## Bacterial therapy in pregnancy to drive the intra-partum colonization of newborns

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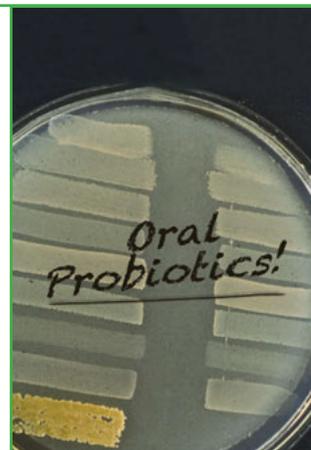
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## Introduction

Recently, a very interesting paper has been published on Nature Medicine. The Authors have clearly shown the relevancy of a close contact between mother and newborn to effect appropriate passage of microbes to her infant [1]. They have demonstrated that it is possible to get partial restoration of the microbiota of cesarean-born infants by exposing them to maternal vaginal fluids. Vaginal microbe transfer to cesarean-born infants makes them "microbially" similar to vaginally delivered infants. Epidemiological studies have clearly shown an association between cesarean-section delivery and increased risk of obesity, asthma, allergies and immune deficiencies [2-5]. The authors' attempts to establish an appropriate microbiota in cesarean-born infants by exposing them to the mothers' vaginal fluids demonstrates the importance of mother-infant microbe transfer and highlights, as a next step, the importance of specifically-directed "manipulation" of the pre-term maternal microbiota in order to further optimize and enrich this transfer process.

## Failure of probiotic therapy

Unfortunately, despite their undoubted commercial success, the use of probiotics has not always lived up to the expectations of those who saw in their application a panacea against multiple ailments. This partial failure of probiotics has several explanations. For example, it was for a long time considered sufficient to consume probiotic microbes incorporated in capsules, tablets or sachets in order to alleviate a wide variety of disorders, especially of the intestinal tract. However, a beneficial outcome can only be achieved by carefully taking into consideration certain specific parameters. The probiotic strain should for example be derived following a careful selection process which includes established steps such as: 1) ability to survive both in gastric and enteric environments 2) a high *in vivo* proliferative potential, 3) capability of adhering to intestinal mucosa and 4) absence of (transferable) antibiotic resistance determinants. However, satisfying these selection criteria alone is not sufficient to establish an effective probiotic. The stability and the dosage of the probiotic strain in the chosen finished delivery format are also important factors. Anyway, the parameter which most certainly will contribute to probiotic therapeutic failure is the absence of colonization. Following their administration, probiotic cells are in fact typically faced with the problem of achieving colonization in tissues that are already highly colonized by the host's indigenous microbes. This process is really difficult. The established resident bacteria leave little or no physical space on the surface of our tissues for newcomers to colonize and only following prolonged periods of administration of high doses of probiotic strains that have been selected for their high adhesion and proliferation indices will there any real prospect of success.

## A totally different moment: the birth

In everyone's life, however, there is one moment, albeit brief, in which this situation is reversed. This is a short time in which colonization does not seem to be so difficult but, on the contrary, takes place with ease. This is the moment of our birth. Infants are known to be microbiologically sterile until a few moments before their birth. During childbirth, and in the period immediately thereafter, the baby is predominantly colonized by the mother's own microbes, these initially being of vaginal and rectal origins, but then also including microbes from the mother's mouth. It is believed that most of the microbes associated with newborns in the first few days after birth directly reflect the composition of the maternal flora. This situation can be considered to have both pros and cons. A cause for concern is that, unfortunately, as well as the commensals, potentially pathogenic microbes can also colonize the infant with relative ease [6, 7]. For example, *Streptococcus agalactiae*, also known as the group B streptococcus, is the leading cause of severe neonatal bacterial infections in developed Countries. Infants can be colonized during passage through the birth canal by group B streptococci that are present in the mother's gastrointestinal and/or genital tract. While vaginal infection in pregnancy is usually asymptomatic, in the newborn group B streptococci can produce extremely serious clinical pictures: early-onset infections are characterized by sepsis, pneumonia, and, less frequently, meningitis; in late-onset infections the main clinical manifestations include osteomyelitis, septic arthritis, cellulitis and other localized infections. Studies of western populations of pregnant women have estimated the prevalence of vaginal group B streptococcus colonization to be 15-25%. Approximately one third of infants of these women are colonized at birth and during the first 7 days of life, about 3% of colonized infants develop early-onset infections that can either be fatal or induce severe consequences. Infections occurring beyond the first 7 days of life however seem not to be related to the mother's *intra-partum* colonization, but rather to group B streptococci acquired in the *post-partum* phase. A recent study of newborns under 3 months of age found that the incidence of streptococcal disease was 0.5 per 1,000 live births [8]. To prevent group B streptococcal disease the method adopted in many countries is based on vaginal-rectal screening performed between weeks 35 and 37 of gestation, with *intra-partum* antibiotic treatment given only for women testing positive. Several randomized clinical trials have shown that this prophylactic approach reduces the risk of early infection from 4.7 to 0.4%.

## The case of *Enterococcus faecium* L3

For the past decade, *Enterococcus faecium*, a Gram positive, non-hemolytic, commensal of the human gut has been the subject of study by a group of Russian researchers. A particular strain, identified later as L3, was shown to release two low molecular weight, thermostable bacteriocins named enterocin A and enterocin B. This strain has been shown to effectively compete with *Streptococcus agalactiae*. Agar co-culture studies clearly showed that L3 kills *S. agalactiae* [9]. Subsequent studies have demonstrated that the L3 antibiotic-like activity can also affect the growth of other potential pathogens of the gut and vagina, including *Escherichia, Shigella, Salmonella, Proteus, Klebsiella, Mycoplasma* and *Candida*. It is possible that these pathogens compete with *Enterococcus faecium* for the same niche. To help illustrate what it could mean to fight for the same ecological niche, Figure 1 displays colonies of enterococcus surrounded by "scorched earth" zones of interference with the growth of other bacteria in a mixed fecal population growing on the surface of MRS agar. Other studies have also shown that administration of L3 in premature infants undergoing antibiotic therapy, is associated with both a significant weight increase and a reduction in the frequency of infections (20.7% in the L3 treated infants versus 53.9% of controls). In these same studies a significant reduction in the persistence of *Clostridium difficile* was also observed. Moreover, in both premature and mature, L3 therapy reduced the risk of dyspeptic disorders and increased the populations of intestinal bifidobacteria and lactobacilli [10]. These results collectively show that L3 administra-
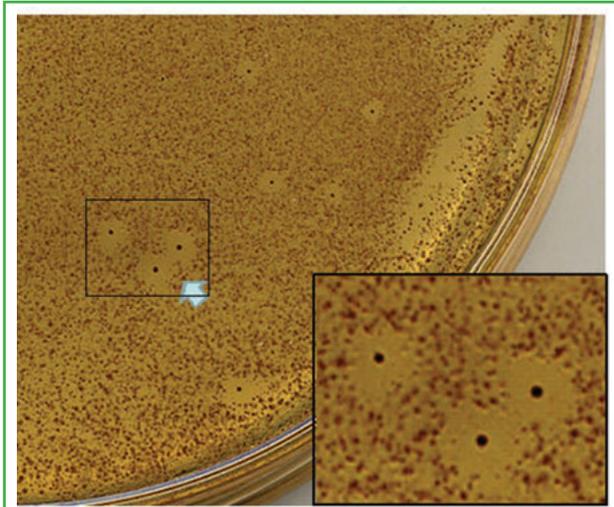
**Figure 1** - Plating of a fecal sample from a healthy 6-month-old breast fed baby on a MRS agar plate anaerobically. No indicator bacteria are added and only endogenous fecal lactic acid bacteria are grown on the plate. Some bacteriocin-producing fecal bacteria inhibit growth of other fecal bacteria. The arrow indicates bacterial growth inhibitory zones due to the bacteriocin producing endogenous lactic acid bacteria (enterococci) present in the feces. The insert shows magnification of some bacteriocin-producing colonies.
(Nes I.F., Diep D.B., Ike Y. Enterococcal Bacteriocins and Antimicrobial Proteins that Contribute to Niche Control. Enterococci 2014; pp1-24)

tion during pregnancy could potentially reduce maternal dysbiosis and the presence of pathogens, thereby reducing the need for intravenous antibiotic *intra-partum* therapy. Further, L3 vertical transmission from mother to newborn during passage through the birth canal could contribute positively to newborn weight gain, in addition to increasing gut levels of *Bifidobacterium* and *Lactobacillus* species and effecting a reduction in neonatal infections, *Clostridium difficile* diarrhea and various dyspeptic manifestations.

## Exploiting childbirth?

The fact that childbirth allows for close contact between a mother who is abundantly colonized by microorganisms and her baby, whose tissues are sterile, but receptive for microbial colonization, could be "exploited" to try to colonize the baby with strains that are selected for particular characteristics that in some way are useful for the baby. In this view, strain L3 is just one example. Strains encoding and producing beta-galactosidase and/or proteases capable of digesting immunogenic milk proteins could be another example [11].

The enzymatic pools created by such strains may be useful, after newborn colonization, to promote the digestion of milk (both maternal and artificial) thereby reducing the risk of development of lactose intolerance and allergy to milk proteins. Similarly, colonization by strains capable of shifting immune reactivity from a primarily Th2 response (allergic) to a Th1 response (non-allergic) could help reduce the incidence of asthma and other allergies. Moreover, strains capable of improving the specific immune response to vaccination could be considered potentially useful. One strain which appears capable of reducing asthma and allergy and of improving the immune response to vaccines is *Bifidobacterium animalis lactis* BB12 [12]. Same concept could be applied for the oral microbiota. In 1983 [13] it was shown that babies are typically colonized by *Streptococcus salivarius* strains derived from their mother. This evidence opens the prospect for colonization of mothers with beneficial probiotic strains of salivarius, such as K12 or M18, in the period immediately preceding delivery as a strategy for achieving natural colonization of the infant from the first days of life.

## Could an exogenous probiotic be "vertically" transmitted?

While it has been shown that vertical transmission of well-established endogenous, microorganisms from mother to newborn regularly occurs during delivery, what is less well known is the extent to which similar "contamination" of babies can occur from strains that have been just recently introduced to the mother's native flora. That is, with strains voluntarily administered during pregnancy for the express purpose of influencing the colonization of the newborn. Although it may seem "a miracle" the phenomenon seems to occur. For several years we have known that certain strains administered to mothers only until the day of birth, were later found in their child's stool, even 24 months after the last maternal self-administration [14]. Obviously this outcome cannot necessarily be expected to apply to all other strains, but it does mean that colonization strategies such as this are indeed possible.

## Acknowledgment

## Conflict of interest

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