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Commentary

Lactobacilli as probiotics against genital infections

Lactobacilli constitute an important part of the normal indigenous alimentary and urogenital tract microbiota of man and animals. The indigenous microbiota is a natural resistance factor against potential pathogenic microorganisms and provides colonization resistance by promoting gut maturation and integrity, steric exclusion, producing autogenic regulation factors (*e.g.* organic acids, hydrogen peroxide and bacteriocins). Lactobacilli thus play an important role in maintaining vaginal health of women[1] and form the dominant species of the vaginal microbiota[2]. When lactobacilli are depleted due to douching , sexual practice and use of antibiotics, other pathogenic organisms such as Gram-negative anaerobes take over and it results in bacterial vaginosis (BV)[3] which is the commonest cause of vaginal discharge occurring in women attending gynaecological clinics in our country. The adverse effects of BV on pregnancy and acquisition of HIV are enormous to women[4]. The hydrogen peroxide producing lactobacilli may have a protective role against vaginal colonization by pathogenic species such as those causing BV and possibly human immunodeficiency virus (HIV), gonorrhoea and herpes simplex virus-2[5,6]. There is therefore growing interest in use of lactobacilli of human origin as probiotics against genital infections. In the development of appropriate biotherapeutic remedy for these conditions, such as probiotic lactobacilli and vaccine development, the identification of the dominant vaginal lactobacilli present is vital.

In recent years there have been significant advances in the characterization and identification of bacteria by molecular methods. Especially, *Lactobacillus* species that are commensals in the human vagina have received considerable attention as a result of their probiotic properties[7]. The new culture-independent techniques, such as pulse field gel electrophoresis (PFGE), ribotyping, 16S rDNA restriction fragment length polymorphism (RFLP), multiplex polymerase chain reaction (PCR), arbitrary primed (AP) PCR or triplet arbitrary primed (TAP) PCR[8], provide more specific methods for detailed investigations at the species and the strain levels and are valuable tools for ecological *Lactobacillus* studies as well.

In the past, the nature of vaginal lactobacilli diversity has been limited due to reliance on culture-dependent methods. The taxonomy of lactobacilli has expanded as a result of genomic sequence analysis. Using DNA homology studies, *L. acidophilus* complex which previously could not be distinguished biochemically, has been subdivided into six distinct species; *L. acidophilus, L. crispatus, L. gasseri, L. gallinarum, L. amylivorus* and *L. johnsonii*[9]. Previous studies indicated the colonization by *L. crispatus* or *L. jensenii,* the most common species of vaginal lactobacilli[10]. Only, recently *L. iners* has been identified in the human vagina[11]. It was found in vaginal samples from Swedish women, but the most frequently occurring species were *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*[12]. The use of 16S rRNA phylogenetic sequence analysis is now accepted as a reliable molecular method for identification of microbial communities.

Various clinical trials have been carried out to assess the utility of lactobacilli in prevention as well as treatment of bacterial vaginosis. A recent study from Italy[13] showed that the use of lactobacilli containing vaginal tablets helped treat 61 per cent of the females suffering from BV in comparison to 19 per cent of the placebo treated patients. In another study from Austria, Petricevic *et al*[14] demonstrated an improvement of the Nugent score by at least five after treating patients of BV with topical *L. casei rhamnosus* (Lcr35). Other authors have

shown similar encouraging results regarding role of lactobacilli as probiotics[15,16]. Studies have also been conducted to show use of different excipients on the survival of probiotic vaginal lactobacilli[17]. The use of genetically engineered lactobacilli has also been studied in delivering HIV inhibitors. Liu *et al*[18] reported on the development of a novel, live microbicide that employs a natural vaginal strain of *L. jensenii* engineered to deliver the potent HIV inhibitor cyanovirin-N (CV-N). Mechanistically, CV-N blocks multiple steps leading to membrane fusion and virus entry. Also encouraging animal data on a lactobacilli-based vaccine against HIV[19] lend credibility to this approach. So to conduct similar studies in India it would be essential to first delineate the common species and strain types in our country.

In this issue, Garg *et al*[20] present a new understanding of the nature of the *Lactobacillus* vaginal microbiota of women in India. It is interesting to note that the species identified from India are different from the ones seen in other countries. The commonest being *L. reuteri* (32.5%) which along with other species like *L. fermentum*, *L. salivarius* an *L. plantarum* constitute 80 per cent of the total number of the identified species. Individual studies in each part of the country would contribute more in understanding the diversity of vaginal flora. As we know that hydrogen peroxide producing lactobacilli are considered more protective, the study would have been wholesome had the authors conducted an *in vitro* study on the hydrogen peroxide production by the isolates as studied by others[21].

A question which still remains unanswered is whether replacement with just a single species would suffice or a balanced mixture of various species as occurs in nature would be more useful. There are a few studies regarding this concept. *In vitro* experiments have shown *L. rhamnosus* GR-1 is particularly effective in inhibiting growth, adhesion and biofilm formation of Gram-negative pathogens and *Candida albicans*, while *L.reuteri* RC-14 is active against Gram-positive pathogens such as staphylococci, enterococci and streptococci[22]. A combination of such type of lactobacilli can be more useful as a probiotic.

In conclusion, in order to clearly delineate the role of probiotic vaginal lactobacilli it is essential to study the various species and strain types in a given geographical area and to further incorporate the most common types in the treatment regimen. This study[20] is an interesting piece of work that adds useful information to the complex scenario of lactobacilli in vaginal health and in therapeutics of various vaginal diseases.

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