Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.7, 2014

The Efficacy of Probiotic Lactobacilli against Female Urogenital Opportunistic Infections

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Abstract
Urogenital opportunistic infections present a leading challenge to the global human health due to the increased cost of healthcare. The biologic balance of the vaginal flora is maintained by the presence of intrinsic probiotic Lactobacilli strains which produce toxigenic compounds that impede the overgrowth of the vaginal ecosystem by opportunistic pathogenic microorganisms. As a result, the present review was carried out to evaluate the efficacy of probiotic lactobacilli inorder to ascertain its usefulness in the prophylaxis and/ treatment of female urogenital opportunistic infections. This study was carried out by evaluating the probiotic role of Lactobacilli by the production of some defense factors such as lactic acid, hydrogen peroxide as well as bacteriocin. The results revealed that, there was an inverse correlation between the opportunistic infective agents and the presence of Lactobacilli isolates evidenced by the fact that the most common opportunistic bacterial isolates such as *Streptococcus agalactiae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, and *Klebsiella oxytoca,* were inhibited by the hydrogen peroxide, lactic acid and bacteriocin, produced by the Lactobacilli isolates investigated. The results obtained from this research portend that, the use of vaginal pessaries containing lactobacilli microspores could help prevent the lactobacilli-opportunistic infective agents' disequilibrium that had posed a new threat to the global female health.
Keywords: Lactobacilli, Probiotic, Opportunistic infection, Microspores.

Introduction

The biologic balance of the vaginal flora is maintained by the presence of some intrinsic probiotic Lactobacilli strains which produce toxigenic compounds that impede the overgrowth of the vaginal ecosystem by opportunistic pathogenic microorganisms. Lactic acid bacteria are a group of cocci or rod-like Gram-positive non-sporing bacteria that elicit bubbles of lactic acid upon carbohydrate fermentation. These bacteria are involved in a variety of food fermentation processes and they are associated with the mucous membranes of animals. *Lactobacilli species* represent the major lactic acid-producing bacteria predominant in the gastric, intestinal as well as the urinary tracts of humans. Although several reports on simultaneous colonization of the human vagina by two different species of *Lactobacillus*, one species has been isolated from the vaginal tract and it is implicated in the prophylaxis of urinary tract infections (Kaewsrichan *et al*., 2006; Pascual *et al*, 2006).

Urogenital infections present with infectious or non-infectious inflammation of the vaginal mucosa, which sometimes involves the vulval compartment. This inflammation often causes burning sensation, irritation, foul-smelling discharge as well as generalized discomfort. Urogenital infections fall into irritant, hormonal, foreign body, sexual transmitted diseases and infective serotypes, which constitute a major health challenge to women.

Infective serotypes may be caused by pathogenic bacteria, fungi, parasites as well as viruses, and the most common bacterial agents causing urogenital infections include *Staphylococcus aureus*, *Echerichia coli*, Group B streptococci, *Listeria monocytogenes, P. aeruginosa*, *K.pneumoniae*, *K. oxytoca, Acinetobacter* spp., *Neisseria gonorrhoea*, among others (Hill, Eschenbach and Holmes, 1984).

There are many sources of exposure to lactobacilli and bifidobacteria. These sources include probiotics, lacto-fermented products such as sauerkraut, yogurt, soft cheese, and cabbage, as well as the host's intrinsic microbial flora (Hammes and Tichaczek, 1994). The probiotic role played by *Lactobacilli species* in the prophylaxis and treatment of opportunistic vaginal infections is achieved by their ability to produce mucosal protective and mucosal aggressive compounds such as lactic acid, bacteriocin and hydrogen peroxide. These properties of Lactobacilli was the reason for the use of this bacteria as probiotic in addition to being a member of normal flora and are generally regarded as safe for use in humans (Hawes *et al*., 1996).

The urinary and genital tracts of a healthy woman constitute a wide range of microbial species, which vary in number depending upon certain extrinsic factors such as the bioburden *status quo* of sexual partners, misuse of antibiotics and spermicidal drugs, as well as intrinsic factors such as age, reproductive phase, among others.

In a normal vaginal ecosystem, the *lactobacilli species* pre-dorminate and they play an ignoble role by inducing the stasis of opportunistic pathogenic microorganisms. Upon lactobacilli species reduction, eradication, replacement by pathogenic microbial seroytypes, or a combination of the three measures, the urinary tract become pre-disposed to opportunistic infections ranging from hosts' increased susceptibility to vulvo-vaginal candidiasis, vaginal trichomonatis, urinary tract infections, genital tract infections, and bacterial vaginosis

(Pascual, 2004).
The association between abnormal vaginal microbial ecosystem and increased risk for sexually transmitted infections, bladder and vaginal infections, have necessitated the need to manage urinary and genital health (Reid *et al.*, 2004).The physiological properties of the probiotic lactobacilli serotypes such as their ability to produce adhesin and lactic acids upon carbohydrate fermentation procedures, bacteriocins, hydrogen peroxide, and biosurfactants, are measures undertaken to restore microbial ecosystem after insult(Pascual *et al*, 2008).
Several clinical assays havedemonstrated that certain *Lactobacillus and Bifidobacterium* species such as *L. acidophilus, L. rhamnosus, L. gasseri, L. casei, L. reuteri, L. delbrueckii, Bulgaricus, L. crispatus, L. plantarum, L. salivarusL. johnsonii, L. gallinarum, L.plantarum, L. fermentum, L. helveticus, L. paracasei, L. lactis, B. bifidum, B. breve, B. infantis, B. longum, B. lactis, B. adolescentis, B.essensis, B.laterosporum, Enterococcus faecalis, E. faecium, Streptococcus salivarus, S. termophilus, Lactococcuslactis, L. lactis, Propionibacteriumfreudenreichii, Pediococcusacidilactici, as well as Leuconostocmesenteroides,* when administeredper-orally or vaginally, would result to the reduction in UTI recurrence, colonization of the vagina, and possibly, the reduction in vaginal coliformic counts (Reid *et al*., 2004).
The Overgrowth of commensal lactobacilli could result from patients with D-lactic acidosis due to short bowel syndrome, consumption of dietary carbohydrates, ingestion of fermentation products containing d-lactate–producing bacteria, as well as the misuse of broad spectrum antibiotic chemotherapy (Bongaerts *et al*., 1997; Coronado, Opal and Yoburn, 1995).
In bid to correct this anomaly, the present review focused on evaluating the efficacy of probiotic lactobacilli so as to ascertain its usefulness in the causal prophylaxis and/ treatment of female urogenital opportunistic infections, that had posed a major challenge to the global female health.

Discussion
Many researches have showed that lactobacilli play a crucial probiotic role in the protection of the vaginal ecosystem from opportunistic colonization by toxigenic pathogens and this could be attributed to two principal mechanisms. These mechanisms involve the production of substances that cause opportunistic infective agents' growth inhibition and the occlusion of pathogenic attachment to the vaginal epithelial cell walls. Although some *Lactobacillus* serotypes elicit these urogenital mucosal protective properties, significant differences among species and among strains from a single species, are not uncommon (Adreu A., 2004).
The relevance of Lactobacilli strains in protecting the vagina from urogenital diseases, has gained their acceptance as a novel probiotic. Investigations into the biochemical mechanisms by which lactic acid bacteria play their probiotic roles, were made. In a study, the ability of Lactobacilli to produce hydrogen peroxide was investigated and the result showed that some isolates do not have the ability to produce hydrogen peroxide on incubation. This result was in agreement with the results obtained by other researchers who found that only one clinical isolate had the ability to generate hydrogen peroxide among all Lactobacilli strains investigated. Therefore, the presence of other opportunistic pathogens in this study could be due to the absence of hydrogen peroxide production by Lactobacilli strains. In converse, other researchers found that 93.3% of women colonized by Lactobacilli were able to produce hydrogen peroxide while 6.7% were non-producers of hydrogen peroxide as part of normal vaginal microflora. Some Lactobacilli isolates showed potent bacteriocin activity against clinical isolates of *E. coli* and *S. aureus*. These results were in concordance with the reports by some researchers that some Lactobacilli strains demonstrated potent antimicrobial properties, possibly due to the presence of minute doses of thermostable, ribosomally-synthesized antimicrobial peptides referred to as bacteriocin. It is suggested that this peptidic compound elicit bactericidal or bacteriostatic effects on microbial strains that were related to the strains that produced them (Shopova, 2003).
The production of lactic acid by vaginal Lactobacilli strains correlates with the work done by Aroutcheva *et al*.(2001), who found that pH changes of the media was attributed to the secretion of lactic acid and other organic acids such as acetic acids, by the lactobacilli strains. It was found that the low vaginal pH is usually attributed to lactic acid producing Lactobacilli. Also, they observed that when the level of oestrogen rises, a cupious quantity of glycogen is deposited in the vaginal ecosystem. Then, the glycogen undergoes anaerobic metabolism in the presence of lactobacilli species to produce lactic acid, which increases the acidic potential of the vaginal epithelium (Eschenbach *et al.,* 2000).
Lactobacilli play an important role in maintaining the vaginal health by production of defense factors and some of these defense factors have an inhibitory effect on some opportunistic pathogenic infective agents. This was confirmed by results mentioned by other researchers who reported that the normal vagina of women of child-bearing potential is predominately colonized with Lactobacilli which produce hydrogen peroxide, bacteriocins and lactic acid. These substances are able to lower the vaginal pH, which create a toxic environment for opportunistic infective agents (Ronnquist, *et al.,* 2006).
Although the mechanisms by which lactobacilli elicit probiotic effects are poorly understood, some researchers advocate that probiotics act through biofilm formation based on auto-aggregation and surface hydrophobicity,

the modulation of the intestinal ecosystem of the host and the capacity to interact with the immune system co-aggregation with certain pathogenic bacteria, and adherence to vaginal epithelial cells, competitive inhibition with toxigenic bacterial strains and exclusion of pathogens from the cell surface, production of antimicrobial substances such as lactic and acetic acids, hydrogen peroxide and bacteriocins, as well as the modulation of the immune system (Almeghaiseeb,2007; Dunne *et al*., 2001).

Probiotic lactobacilli compete with opportunistic pathogens for nutrients and ecological space. Some bacteria can inhibit and prevent the colonization by pathogenic microorganisms by a mechanism of steric hindrance or occlusion of specific receptors (Johannsen, 2003).

The modes of action of probiotics have been suggested to be through a directeffect on other microorganisms, commensal and/or pathogenic ones, modulation of the host's defense mechanisms including the innate as well as the acquired immune system, or on actions affecting microbial products like toxins, host products such as bile salts and food ingredients (Oeslschaeger, 2010).

The Probiotic effects of lactobacilli on other microorganisms involve the production of antimicrobial inhibitory substances such as organic acids, bacteriocins, hydrogen peroxide and biosurfactants, secretion of organic acids such as lactic and acetic acids, among other mechanisms (Rolfe, 2000).

Hydrogen peroxide is produced by some lactobacilli strains in the presence of atmospheric oxygen. Lactobacilli strains do not produce catalase, hence, they cannot degrade hydrogen peroxide that, after accumulation, develops its oxidative properties with the production of powerful oxidizing moieties such as singlet oxygen, superoxide radicals, and the hydroxyl radical. Reactive oxygenspecies can cause irreversible damage to a number of cell components such as enzymes, DNA and cellular membranes (Dalié *et al.*, 2010).

Hydrogen peroxide production by the *Lactobacillus* species is considered to represent a nonspecific antimicrobial defense mechanism of the normal vaginal ecosystem. Biosurfactants are microbial amphiphilic and polyphilic polymeric substances that interact with interfaces in a heterogeneous system. Several biosurfactants exhibit antibacterial, antifungal and antiviral activities which inhibit the adhesion of a broad spectrum of urogenital pathogenic microbial strains. These molecules impair with interfacial hydrophobicity and therefore inhibit the adhesion of pathogenic microbes to their sites of infection. Studies have shown that the release of biosurfactants by probiotic lactic acid bacteria *in vivo* could be a possible defence mechanism against opportunistic colonizing microbial strains in the urinary and genital tracts (Rodrigues *et al*., 2006; Gudin *et al*., 2010).

Recent studies have shown the immuno-regulatory potential of probiotic microbes for the prophylaxis and therapeutic effects on diseases. The epithelial barrier consists of a dense mucous layer containing secretory immunoglobulin A and antimicrobial peptides as well as dynamic functional complexes that regulate cellular permeability (Ohland and MacNaughton, 2010).

When factors such as chronic psychological stress, epithelial ion secretion and enhanced cellular permeability, disrupt epithelial membrane barrier, the binding of luminal bacteria to surface epithelia increases, the uptake of luminal antigens through follicle associated epithelium increases and hence, mucosal inflammation (Zareie *et al.*, 2006). It is postulated that the consumption of probiotic lactobacilli strains could improve upon the intestinal barrier integrity and the modulation of mucin production. The stimulation and modulation of the mucosal immune system by probiotic lactobacilli species reduce the production of pro-inflammatory cytokines, increase in production of anti-inflammatory cytokines, and host defence peptides, enhances immunoglobulin A defence and influence on dendritic cell maturation (Devine and Marsh, 2009).

Recent research suggests that probiotic lactobacilli strains could play a crucial role in lowering the escalating incidence of vaginal candidiasis, bacterial vaginosis and recurrent lower urinary tract infections, that are resistant to the available allopathic chemotherapeutic agents.

Conclusion

The evaluation of the probiotic role of Lactobacilli by the production of some defense factors such as lactic acid, hydrogen peroxide as well as bacteriocin, so as to ascertain its usefulness in the prophylaxis and/ treatment of female urogenital opportunistic infections was investigated. The results showed that the presence of some *Lactobacilli species* in the vaginal ecosystem deterred the opportunistic colonization by other pathogenic microorganisms. Hence, there was an inverse correlation between the opportunistic infective agents and the presence of Lactobacilli isolates. The results obtained from the research carried out by Andreu, (2004), Pascual *et al.,* (2008), and Ronnquist *et al.,* (2006), shows that the use of vaginal pessaries containing lactobacilli microspores could help prevent the lactobacilli-opportunistic infective agents' disequilibrium that had posed a new threat to the global female health. It is suggested that the safety profile of such probiotic bacteria be carried out so as to provide safe, cost effective and efficacious lacto-fermented probiotics for the prophylaxis and treatment of opportunistic infections.

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