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Review

Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant micro-organism and is the principal nosocomial pathogen worldwide. Following initial in vitro experiments demonstrating that *Lactobacillus acidophilus* CL1285® and *Lactobacillus casei* LBC80R® commercial strains exhibit antibacterial activity against clinical MRSA isolates, we conducted a literature search to find any evidence of probiotic efficacy in decolonisation or treatment of *S. aureus* infection. As summarised below, many strains of lactobacilli and bifidobacteria isolated from a variety of sources inhibited the growth of *S. aureus* and clinical isolates of MRSA in vitro. The most active strains were *Lactobacillus reuteri*, *Lactobacillus rhamnosus* GG, *Propionibacterium freudenreichii*, *Propionibacterium acnes*, *Lactobacillus paracasei*, *L. acidophilus*, *L. casei*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus fermentum* and *Lactococcus lactis*. Their effects were mediated both by direct cell competitive exclusion as well as production of acids or bacteriocin-like inhibitors. *L. acidophilus* also inhibited *S. aureus* biofilm formation and lipase production. In vitro antimicrobial activity did not necessarily assure efficacy in vivo in animal infectious models, e.g. *S. aureus* 8325–4 was most sensitive in vitro to *L. acidophilus*, whilst in vivo *Bifidobacterium bifidum* best inhibited experimental intravaginal staphylococcosis in mice. On the other hand, *L. plantarum*, which showed the highest inhibition activity against *S. aureus* in vitro, was also very effective topically in preventing skin wound infection with *S. aureus* in mice. Very few clinical data were found on the interactions between probiotics and MRSA, but the few identified clinical cases pointed to the feasibility of elimination or reduction of MRSA colonisation with probiotic use.

1. Introduction

Electronic databases, including PubMed, Google Scholar, Cochrane Trials Register, and government and company web pages, were searched using the terms 'probiotic', 'lactobacilli', '*Lactobacillus*', 'methicillin-resistant *Staphylococcus aureus*', 'MRSA' and '*Staphylococcus aureus*' in order to review current scientific evidence for the rational use of probiotics in decolonisation and treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Citations from the identified articles were also utilised.

The human colon contains as many as 10^{12} bacteria/g of contents and >1000 bacterial species [1,2]. The human mouth has one of the most diverse microbiomes in the body, and individuals' oral microbiomes are highly specific at the species level [3]. The skin is an ecosystem harbouring enormous variability of microbial communities that live in a range of physiologically and topographically distinct niches [4,5] and there is increasing evidence that skin diseases such as atopic dermatitis and diabetic wounds are associated with a shift in the cutaneous microbiota [6,7].

These indigenous bacteria are important for host defence because they inhibit the growth of potentially pathogenic micro-organisms [8]. This defence mechanism, termed 'colonisation resistance', can be applied to the prevention of colonisation by exogenously introduced organisms and to the prevention of overgrowth by potential pathogens. Multiple mechanisms may contribute to the inhibition of pathogens, including depletion of nutrients, prevention of adherence to sites within the mucosa, or the production of inhibitory substances or conditions.

MRSA present on the skin and in the nares can be inadvertently ingested. In a healthy carrier with uncompromised natural gut microbiota it may be innocuous, but in an immunocompromised patient harbouring a disrupted microbiota as a result of antibiotic therapy it can cause infection. Use of antibiotics, immunosuppressive therapy or irradiation may cause alterations in the composition and have an effect on the commensal microbiota. Therefore, the introduction of beneficial bacterial species into the gastrointestinal tract may be a way to re-establish the microbial equilibrium and to prevent disease [9]. Following the same underlying principle, if

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MRSA can be displaced from the nares and skin by topical application of beneficial bacterial species in a pharmaceutically acceptable carrier, decolonisation of MRSA by probiotics may prevent MRSA spread from healthy carriers to susceptible patients.

2. Probiotics

The World Health Organization (WHO) defines probiotics as 'live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host'. A group of requirements has been identified for a micro-organism to be defined as a probiotic [10].

Bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus* as well as some fungal strains belonging to *Saccharomyces* [11–13]. As the name implies, they all produce lactate and lactic acid as a final metabolite of sugar fermentation. *Lactobacillus rhamnosus* GG is the first probiotic most extensively studied to date [14,15].

Lactobacilli, bifidobacteria and lactococci are generally regarded as safe because of their long history of use in the food and dairy industries [16]. Although rare cases of bacteraemia or fungaemia were reported, there is no evidence from population-based studies of any increased risk of bacteraemia or endocarditis due to probiotics [17,18].

The mechanisms by which probiotics exert their effects are closely related to the properties, manufacturing and formulation of the selected strains [19], but key proposed mechanisms are common for all strains and may involve any or all of the following [20]:

- prevention of gastrointestinal tract colonisation by pathogens through competition with pathogens for adhesion/attachment sites and/or nutrients and growth factors [21,22];
- production of organic acids that lower the intestinal pH and thereby inhibit the growth of pathogens [23] and increase peristalsis, thereby indirectly removing pathogens [24];
- production of inhibitory substances such as bacteriocins and other toxic primary metabolites detrimental to pathogens [25–29];
- modulation of the host immune system [22,28,30–32]; and
- inhibition of bacterial toxins [33].

3. Meticillin-resistant *S. aureus*

S. aureus is a Gram-positive cocci distinguished by its tendency to cluster under microscopic examination and its positive result on coagulase testing. It thrives on human skin and mucous membranes, grows rapidly under either aerobic or anaerobic conditions, forms biofilms, and can be carried by its host for a long period of time without causing clinical consequences. MRSA is a multidrug-resistant micro-organism and is the principal nosocomial pathogen worldwide. Its colonisation and infection rates in acute and non-acute care facilities and in the community have increased dramatically over the past two decades [34,35]. Although more recent data from the USA show stabilisation of this trend, the total number of MRSA-related hospitalisations increased to reach 463 017 in 2009 [36].

Colonisation indicates the presence of the organism without symptoms of illness. *S. aureus* colonisation can occur in the nares, trachea, skin folds, rectum, or in an open wound such as a decubitus ulcer. Studies have shown that ca. 80% of the population could be nasally colonised by *S. aureus* and that colonisation increases the risk of developing more serious *S. aureus* infections, particularly in patients with concomitant human immunodeficiency virus (HIV), immunocompromised patients, patients with intravascular devices, wound patients, in patients undergoing surgical procedures or transplantation, and in patients on dialysis [37,38]. Hospital workers are more likely to be colonised than persons in the general population because of increased exposure.

There is increasing evidence that *S. aureus* may adhere to mucus and colonise the intestinal tract [39], which may pose an increased risk of infection in some groups of hospitalised patients [40,41].

Infection is defined as tissue invasion by *S. aureus* with subsequent clinical symptoms ranging from superficial skin lesions such as boils to systemic manifestations such as fever, malaise and leukocytosis. *S. aureus* is the causative agent of serious infections such as pneumonia, meningitis, endocarditis and osteomyelitis [42]. *S. aureus* exotoxins also cause disease syndromes such as bullous impetigo, scalded skin syndrome and toxic shock syndrome. Whilst outbreaks of cutaneous infections in otherwise healthy people may be managed well without antibiotics, in compromised individuals staphylococci are an important cause of life-threatening nosocomial infections such as ventilator-associated pneumonia [43].

MRSA that is resistant to the synthetic penicillins (meticillin, oxacillin and nafcillin) is also resistant to cephalosporins and sometimes to other antibiotics (erythromycin, clindamycin, aminoglycosides and quinolones).

Because of its resistance to many antibiotics, management of MRSA infections requires more complicated, toxic and expensive treatment. In Canada, the cost per infected MRSA patient, which averaged $12 216 in 2004, is now $14 485 [44]. The standard antibiotic therapy for MRSA infections is intravenous vancomycin, but this can have serious side effects such as ototoxicity, nephrotoxicity, and allergic reactions such as fever and rash. Several newer agents against MRSA have recently been introduced or are under clinical development, but resistance to these new classes of antibiotics has already been reported [45,46].

4. Non-clinical studies of probiotic effects on *S. aureus*

Studies on the antagonistic interactions between lactic acid bacteria (LAB) and *S. aureus* have been carried out in various laboratories worldwide over the past decades [47]; they are summarised below by probiotic species studied. The results indicate that they inhibit *S. aureus* and/or MRSA growth by either or both competition with pathogens for adhesion/attachment sites and nutrients and secretion of inhibitory substances.

4.1. *Lactobacillus reuteri*

Prince et al. utilised a primary human keratinocyte culture to investigate whether *L. reuteri* ATCC 55730, *L. rhamnosus* AC413 and *Lactobacillus salivarius* UCC118 could inhibit *S. aureus* infection [48]. They demonstrated that both *L. reuteri* and *L. rhamnosus*, but not *L. salivarius*, reduced *S.-aureus*-induced keratinocyte cell death both in undifferentiated and differentiated keratinocytes. Keratinocyte survival was significantly higher if the probiotic was applied prior to or simultaneously with *S. aureus* infection but not when added after infection had commenced. The protective effect was not dependent on the production of inhibitory substances. *L. reuteri* inhibited adherence of *S. aureus* to keratinocytes by competitive exclusion. Since *S. aureus* utilises $\alpha_5\beta_1$ integrin to adhere to keratinocytes, blocking of this integrin resulted in a protective effect similar to that observed with probiotics.

A study by Vesterlund et al. showed that *S. aureus* adhered to mucus from resected human intestinal tissue [39]. In displacement assays, the amount of adherent *S. aureus* in human intestinal mucus was reduced by 39–44% by *L. rhamnosus* GG, *Lactococcus* (*Lc.*) *lactis* subsp. *lactis* and *Propionibacterium freudenreichii* subsp. *shermanii*.

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