Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Biotherapeutic agents and vaginal health

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Keywords
biotherapeutic agents, prebiotics, probiotics, vaginal health, vaginal microbiota.

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2015/1880: received 15 September 2015, revised 22 December 2015 and accepted 7 January 2016

doi:10.1111/jam.13054

Summary

Treatment of vaginal infection requires different drugs although the recurrence rate post treatment remains high due to adverse effects on the beneficial microbiota. Thus, there are clear clinical advantages for the use of biotherapeutic agents (prebiotics and/or probiotics) for treating these infections. Pre- and probiotic beneficial effects can be delivered topically or systemically. In general, both approaches have the potential to optimize, maintain and restore the ecology of the vaginal ecosystem. Specific carbohydrates provide a therapeutic approach for controlling infections by stimulating the growth of the indigenous lactobacilli but inhibiting the growth and adhesion of pathogens to the vaginal epithelial cells. Overall, little evidence exists to promote the prevention or treatment of vaginal disease with prebiotic carbohydrates in formulations such as pessaries, creams or douches. However, recent reports have promoted prebiotic applications in ecosystems other than the gut and include the mouth, skin and vagina. This review focuses on the utilization of pre- and probiotics for vaginal health.

Introduction

Detrimental changes to the vaginal ecosystem are caused by different micro-organisms. Candidiasis (Candidosis) is caused usually by *Candida albicans*, bacterial vaginosis (BV) by the replacement of normal vaginal flora 'lactobacilli' by excess anaerobic bacteria including *Prevotella* sp., *Mobiluncus* sp., *Gardnerella vaginalis, Ureaplasma, Mycoplasma* and other fastidious or uncultivated anaerobes while trichomoniasis is caused by (the protozoan) *Trichomonas vaginalis* (Sobel and Chaim 1996; Sherrard *et al.* 2011). Any vaginal infections are associated with significant changes to the endogenous vaginal microbiota profile – decreasing the growth of lactobacilli but increasing the growth of anaerobic bacteria (Faro 2000) such as for BV. The anaerobic bacteria include *G. vaginalis*, *Mycoplasma hominis*, *Prevotella* and *peptostreptococcus* species (Ziyadi *et al.* 2016). However, in the case of vulvo-vaginal candidiasis (VVC), it appears that women with VVC do not lack lactobacilli and are not colonized with unusual species of lactobacilli (Sobel and Chaim 1996). Table 1 shows the symptoms and clinical signs of these conditions.

International studies have revealed that *Lactobacillus* species are the dominant vaginal bacteria in a majority of women (Ravel *et al.* 2011; Ma *et al.* 2012). However, some healthy individuals carry a vaginal microbiota lacking significant numbers of *Lactobacillus* spp. and harbour a diverse population of facultative and strictly anaerobic microbiota (Ma *et al.* 2012). These organisms include Group B streptococci, *Escherichia coli*, *Staphylococcus aureus* and *T. vaginalis* (whose presence may be described as a quiescent infection) (Donders *et al.* 2002; Ravel *et al.* 2011; Hickey *et al.* 2012). In health, the lactic acid bacteria have the ability to maintain an acidic environment (pH 3·5–4·5) in the vagina and produce bacteriocins and hydrogen peroxide (Tomás *et al.* 2003) which prevent any overgrowth of pathogens (Wilson 2004).

Most vaginal infections are treated with common and inexpensive antimicrobial agents, but frequent recurrences and chronic infections are common, in particular with candidiasis (Rodgers and Beardall 1999). Vaginal treatment drugs can be purchased easily over-the-counter (OTC) or be used as prescription only medicines (POM or Rx USA) but little is reported about the extent and outcome of self-medication (Sihvo *et al.* 2000). This usage results in resistant micro-organisms – putting patients at risk from pathogen overgrowth (Elmer *et al.* 1996). This is not surprising considering the diversity of

Table 1 Symptoms and clinical signs of bacterial vaginosis, candidiasis and trichomoniasis

	Bacterial vaginosis	Candidiasis	Trichomoniasis
Symptoms	Approx. 50% asymptomatic	10–20% asymptomatic	10–50% asymptomatic
	Offensive fishy smelling discharge	Vulval itching	Offensive vaginal discharge
		Vulval soreness	Vulval itching/irritation
		Vaginal discharge (nonoffensive)	Dysuria
		Superficial dyspareunia	Rarely low abdominal discomfort
Clinical signs	Thin white homogenous discharge, coating walls of vagina and vestibule	Vulval erythema	Vulval erythema
	Absence of vaginitis	Vulval fissuring	Vaginitis
		Vaginal discharge may be curdy (nonoffensive)	Vaginal discharge in up to 70% frothy and yellow in 10–30%
		Satellite skin lesions	Approx. 2% 'strawberry' cervix visible to naked eye
		Vulval oedema	5–15% no abnormal signs

Adapted from Sherrard *et al.* (2011).

the vaginal microflora (Larsen 1994; Larsen and Monif 2001). In healthy women, most of the vaginal micro-organisms are lactobacilli (Klebanoff *et al.* 1991) as mentioned previously. Their very existence is critical for optimal vaginal health. Thus, there are clear clinical advantages to use biotherapeutic agents (prebiotics and probiotics) for the treatment and prevention of vaginal infections.

This review attempts to focus on the possibility of prebiotics and/or probiotics as a prophylactic or biotherapeutic tool for treating vaginal infections.

Biotherapeutics

Biotherapeutic agents have been defined by McFarland and Elmer (1995) as living micro-organisms used to prevent or treat human disease by interacting with the natural microbial ecology of the host. Gut health bacteria or 'probiotics' have been aimed traditionally at treating various types of gastrointestinal disorders but, more recently, a few reports have extended their use towards vaginal infections (Elmer *et al.* 1996; Sarkisov *et al.* 2000; Lenoir-Wijnkoop *et al.* 2007). It is important in reality to expand the definition of a biotherapeutic agent and in particular consider applications of both prebiotics and/or probiotics for vaginal infection control.

In a general sense, functional foods containing biotherapeutic agents have been employed for self-care and complementary medicine for many years (Sarkar 2007). These products may have a positive effect with respect to the systemic control of vaginal infections (Elmer *et al.* 1996; Sarkisov *et al.* 2000). Both prebiotic carbohydrates (Sutherland *et al.* 2008) and probiotic bacteria (Maggi *et al.* 2000; Reid *et al.* 2003) perform this role – indirectly or directly. Both polysaccharide and oligosaccharide carbohydrates can be used for this purpose (Rousseau *et al.* 2005; Bou-Antoun 2008; Coste *et al.* 2012; Linhares *et al.* 2013; Hou *et al.* 2014). Interest in probiotics as components of functional foods provides potential for manipulation of the gut microbiota (Shanahan *et al.* 2009).

Prebiotics

Very little evidence exists to promote the prevention or treatment of vaginal disease with prebiotic carbohydrates (topically) in formulations such as pessaries, creams or douches and any relevant dose-dependent issues. However, recent reports have promoted prebiotic applications in ecosystems other than the gut (Gibson and Roberfroid 1995; Roberfroid 2007) and include oral hygiene (Tester and Al-Ghazzewi 2011; Maitra *et al.* 2013), skin care (Al-Ghazzewi and Tester 2010) and vaginal health (Sutherland *et al.* 2008; Tester *et al.* 2012). Perhaps these topical applications of prebiotics could more properly be defined as epibiotic prebiotics or epibiotics.

Prebiotics stimulate the growth of the body's indigenous lactobacilli. They have the potential to optimize, maintain and restore the flora of the vaginal ecosystem (Cocolin *et al.* 2007). To date, vaginal infection treatments have not focussed extensively on the natural restoration of a vaginal acidic environment – allowing the proliferation of lactic acid bacteria (Haya *et al.* 2014). This reflects on the high rate of relapse occurring after treatment.

Glycogen can provide a source of energy for the microbial flora residing in the vagina and when depolymerized (see below) is metabolized by the lactobacilli readily (Kumar *et al.* 2011; Mirmonsef *et al.* 2014). However, the glycogen would not be considered to function like a traditional prebiotic – rather as a source of carbon. The vaginal glycogen – under hormonal control – is utilized by the lactic acid bacteria to generate lactic acid (Nasiou-

dis *et al.* 2015) which is (in part) responsible for regulating the acidic (anti-pathogenic) vaginal environment (Turovskiy *et al.* 2011). The lactic acid bacteria (LAB) require α-amylase to be present in the vagina to depolymerize the high molecular weight glycogen molecule before they can utilize it (Spear *et al.* 2015). This enzyme depolymerizes glycogen sufficiently so that can be utilized by *Lactobacillus* spp. (Spear *et al.* 2014). Although the vaginal ecosystem is at most times acidic, the activity of α-amylase at low pH is reduced, but to detectable levels, which may contribute to helping maintain *Lactobacillus* growth at a limited but sustained rate (Spear *et al.* 2015). It is uncertain how this provides nutrient selectivity to the LAB *vs* the pathogens in the vagina.

Specific nonendogenous carbohydrates provide an alternative therapeutic approach for controlling microbial infections by inhibiting the adhesion of pathogens to the vaginal epithelial cells (Al-Ghazzewi and Tester 2014b) and this complements any 'prebiotic' role. Mannose-rich carbohydrates are very effective in this respect (Tester and Al-Ghazzewi 2016). Rajan *et al.* (1999) reported that the adherence of type 1-piliated *E. coli* to carbohydrate on the epithelia of the vaginal mucosa plays a major role in the pathogenesis of ascending urinary tract infections in women. The mannose-rich carbohydrates (e.g. hydrolysed glucomannans) are able to provide additional topical biological activity – for example, accelerate wound healing (Al-Ghazzewi *et al.* 2015) – by promoting accumulation of fibroblasts and the production of collagen (Shahbuddin *et al.* 2013). This combined efficacy of the glucomannans could provide major health benefits to infection control in the vagina (Fig. 1).

The properties of the hydrolysed glucomannan (Fig. 1) include:-

i Prebiotic
ii Pathogen binding
iii Stimulation of local immune system
iv Stimulation of healing

All these features provide therapeutic efficacy – without any specific drug utilization. Hydrolysed glucomannan (GMH) promotes the growth, metabolism and antimicrobial properties of probiotic micro-organisms (Al-Ghazzewi *et al.* 2007) including vaginal healthy lactobacilli strains (Sutherland *et al.* 2008) – supporting the potential application of GMH for vaginal therapy. Tester *et al.* (2012) studied the synbiotic ability of GMH to recover the healthy microflora of vaginas treated with antifungal agents. The authors reported that the introduction of GMH into the vagina helped the recovery and optimization of a healthy vaginal microbiology and thus helped prevent further infection.

Table 2 shows the beneficial carbohydrates or prebiotics reported for use in vaginal therapy. Carbohydrates such as fructo-oligosaccharides and gluco-oligosaccharides have been used as prebiotics for intestinal health. Rousseau *et al.* (2005) reported that both these carbohydrates promote selectively the growth of vaginal lactobacilli *in vitro*. Coste *et al.* (2012) found that gels containing gluco-oligosaccharides improved the recovery of normal vaginal flora and maintained the optimal pH in patients treated previously with metronidazole. Alginate oligosaccharides have been reported to improve vaginal health (Hou *et al.* 2014).

It is evident from Table 2 that different oligosaccharides have been employed to stimulate the growth of the desirable vaginal microflora and/or control the growth of pathogens. Some are used as pathogen barriers. Sulphated

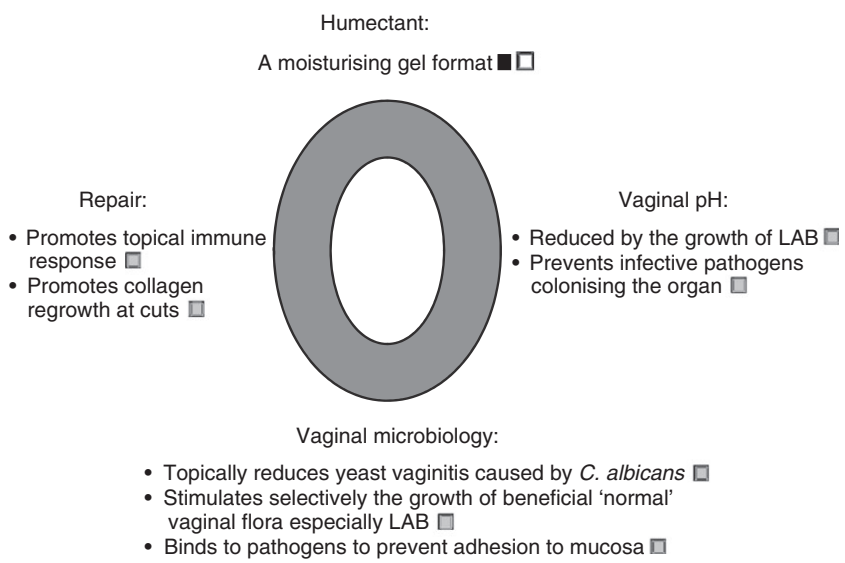

Figure 1 Schematic role of glucomannans in vaginal health. Native (■) lightly hydrolysed. (□) and extensively hydrolysed (▩) glucomannan. (Adapted from Tester and Al-Ghazzewi 2016).

Table 2 Beneficial carbohydrates or prebiotics reported for use in vaginal therapy

Carbohydrate	Beneficial role	Type of study	Reference
Gluco-oligosaccharides, Fructo-oligosaccharides	Stimulate the growth of LAB	*In vitro*	Rousseau *et al.* (2005)
Gluco-oligosaccharides, Fructo-oligosaccharides, Galacto-oligosaccharides	Promote growth of LAB	*In vitro*	Bou-Antoun (2008)
Gluco-oligosaccharides	Improves the recovery of normal vaginal flora	Clinical trial	Coste *et al.* (2012)
Glucomannan hydrolysates (GMH)	Stimulate the growth of LAB	Clinical trial	Tester *et al.* (2012)
	Inhibit pathogen adhesion	*In vitro*	Sutherland *et al.* (2008)
Glycogen	Carbon source for LAB – requires α-amylase for depolymerization	Review	Kumar *et al.* (2011)
		Clinical trial	Mirmonsef *et al.* (2014)
Alginate oligosaccharide	Antibacterial agent	Clinical trial	Hou *et al.* (2014)
	Reduces the pH		
Dextrin sulphate	Restricts cellular adhesion (microbicide)	Clinical trial	Low-Beer *et al.* (2002)
		Clinical trial	Bakobaki *et al.* (2005)
		Clinical trial	D'Cruz and Uckun (2005)

LAB, lactic acid bacteria.

dextrins have been used as biocides (Low-Beer *et al.* 2002; Bakobaki *et al.* 2005; D'Cruz and Uckun 2005) for example. Their charged structure apparently restricts cellular adhesion and subsequently cell entry of viruses (e.g. HIV) on/in the vaginal epithelia (D'Cruz and Uckun 2005).

Probiotics

Probiotic approaches to vaginal infections have been both oral and topical (Reid *et al.* 2001, 2003; Anukam *et al.* 2006a,b; Petricevic and Witt 2008; Hemmerling *et al.* 2010). Natural remedies to treat (and potentially prevent the recurrences of) undesirable yeast infections of the vagina have been used for many years (although not necessarily 'dose' dependent). These include in particular, yoghurts where the creamy acidic lactic acid bacteria-rich products provide comfort (Neri *et al.* 1993; Hantoushzadeh *et al.* 2012). The probiotic bacteria in yoghurts are typically *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. In addition, other lactobacilli and bifidobacteria are also added occasionally during or after culturing yoghurt such as within 'enriched' bio-yoghurt. The use of yoghurts is based principally on the notion that the probiotics within them can act a biotherapeutic factors and grow within the vaginal cavity.

Certain species of lactobacilli are dominant in the vagina and possess a strong antimicrobial activity (Chang *et al.* 2001), although their distribution and abundance may differ depending on factors such as local environment, race or geography (Jin *et al.* 2007). These organisms include *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Lactobacillus jensenii*, *Lactobacillus crispatus*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Lactobacillus vaginalis* (Jin *et al.* 2007; Martínez-Peña *et al.* 2013). Other literature reports indicate that four main vaginal species dominate: *Lact. crispatus*, *Lactobacillus iners*, *Lact. jensenii* and *Lact. gasseri*, along with other lactobacilli to lesser extent, as *Lact. acidophilus*, *Lactobacillus ruminis*, *Lact. rhamnosus* and *Lact. vaginalis* (Ravel *et al.* 2011; Douillard and de Vos 2014; Nader-Macías and Tomás 2015). The natural vaginal ecosystem in a healthy vagina may be disturbed – creating infection and discomfort. The dominant bacteria in unhealthy vaginal ecosystems are presented in Table 3.

Table 3 Dominant bacteria of an unhealthy vaginal ecosystem

Bacteria	Vaginal complications
Gardnerella vaginalis	Causative of bacterial vaginosis
Escherichia coli	Cause infection by triggering vaginal inflammatory immune response and displace vaginal healthy lactobacilli
Streptococcus agalactiae	
Enterococcus faecalis	
Prevotella melaninogenicus	Cause the host's immune system to generate substances with tissue destroying potential and displace vaginal healthy lactobacilli
Bacteroides fragilis	Endogenous infections and displace vaginal healthy lactobacilli
Peptostreptococcus anaerobius	Infection
Eubacterium	Potential pathogen
Bifidobacterium	No role in infection of vaginal tract
Clostridium	Infection
Mycoplasma hominis	Possible infection but no evidence of regular vaginal pathogen

Modified from Faro (2003).

Probiotic bacteria efficacy is concerned traditionally with the gut of the host but gut origin beneficial effects can be derived indirectly through ingestion (Tester and

Al-Ghazzewi 2013). Several studies have assessed the efficacy of probiotics for vaginal infection therapy (Sieber and Dietz 1998; Maggi *et al.* 2000; McLean and Rosenstein 2000; Reid *et al.* 2001, 2003; Mastromarino *et al.* 2002; Reid and Burton 2002; Antonio and Hillier 2003; Strus *et al.* 2005). The effectiveness of consuming probiotics to prevent/treat yeast infections is very inconsistent (Falagas *et al.* 2006). Abad and Safdar (2009) discussed the efficacy of probiotics for the prevention or treatment of three urological infections (bacterial vaginosis, vulvovaginal candidiasis, urinary tract infection). The authors indicated that lactobacilli can help treat bacterial vaginosis, although the bacteria have no clear benefit for candidiasis or urinary tract infection. Wagner *et al.* (1997) reported that probiotics – especially *Bifidobacterium animalis* – inhibited *C. albicans* growth, stimulated the mucosal and systemic immune system and provided nutrient competition in immunodeficient mice (Saier and Mansour 2005). Furthermore, probiotics confer health benefits and inhibit undesirable micro-organisms where efficacy is due partly to the origin of the strains. The mechanisms by which probiotics work include: (i) competitive exclusion of the pathogens from nutrients, (ii) interfering with pathogens adhesion, (iii) production of antimicrobial metabolites such as bacteriocin, H_2O_2 and lactic acid and (iv) modulation of mucosal immune functions (Menard 2011; Al-Ghazzewi and Tester 2014a). In a randomized double-blind placebo-controlled clinical trial, Guéniche *et al.* (2009) reported that certain probiotics such as *Lactobacillus johnsonii* NCC 533 (La1) can modulate skin immune systems leading to preservation of skin homoeostasis. Commercially available probiotics containing vaginal therapy products are based on either probiotics alone (Cavaliere *et al.* 1998) or in combination with specific drugs (Fromtling 1988; Darwish *et al.* 2007). It is assumed in this therapeutic approach that the drugs do not destroy the probiotic bacteria.

Synbiotics

Both prebiotics and probiotics complement each other when used to improve health. A combination of the two concepts is defined as synbiotic (Gibson and Roberfroid 1995; Fooks and Gibson 2002), where prebiotics can increase the survival of the probiotic strains. For example, soybean oligosaccharide (SOS), fructo-oligosaccharide (FOS) or inulin have been found to enhance the survival and prolong the retention period of *Lact. acidophilus* LAFTI L10 (L10), *Bifidobacterium lactis* LAFTI B94 (B94) or *Lactobacillus casei* L26 LAFTI (L26) *in vivo* (Su *et al.* 2007). Ritchie and Romanuk (2012) reported that synbiotics have beneficial effects in the treatment and prevention of gastrointestinal diseases, although, others have claimed the function extends beyond the gut by inducing systemic effects on for example the skin (Ouwehand *et al.* 2002; Suzuki *et al.* 2010), mouth where they protect against dental caries (Maitra *et al.* 2013) and vagina (Sutherland *et al.* 2008). In the vaginal ecosystem, it is presumed that synbiotics have the capacity to optimize, maintain and restore the natural microbiota of the vagina.

Pro-prebiotics dosage for vaginal therapy

Probiotics such as *Lact. rhamnosus* GR-1, *Lact. rhamnosus* Lcr 35, *Lact. reuteri* RC-14, and *Lact. crispatus* CTV-05 taken orally or vaginally in various doses can improve the vaginal flora without any side effects (Reid *et al.* 2001, 2003; Petricevic and Witt 2008; Hemmerling *et al.* 2010). Other strains such as *Lact. rhamnosus* L60 and *Lact. fermentum* L23 have been considered for probiotic development due to their specific characteristics including the production of bacteriocins, adherence properties etc. (Ruiz *et al.* 2009). A randomized study compared the efficacy of vaginal probiotics with vaginal metronidazole and showed that two intravaginal capsules of probiotics containing 1×10^9 *Lact. rhamnosus* GR-1 and 1×10^9 *Lact. reuteri* RC-14 taken once/day for 5 days proved to be more effective than 0·75% metronidazole vaginal gel applied twice daily for 5 days (Anukam *et al.* 2006b). However, another randomized trial showed that there was no difference in BV treatment of patients administered 1×10^7 *Lact. acidophilus* and 0·03 mg estriol with vaginal metronidazole at 3–7 days (Donders *et al.* 2010). This shows that some ambiguity exists with respect to intravaginal probiotic bacteria use and applications.

Probiotics can be used as complementary to traditional therapies to improve the treatment of vaginal infections and reduce recurrence of such episodes (Anukam *et al.* 2006a; Hummelen *et al.* 2010). One probiotic capsule (1×10^9 *Lact. rhamnosus* GR-1 and 1×10^9 *Lact. reuteri* RC-14) taken orally twice daily for 30 days in combination with metronidazole (500 mg twice daily for 7 days), showed a significant increase in efficacy compared with metronidazole alone at a 30-day follow-up (Anukam *et al.* 2006a). A single dose of tinidazole (2 g) supplemented with two capsules containing *Lact. rhamnosus* GR-1 and *Lact. reuteri* RC-14 daily for 4 weeks showed a significant increase in efficacy (normal vaginal flora) in the probiotic group (88%) compared with tinidazole (50%) alone (Hummelen *et al.* 2010). This approach has the potential to be effective only if the probiotics are resistant to the antimicrobial agents administered.

Probiotics can be prophylactic in healthy subjects with a history of recurrent BV. Ya *et al.* (2010) conducted a

randomized, double-blind, placebo-controlled trial on healthy women who had suffered more than two BV episodes in the previous year. Subjects received either one vaginal capsule of probiotics (eight billion colony-forming units of *Lact. rhamnosus*, *Lact. acidophilus*, and *Strep. thermophilus*) or a placebo on a 7 days on, 7 days off, 7 days on regimen. The authors concluded that lower rates of BV incidence were reported during the 2 months after probiotic treatment (16% for the probiotic and 45% for the placebo group).

In terms of prebiotic 'dosage', there is little data in the literature concerning oligosaccharide applications for vaginal therapy. Coste *et al.* (2012) conducted a randomized double-blind study on patients with recurrent bacterial vaginosis (previously treated with metronidazole) using gels containing gluco-oligosaccharides (300 mg) once daily for 16 days. The authors reported that the prebiotic improved the recovery of normal vaginal flora and maintained the optimal pH. Tester *et al.* (2012) evaluated konjac glucomannan hydrolysates (GMH) on recovering healthy microbiota in infected vaginas treated with an antifungal agent. Patients were assigned randomly into two groups to receive a standard antifungal treatment or a standard antifungal treatment plus pessary capsules containing 200 mg GMH (twice a week for 30 days). The authors reported an improvement of vaginal health recovery (post antifungal treatment for *Candida* infection) especially healthy microbiota due to the presence of GMH in the vagina. Earlier, Sutherland *et al.* (2008) compared the glucomannan hydrolysates with inulin and glucose (at doses 0·1, 0·5, 1·0 and 2·0% w/v) for its capacity to support the growth of probiotic bacteria but inhibit the growth of *C. albicans in vitro*. The authors concluded that inhibition of *C. albicans* growth was higher with the glucomannan than that of glucose or inulin in the presence of lactic acid bacteria.

Pharmabiotics

These are bacteria of human origin, or their products with a proven pharmacological role in health or disease (Shanahan *et al.* 2009; Hill 2010). Probiotics show preventative or curative factors for human health and come under two regulatory frameworks in Europe: (i) food or (ii) pharmaceutical. In order to introduce a probiotic product to the market, there are three principal regulatory options. The first is as functional foods or food supplements. In this case manufacturers need to comply with Regulation 1924/2006 (EC) on nutrition and health Claims. Until now nearly every application submitted under this option has been rejected (Cordaillat-Simmons 2014) by the European Food Safety Authority (EFSA) due to (i) failing to provide the exact mechanism of action of the probiotic strains in question or (ii) lack of clinical data. The other two regulatory options for probiotics are secondly as medical devices or thirdly as medicinal products. However, the European Commission has proposed a new regulation on medical devices in which living micro-organisms are excluded (proposed Regulation 2012/0266(COD). Thus, the only viable option remains as medicinal products which have the capacity to modulate the human system and have a positive effect (prevention/cure) on human disease.

Conclusions and outlook

There is a limited knowledge regarding the use of biotherapeutic agents (pre- and probiotics) for preventing/treating vaginal health. However, recent research interest has started to change this. Most vaginal infections are treated with drugs but frequent recurrences and chronic infections are common due to the adverse effects on the indigenous lactobacilli. Pre- and probiotics have the potential to optimize, maintain and restore the microflora of the vaginal ecosystem. Hence, biotherapeutics provide an alternative approach to reducing vaginal infections and promoting consumer health.

Conflict of Interest

No conflict of interest declared.

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