirth J & Fisher R (2010). Interplay of cytokine polymorphisms and bacterial vaginosis in the etiology of preterm delivery. *J Reprod Immunol* **87**, 82–89.

Kau AL, Ahern PP, Griffin NW, Goodman AL & Gordon JI (2011). Human nutrition, the gut microbiome and the immune system. *Nature* **474**, 327–336.

Keane FE, Ison CA & Taylor-Robinson D (1997). A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD AIDS* **8**, 489–494.

King AE, Wheelhouse N, Cameron S, McDonald SE, Lee K-F, Entrican G, Critchley HOD & Horne AW (2009). Expression of secretory leukocyte protease inhibitor and elafin in human fallopian tube and in an in-vitro model of *Chlamydia trachomatis* infection. *Hum Reprod* **24**, 679–686.

Lewis WG, Robinson LS, Gilbert NM, Perry JC & Lewis AL (2013). Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted *Actinobacterium Gardnerella vaginalis*. *J Biol Chem* **288**, 12067–12079.

Lewis WG, Robinson LS, Perry J, Bick JL, Peipert JF, Allsworth JE & Lewis AL (2012). Hydrolysis of secreted sialoglycoprotein immunoglobulin A (IgA) in ex vivo and biochemical models of bacterial vaginosis. *J Biol Chem* **287**, 2079–2089.

Libby EK, Pascal KE, Mordechai E, Adelson ME & Trama JP (2008). *Atopobium vaginae* triggers an innate immune response in an in vitro model of bacterial vaginosis. *Microbes Infect* **10**, 439–446.

Linhares IM, Summers PR, Larsen B, Giraldo PC & Witkin SS (2011). Contemporary perspectives on vaginal pH and lactobacilli. *Am J Obstet Gynecol* **204**, 120.e1–120.e5.

Ma B, Forney LJ & Ravel J (2012). Vaginal microbiome: rethinking health and disease. *Annu Rev Microbiol* **66**, 371–389.

Mackelprang RD, Scoville CW, Cohen CR, Ondondo RO, Bigham AW, Celum C, Campbell MS, Essex M, Wald A, Kiarie J, Ronald A, Gray G & Lingappa JR (2015). Toll-like receptor gene variants and bacterial vaginosis among HIV-1 infected and uninfected African women. *Genes Immun* **16**, 362–325.

Macklaim JM, Fernandes AD, Di Bella JM, Hammond J-A, Reid G & Gloor GB (2013). Comparative meta-RNA-seq of the vaginal microbiota and differential expression by *Lactobacillus iners* in health and dysbiosis. *Microbiome* **1**, 12.

Macklaim JM, Gloor GB, Anukam KC, Cribby S & Reid G (2011). At the crossroads of vaginal health and disease, the genome sequence of *Lactobacillus iners* AB-1. *Proc Natl Acad Sci USA* **108** Suppl 1, 4688–4695.

Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA & Axel R (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**, 333–348.

Martin DH (2012). The microbiota of the vagina and its influence on women's health and disease. *Am J Med Sci* **343**, 2–9.

Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J & Kreiss J (1999). Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* **180**, 1863–1868.

Matsuki T, Pédron T, Regnault B, Mulet C, Hara T & Sansonetti PJ (2013). Epithelial cell proliferation arrest induced by lactate and acetate from lactobacillus casei and bifidobacterium breve. *PLoS One* **8**, e63053.

McDonald HM, O'Loughlin JA, Jolley P, Vigneswaran R & McDonald PJ (1992). Prenatal microbiological risk factors associated with preterm birth. *Br J Obstet Gynaecol* **99**, 190–196.

Meis PJ, Goldenberg RL, Mercer B, Moawad A, Das A, McNellis D, Johnson F, Iams JD, Thom E & Andrews WW; National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network (1995). The preterm prediction study: significance of vaginal infections. *Am J Obstet Gynecol* **173**, 1231–1235.

Mirmonsef P, Zariffard MR, Gilbert D, Makinde H, Landay AL & Spear GT (2011). Short-chain fatty acids induce pro-inflammatory cytokine production alone and in combination with toll-like receptor ligands. *Am J Reprod Immunol* **67**, 391–400.

Mitchell C & Marrazzo J (2014). Bacterial vaginosis and the cervicovaginal immune response. *Am J Reprod Immunol* **71**, 555–563.

Moncla BJ, Chappell C, Debo BM, Macio IS, Bunge KE & Hillier SL (2014). The effects of hormones and vaginal microflora on the content of MUC1, MUC4, MUC5AC and MUC7 in the cervicovaginal fluid (CVF). *AIDS Res Hum Retroviruses* **30** Suppl 1, A29.

Moncla BJ, Mietzner TA & Hillier SL (2012). In vitro activity of cationic peptides against *Neisseria gonorrhoeae* and vaginal *Lactobacillus* species: The effect of divalent cations. *Advances in Bioscience and Biotechnology* **3**, 249–255.

Mossop H, Linhares IM, Bongiovanni AM, Ledger WJ & Witkin SS (2011). Influence of lactic acid on endogenous and viral RNA-induced immune mediator production by vaginal epithelial cells. *Obstet Gynecol* **118**, 840–846.

Münk C, Wei G, Yang OO, Waring AJ, Wang W, Hong T, Lehrer RI, Landau NR & Cole AM (2003). The theta-defensin, retrocyclin, inhibits HIV-1 entry. *AIDS Res Hum Retroviruses* **19**, 875–881.

Nasioudis D, Beghini J, Bongiovanni AM, Giraldo PC, Linhares IM & Witkin SS (2015). α-Amylase in vaginal fluid: association with conditions favorable to dominance of Lactobacillus. *Reprod Sci* **22**, 1393–1398.

Nelson DB, Hanlon A, Nachamkin I, Haggerty C, Mastrogiannis DS, Liu C & Fredricks DN (2014). Early pregnancy changes in bacterial vaginosis-associated bacteria and preterm delivery. *Paediatr Perinat Epidemiol* **28**, 88–96.

Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ & Turner MW (2000). Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* **68**, 688–693.

Nguyen PV, Kafka JK, Ferreira VH, Roth K & Kaushic C (2014). Innate and adaptive immune responses in male and female reproductive tracts in homeostasis and following HIV infection. *Cell Mol Immunol* **11**, 410–427.

Nilsson K, Risberg B & Heimer G (1995). The vaginal epithelium in the postmenopause–cytology, histology and pH as methods of assessment. *Maturitas* **21**, 51–56.

Nugent RP, Krohn MA & Hillier SL (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* **29**, 297–301.

Nunn K, Wang Y-Y, Harit D, Cone R & Lai S (2014). Influence of vaginal microbiota on the diffusional barrier properties of cervicovaginal mucus. *AIDS Res Hum Retroviruses* **30** Suppl 1, A234.

Nunn KL, Wang Y-Y, Harit D, Humphrys MS, Ma B, Cone R, Ravel J & Lai SK (2015). Enhanced trapping of HIV-1 by human cervicovaginal mucus is associated with *Lactobacillus crispatus*-dominant microbiota. *MBio* **6**, e01084–15.

O'Hanlon DE, Moench TR & Cone RA (2011). In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC Infect Dis* **11**, 200.

O'Hanlon DE, Moench TR & Cone RA (2013). Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS One* **8**, e80074.

Orfanelli T, Jayaram A, Doulaveris G, Forney LJ, Ledger WJ & Witkin SS (2014). Human epididymis protein 4 and secretory leukocyte protease inhibitor in vaginal fluid: relation to vaginal components and bacterial composition. *Reprod Sci* **21**, 538–542.

Pandit H, Gopal S, Sonawani A, Yadav AK, Qaseem AS, Warke H, Patil A, Gajbhiye R, Kulkarni V, Al-Mozaini MA, Idicula-Thomas S, Kishore U & Madan T (2014). Surfactant protein D inhibits HIV-1 infection of target cells via interference with gp120-CD4 interaction and modulates pro-inflammatory cytokine production. *PLoS One* **9**, e102395-11.

Peters SE, Beck-Sagué CM, Farshy CE, Gibson I, Kubota KA, Solomon F, Morse SA, Sievert AJ & Black CM (2000). Behaviors associated with *Neisseria gonorrhoeae* and *Chlamydia trachomatis*: cervical infection among young women attending adolescent clinics. *Clin Pediatr (Phila)* **39**, 173–177.

Petrova MI, Lievens E, Malik S, Imholz N & Lebeer S (2015). Lactobacillus species as biomarkers and agents that can promote various aspects of vaginal health. *Front Physiol* **6**, 81.

Pyles RB, Vincent KL, Baum MM, Elsom B, Miller AL, Maxwell C, Eaves-Pyles TD, Li G, Popov VL, Nusbaum RJ & Ferguson MR (2014). Cultivated vaginal microbiomes alter HIV-1 infection and antiretroviral efficacy in colonized epithelial multilayer cultures. *PLoS One* **9**, e93419.

Ravel J, Brotman RM, Gajer P, Ma B, Nandy M, Fadrosh DW, Sakamoto J, Koenig SS, Fu L, Zhou X, Hickey RJ, Schwebke JR & Forney LJ (2013). Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**, 29.

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L & Forney LJ (2011). Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108** Suppl 1, 4680–4687.

Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Bieda J, Chaemsaithong P, Miranda J, Chaiworapongsa T & Ravel J (2014). The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome* **2**, 18.

Rose WA, McGowin CL, Spagnuolo RA, Eaves-Pyles TD, Popov VL & Pyles RB (2012). Commensal bacteria modulate innate immune responses of vaginal epithelial cell multilayer cultures. *PLoS One* **7**, e32728.

Royse KE, Kempf M-C, McGwin G, Wilson CM, Tang J & Shrestha S (2012). Toll-like receptor gene variants associated with bacterial vaginosis among HIV-1 infected adolescents. *J Reprod Immunol* **96**, 84–89.

Ryckman KK, Williams SM, Krohn MA & Simhan HN (2011). Interaction between interleukin-1 receptor 2 and Toll-like receptor 4, and cervical cytokines. *J Reprod Immunol* **90**, 220–226.

Schwebke JR (2009). New concepts in the etiology of bacterial vaginosis. *Curr Infect Dis Rep* **11**, 143–147.

Schwebke JR, Richey CM & Weiss2 HL (1999). Correlation of behaviors with microbiological changes in vaginal flora. *J Infect Dis* **180**, 1632–1636.

Spear GT, French AL, Gilbert D, Zariffard MR, Mirmonsef P, Sullivan TH, Spear WW, Landay A, Micci S, Lee B-H & Hamaker BR (2014). Human α-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis* **210**, 1019–1028.

Srinivasan S, Liu C, Mitchell CM, Fiedler TL, Thomas KK, Agnew KJ, Marrazzo JM & Fredricks DN (2010). Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. *PLoS One* **5**, e10197.

Stewart-Tull DE (1964). Evidence that vaginal lactobacilli do not ferment glycogen. *Am J Obstet Gynecol* **88**, 676–679.

Stoyancheva G, Marzotto M, Dellaglio F & Torriani S (2014). Bacteriocin production and gene sequencing analysis from vaginal Lactobacillus strains. *Arch Microbiol* **196**, 645–653.

Taha TE, Hoover DR, Dallabetta GA, Kumwenda NI, Mtimavalye LA, Yang LP, Liomba GN, Broadhead RL, Chiphangwi JD & Miotti PG (1998). Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS* **12**, 1699–1706.

Tramont EC (1977). Inhibition of adherence of *Neisseria gonorrhoeae* by human genital secretions. *J Clin Invest* **59**, 117–124.

Turner MW (2003). The role of mannose-binding lectin in health and disease. *Mol Immunol* **40**, 423–429.

Tyutyunnik VL, Kan NE, Lomova NA, Karapetyan TE, Kogan EA & Shchyogolev AI (2014). Role of innate immunity in pregnant patients with vulvovaginal infections in the development of intrauterine infection in the newborn. *Bull Exp Biol Med* **158**, 74–76.

Usluogullari B, Gumus I, Gunduz E, Kaygusuz I, Simavli S, Acar M, Oznur M, Gunduz M & Kafali H (2014). The role of human dectin-1 Y238X gene polymorphism in recurrent vulvovaginal candidiasis infections. *Mol Biol Rep* **41**, 6763–6768.

Valore EV, Park CH, Igreti SL & Ganz T (2002). Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol* **187**, 561–568.

Valore EV, Wiley DJ & Ganz T (2006). Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infect Immun* **74**, 5693–5702.

van Oostrum N, De Sutter P, Meys J & Verstraelen H (2013). Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis. *Hum Reprod* **28**, 1809–1815.

Wang W, Cole AM, Hong T & Waring AJ (2003). Retrocyclin, an antiretroviral θ-defensin, is a lectin. *J Immunol* **170**, 4708–4716.

Wang W, Owen SM, Rudolph DL, Cole AM, Hong T, Waring AJ, Lal RB & Lehrer RI (2004). Activity of alpha- and theta-defensins against primary isolates of HIV-1. *J Immunol* **173**, 515–520.

Wang Y-Y, Kannan A, Nunn KL, Murphy MA, Subramani DB, Moench T, Cone R & Lai SK (2014). IgG in cervicovaginal mucus traps HSV and prevents vaginal Herpes infections. *Mucosal Immunol* **7**, 1036–1044.

Wilson SS, Wiens ME & Smith JG (2013). Antiviral mechanisms of human defensins. *J Mol Biol* **425**, 4965–4980.

Wira CR, Fahey JV, Sentman CL, Pioli PA & Shen L (2005). Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev* **206**, 306–335.

Witkin SS (2014). The vaginal microbiome, vaginal anti-microbial defence mechanisms and the clinical challenge of reducing infection-related preterm birth. *BJOG* **122**, 213–218.

Witkin SS, Alvi S, Bongiovanni AM, Linhares IM & Ledger WJ (2011). Lactic acid stimulates interleukin-23 production by peripheral blood mononuclear cells exposed to bacterial lipopolysaccharide. *FEMS Immunol Med Microbiol* **61**, 153–158.

Witkin SS, Linhares IM & Giraldo P (2007*a*). Bacterial flora of the female genital tract: function and immune regulation. *Best Pract Res Clin Obstet Gynaecol* **21**, 347–354.

Witkin SS, Linhares IM, Giraldo P & Ledger WJ (2007*b*). An altered immunity hypothesis for the development of symptomatic bacterial vaginosis. *Clin Infect Dis* **44**, 554–557.

Witkin SS, Mendes-Soares H, Linhares IM, Jayaram A, Ledger WJ & Forney LJ (2013). Influence of vaginal bacteria and D-and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. *MBio* **4**, e00460-13.

Wu Z, Cocchi F, Gentles D, Ericksen B, Lubkowski J, Devico A, Lehrer RI & Lu W (2005). Human neutrophil alpha-defensin 4 inhibits HIV-1 infection in vitro. *FEBS Lett* **579**, 162–166.

Yarbrough VL, Winkle S & Herbst-Kralovetz MM (2015). Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. *Hum Reprod Update* **21**, 353–377.

Zheng J, Gänzle MG, Lin XB, Ruan L & Sun M (2015). Diversity and dynamics of bacteriocins from human microbiome. *Environ Microbiol* **17**, 2133–2143.

Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR & Forney LJ (2004). Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology* **150**, 2565–2573.

Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P, Foster JA & Forney LJ (2007). Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *ISME J* **1**, 121–133.

Zilnyte M, Venclovas Č, Zvirbliene A & Pleckaityte M (2015). The cytolytic activity of vaginolysin strictly depends on cholesterol and is potentiated by human CD59. *Toxins* **7**, 110–128.

Additional information

Competing interests

None declared.

Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported in part by the National Institute of Allergy and Infectious Diseases and the National Institute for General Medical Sciences of the National Institutes of Health under awards number R01GM103604, R01AI089878, R21AI107224, R01AI116799 and U19AI084044.