

Probiotics to prevent the need for, and augment the use of, antibiotics

Gregor Reid BSc (Hons) PhD MBA^{1,2}

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Although humans and microbes are inseparable, our knowledge and understanding of the majority of microbes that help keep us alive and well is in desperate need of further investigation. Of the organisms that influence humans before birth and inhabit various niches from birth to old age, we know little about their identity, origin, metabolic properties, attributes and mechanisms of interactions with the host and surrounding microbes. The use of probiotics ("live microorganisms which when administered in adequate amounts confer a health benefit on the host") has re-emerged as a means to restore and boost the beneficial microbes in our bodies. The timing of resurgent interest in this ancient field coincides with the need to augment or replace antibiotics whose side effects are unwelcome and whose efficacy is diminishing due to drug resistance. Evidence that probiotic strains can act as adjuncts to antibiotic therapy by reducing adverse effects, improving antibiotic function and enhancing mucosal immunity is mounting. It is to our discredit that basic research on microbial ecology has been stalled in Canada for the past 20 years. If supported, research into indigenous and probiotic microbes will form an important part of future research that sheds light on health, disease and a basic understanding of life itself. In some cases, probiotics will be the difference between a good quality of life and a bad one, or perhaps even life over death. Improvements in clinical studies, manufacturing and regulatory standards must coincide with this progress to ensure that physicians and consumers have reliable, proven products for safe and efficacious use.

Key Words: *Antibacterial; Antibiotics; Biofilms; Immunity; Infection; Probiotics*

The resurgence of interest in probiotics has been accompanied by many review papers and the use of different definitions. The joint expert panel of the Food and Agriculture Organization of the United Nations and the World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (1). This definition is preferred because it embraces the historical spirit of the term, and is inclusive of intestinal and other forms of probiotics. The expert panel's report was later accompanied by guidelines that outlined the evidence required for a product to be called a probiotic (2). Although the document was distributed to all member nations, it has not yet been adopted in practical terms in countries such as Canada. Thus, unproven and

Les probiotiques pour prévenir le besoin d'antibiotiques et pour en augmenter l'usage

Bien que les humains et les microbes soient inséparables, il faut absolument faire des recherches approfondies afin de mieux connaître et de mieux comprendre la plupart des microbes qui contribuent à nous maintenir en vie et en santé. Nous ne savons pas grand-chose de l'identité, de l'origine, des propriétés métaboliques, des caractéristiques et des mécanismes d'interaction avec l'hôte et les microbes avoisinants des organismes qui influent sur les humains avant la naissance et qui logent dans les diverses niches de l'organisme entre la naissance et le troisième âge. Le recours aux probiotiques (des microorganismes vivants qui, quand ils sont administrés en quantité adéquate, sont bénéfiques à la santé de l'hôte), est réapparu comme moyen de restaurer et de stimuler les microbes bénéfiques de l'organisme. Ce regain d'intérêt envers cet ancien domaine coïncide avec le besoin d'accroître ou de remplacer les antibiotiques, dont les effets secondaires sont importuns et dont l'efficacité est limitée à cause de la résistance aux médicaments. Les données probantes s'accumulent pour démontrer que les souches de probiotiques peuvent agir comme adjuvants à l'antibiothérapie en limitant les effets indésirables, en améliorant la fonction des antibiotiques et en améliorant l'immunité muqueuse. C'est une honte que les recherches fondamentales sur l'écologie microbienne soient en perte de vitesse depuis 20 ans. Si elles sont soutenues, les recherches sur les microbes indigènes et probiotiques formeront une partie importante des futures recherches qui jetteront la lumière sur la santé, la maladie et la vie même. Dans certains cas, les probiotiques feront la différence entre une bonne et une mauvaise qualité de vie ou peut-être entre la mort et la vie. L'amélioration des essais cliniques, la fabrication et les normes de réglementation doivent s'ajouter à ce progrès pour que les médecins et les consommateurs aient des produits fiables et démontrés, pour un usage sécuritaire et efficace.

unreliable products remain easily accessible (3), making it difficult for physicians to see the potential benefits of probiotics or use them with confidence. By allowing products to be called probiotics, Health Canada is failing to institute the joint standards of the Food and Agriculture Organization of the United Nations and the World Health Organization; as a result, some companies do not identify the types and numbers of viable organisms in their products at the time of use, possibly leading people into thinking their products have actually been clinically tested and proven to confer specific health benefits when, in too many cases, no such evidence exists. The end result is the lack of credibility for the field of probiotics and uncertainty as to which products may benefit patients.

¹Department of Microbiology and Immunology, University of Western Ontario; ²Canadian Research and Development Centre for Probiotics, The Lawson Health Research Institute, London, Ontario

Correspondence: Dr Gregor Reid, Canadian Research and Development Centre for Probiotics, F2-116, The Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario N6A 4V2. Telephone 519-646-6100 ext 65256, fax 519-646-6031, e-mail gregor@uwo.ca

The use of products with no basis for ameliorating a condition may lead to the perception that probiotics cannot, for example, prevent urinary tract infections (4), or alleviate irritable bowel syndrome (5) or necrotizing enterocolitis (6), when the use of reliably documented and prepared strains may provide clinical benefits (7-9). Each year, Canadians spend an estimated \$2 billion per year on alternative therapies, perhaps \$10 million to \$15 million of which is spent on 'probiotic' products that are unlikely to provide desirable benefits.

ORIGINS OF PROBIOTICS

Many reports credit the Russian researcher, Dr Élie Metchnikoff, for influencing the development of probiotics, but the rationale for this approach dates further back to the origins of human life itself. We evolved from single-cell organisms, and throughout evolution, microbes could have terminated our existence had they chosen to do so. People have 10 times more bacteria in their bodies than human cells, and no sterile person has survived on this planet. In other words, we cannot separate microbes from humans. With so many organisms associated with us, including species capable of killing us, why do we live and retain a semblance of health for so long?

Microbiological studies, especially over the past century, have almost exclusively investigated harmful bacteria. Although these studies are necessary, they have been to the exclusion of fundamental studies on the human microbiota, so much so that we do not know the role bacteria play during our fetal development, which ones we inherit, from where, and at what time following birth, and which ones we lack or have in numbers that are insufficient for specific health benefits. Only recently have studies uncovered the role that some species play in intestinal angiogenesis (10), establishment of the immune system (11), processing of fat (12) and potentially even long life (13). Probiotics are simply a means to restore microbial numbers inside the body, because many die or are excreted daily in stool. Selection of probiotic strains, and their application in the most appropriate manner, can only be performed if we understand the fundamental origins of our microbiota and their mechanisms of action. Thus, microbial ecology studies are critical if we are to optimally apply probiotics or modulate the indigenous microbiota in favour of sustained health.

ANTIBIOTIC PROBLEM AREAS

Collectively, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, vancomycin-resistant enterococci and uropathogenic *Klebsiella* species that produce extended spectrum beta-lactamases (14-16) cause the majority of infections and pose the biggest problems because of mounting drug resistance. The major problems associated with antibiotics, in addition to the side effects they cause through destruction of the normal microbiota (17,18), are that they are overused along the food chain (19), especially in livestock (20), and in preventing infections (21-25), and are misused by patients (22). It is not surprising to find drug-resistant rates for fluoroquinolones, for example, over 30% (26). In hospitals, the use of antibiotics before and after a range of surgical procedures is now being more limited, particularly because in many cases they are not necessary to protect the host (27). Often in general practice, broad-spectrum agents have been used empirically as a first-line treatment instead of taking steps to determine the causative organism and then use more targeted antibiotics (28). In recent years, with the antibiotic pipeline drying up, physicians have made concerted efforts to more rationally use the

current drugs. Nevertheless, new approaches that augment the current arsenal of antibiotics would be welcomed.

EVIDENCE THAT PROBIOTICS CAN AUGMENT ANTIBIOTIC ACTION

There are perhaps three areas in which probiotics may act as adjuncts to antibiotics. Probiotics may:

- Reduce the risk of antibiotic-induced superinfections in the gut and the vagina;
- Secrete antibacterial substances that lower pathogenic bacterial populations locally and at distant mucosal sites, and disrupt biofilms, making it easier for antibiotics to function; and
- Enhance generalized mucosal immunity, which in turn aids in the eradication of the organisms at the mucosal site.

Diarrhea and superinfections

The bulk of evidence supporting the idea that probiotics strains are beneficial comes from studies of patients suffering from diarrhea (29-31). The use of probiotics to prevent diarrhea or vaginal infections following antibiotic use makes sense conceptually if the drug adversely affects the intestinal and/or vaginal microbiota, and evidence has been found to support this application (32-34). The currently held belief is that probiotics simply substitute for the microbes that have been destroyed by the antibiotics. This is likely an oversimplification, given that probiotics do not colonize for long periods, and they could act by a number of mechanisms, including indirectly reducing the excess electrolyte release, acting on the physiological process of gut motility, signalling downregulation of toxin release by *Clostridium difficile* or inhibiting growth of yeast and other opportunistic pathogens. Some probiotic *Lactobacillus* strains may inhibit or kill intestinal pathogens, including viruses (35,36), and downregulate toxin release in *Escherichia coli* O157:H7 (Griffiths MW, personal communication), as well as staphylococcal exotoxin (37). In the case of *E coli* O157:H7, antibiotics are not a treatment option because they cause the release of the toxin, and thus, if probiotic organisms functioned in vivo in the same way as suggested by in vitro experiments, this would certainly augment the anti-infective armamentarium. The following five examples are provided to illustrate the evidence for and against the augmentation of antibiotics with the use of probiotics.

Forty-seven *Helicobacter pylori* subjects were randomly assigned to receive probiotic therapy (*Lactobacillus rhamnosus* GG, *L rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* subspecies *shermanii* JS) or a placebo during *H pylori* antibiotic treatment and for three weeks thereafter. No significant differences in individual symptoms were found between the two groups. However, the probiotic group showed fewer treatment-related symptoms. The *H pylori* eradication rate was nonsignificantly higher in the group receiving probiotic therapy than placebo (91% versus 79%, $P=0.42$) (38).

In a double-blind, randomized, controlled study (39), 80 infants (six to 36 months of age) were randomly assigned to receive a commercial formula containing 10^7 viable cells of *Bifidobacterium lactis* and 10^6 viable cells of *Streptococcus thermophilus* at the initiation of antibiotics for 15 days. There was a significant difference in the incidence of antibiotic-associated diarrhea in children receiving probiotic-supplemented formula (16%) compared with nonsupplemented formula (31%).

In a double-blind, randomized clinical trial of patients with acute intestinal amoebiasis, 27 patients were given metronidazole (750 mg three times a day) and iodoquinol (630 mg three times a day) for 10 days (group 1), and 27 patients were also given *Saccharomyces boulardii* (250 mg three times a day) orally (group 2). Diarrhea lasted for 48.0±18.5 h in group 1 versus 12.0±3.7 h ($P<0.0001$) in group 2. Durations of fever and abdominal pain for group 1 were 24.0±8.8 h and 24.0±7.3 h, respectively, and the durations of fever and abdominal pain for group 2 were 12.0±5.3 h and 12.0±3.2 h, respectively ($P<0.001$). At week 4, amoebic cysts were detected in five patients (18.5%) from group 1, but none from group 2 ($P<0.02$) (40).

In another assessment of *S. boulardii* efficacy, 269 children (six months to 14 years of age) with otitis media and/or respiratory tract infections were enrolled in a double-blind, randomized, placebo-controlled trial in which they received standard antibiotic treatment plus 250 mg of *S. boulardii* or a placebo orally, twice daily, for the duration of the antibiotic treatment. Patients who received *S. boulardii* had a lower prevalence of diarrhea (three or more loose or watery stools per day for at least 48 h, occurring during or up to two weeks after the antibiotic therapy) than those who received placebo (nine of 119 [8%] versus 29 of 127 [23%], respectively). *S. boulardii* also reduced the risk of antibiotic-associated diarrhea (caused by *C. difficile* or otherwise unexplained diarrhea) compared with placebo (four of 119 [3.4%] patients versus 22 of 127 [17.3%] patients, respectively) (41).

One hundred thirty-eight patients who received antibiotic therapy were randomly assigned to receive either a probiotic containing both *Lactobacillus* and *Bifidobacterium*, or placebo for 20 days. The trial probiotic or placebo was taken within 72 h of administration of antibiotics. On the basis of development of diarrhea, the incidence of samples positive for *C. difficile*-associated toxins was 2.9% in the probiotic group compared with 7.25% in the placebo-control group. When samples from all patients were tested (rather than patients who developed diarrhea), 46% of probiotic patients were toxin-positive compared with 78% of the placebo group (42).

The use of probiotics in hospitals in Canada has been limited to some extent to prevent *C. difficile* infections, and more recently to manage constipation. In Germany, a series of studies showed that the use of *Lactobacillus plantarum* 299v with oat fibre could significantly lower infections associated with liver transplants, abdominal surgery and pancreatitis (43-45). Given the morbidity and mortality rates associated with these and other serious conditions requiring hospitalization, there is good reason to consider probiotic foods as part of the overall care of the patient. This must be balanced with safety issues of giving live bacteria to seriously ill patients, but complications caused by the probiotics themselves are rare and in most cases, these are handled effectively with antibiotic treatment (46). Indeed, patients receiving steroids for ulcerative colitis have been shown to benefit from the intake of VSL#3, a probiotic with an extremely high viable count (more than 10^{11} cells) (47).

Antibacterial and biofilm effects

The secretion of antimicrobial compounds, including organic acids, has been a well documented attribute of probiotic bacteria. Their role in conferring distant side effects, such as in the bladder and the respiratory tract, following oral administration, remains to be determined (48-51), but when in direct contact with pathogens, compounds such as lactic acid, hydrogen peroxide and bacteriocins may have adverse effects on their growth, adhesion

and biofilm spread (52-54). To date, of the many bacteriocins discovered in lactic acid bacteria, only nisin has been commercially available, but it is not used in clinical settings. The *Lactobacillus* strain GR-1 can generate oxidative stress responses that inhibit *Candida albicans*, prevent its biofilm formation, and depending on the conditions, can actually kill the fungus (55). The ability to stress the outer membrane of *E. coli* has also recently been discovered in our study group, in part due to its lactic acid and hydrogen peroxide secretion (unpublished data). This may lead to detachment of pathogens from surfaces (53) and altered expression of cell membrane proteins involved in the structural stability in *E. coli* (unpublished data).

One net effect of stressing bacteria may be an increased ability of antibiotics to function. In a recent randomized, placebo-controlled study (56) of 106 women treated with metronidazole administered orally for seven days to treat bacterial vaginosis, plus a 30-day course of probiotic *Lactobacillus* GR-1 and *Lactobacillus* RC-14, or placebo, the 30-day cure rate was doubled by the administration of the lactobacilli. This augmentation of antibiotic efficacy has been further examined in vitro, and the dose of amoxicillin required to kill uropathogenic *E. coli* was halved with the coinclusion of supernatant from *Lactobacillus* GR-1 (unpublished data). Similar effects arise from lactic acid use. While this may benefit patients when the lactobacilli are in close proximity to the pathogen, such as in the mouth, intestine and vagina, it is not clear if and how it can augment antibiotic function at distant sites.

Enhancing generalized mucosal immunity

It is clear from a growing number of investigations that probiotic organisms modulate immunity. This may play a role in eradicating pathogens from local and distant mucosal sites (49,51,58-60). Intriguingly, potentially pathogenic bacteria living in the nose and possibly inducing allergic reactions as well as being a source of other infections (61), can have their cell counts significantly lowered by ingestion of a probiotic drink containing *Lactobacillus* GG, *Bifidobacterium* B420, *Lactobacillus acidophilus* 145 and *S. thermophilus* (62), which implies some sort of immunological interference. It is known that pathogenic *Staphylococcus epidermidis* has evolved phenol-soluble modulins, which help detach the organisms, and induce inflammation in the switch between aggressive and quiescent modes of infection (63). However, the ability of lactobacilli or other nonpathogenic species to induce a switch in pathogen virulence and/or biofilm formation remains to be uncovered. One possible mechanism is by the use of autoinducer quorum-sensing molecules, shown recently to be produced by probiotic *Lactobacillus* strains (unpublished data). Coupled with anti-inflammatory mediators, such as the one produced by *L. rhamnosus* GR-1 that suppresses interleukin-12 and tumour necrosis factor- α production through inducing secretion of granulocyte colony-stimulating factor (64), quorum sensing may indeed play an important role in preventing or ameliorating inflammation and associated diseases.

CONCLUSION

Clearly, this exciting field is at a new beginning. If supported, research into indigenous and probiotic microbes will form an important part of future research that sheds light on health, disease and a basic understanding of life itself.

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