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OPINION

Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis

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Summary The adaptation of strict hygienic practices by healthcare personnel as well as the implementation of appropriate cleaning and disinfection measures form the basis of infection control policies. However, nosocomial infections constitute a considerable problem even in hospitals with meticulous infection control programmes. This should prompt biomedical researchers to evaluate the efficacy and safety of novel infection control measures. There is preliminary evidence that probiotic type micro-organisms may antagonise the growth of nosocomial pathogens on inanimate surfaces. We therefore propose the hypothesis that environmental probiotic organisms may represent a safe and effective intervention for infection control purposes. We suggest that probiotics or their products (biosurfactants), could be applied to patient care equipment, such as tubes or catheters, with the aim of decreasing colonisation of sites by nosocomial pathogens. This could potentially impede a central step in the pathogenesis of nosocomial infections.

Colonisation of environmental sites and medical devices in healthcare facilities with pathogenic micro-organisms is an important step in the development and spread of serious nosocomial infections.[1] Several approaches have been adopted in order to limit pathogen colonisation. Strict hygienic practices by healthcare personnel — such as basic hand washing — along with regular disinfection of the hospital environment are considered by some of basic importance. However, it should be noted that routine disinfection of the hospital environment is controversial.[2–4] Since nosocomial infections remain an important problem even for

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hospitals with well-organised and decisively implemented infection control programmes, studies of innovative infection control measures are warranted.

There is preliminary evidence that probiotic type micro-organisms, defined by Schrezenmeir *et al.* as, 'a preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonisation) in a compartment of the host and by that exert beneficial health effects in the host', may antagonise the growth of nosocomial pathogens on inanimate surfaces.[5]

In this article we hypothesise that environmental probiotic micro-organisms and biosurfactant products may have an important role in future methods of infection control, and we consider the feasibility, effectiveness and safety of this proposed approach.

The study of probiotics has gained interest in recent medical literature in relation to increasing antimicrobial resistance of bacteria worldwide. Evidence suggests that probiotic organisms may have a role in lowering the incidence or the duration of antibiotic-related diarrhoea, contributing to the prevention or treatment of vaginal candidiasis, bacterial vaginosis and recurrent lower urinary tract infections. Furthermore, they encourage improved immunological defence responses and a decrease in the activity of numerous toxic antimetabolites in the human body.[6–8]

Potentially pathogenic micro-organisms can colonise environmental surfaces in the hospital environment and so act as a source for outbreaks of nosocomial infection. Kramer *et al.* have presented evidence that the majority of Gram-positive bacteria, including *Staphylococcus aureus* and *Enterococcus* spp., are able to survive for months on dry surfaces.[4] Gram-negative bacteria, such as *Klebsiella* spp., *Escherichia coli*, and *Acinetobacter* spp. can also survive for a relatively long time on inanimate surfaces, while common fungi such as *Candida* spp. have similar properties. Environmental conditions such as low temperature or humidity appear to be crucial for the persistence of these organisms on inanimate surfaces.[4]

Certain micro-organisms, like *S. aureus* and *E. coli*, are able to adhere to inanimate surfaces by forming biofilms, which consist of an extracellular matrix of polysaccharides.[9–11] Biofilm formation provides these micro-organisms with a survival advantage against their planktonic (free-floating) competitors, and is an optimal environment for proliferation, gene transfer, and quorum sensing within the bacterial population. However, neighbouring competitive species are able to antagonise biofilm formation, by excreting inhibitory It has been suggested that applying such substances to artificial inanimate surfaces could limit the original development of biofilm.[12,13]

In this respect, it has been shown that probiotic organsims, such as *Lactobacillus* spp., can produce multifunctional molecules, known as biosurfactants, which have antagonistic antiadhesive properties against microbial pathogens.[14] Biosurfactants, which are amphipathic molecules, have so far found limited application in biomedical sciences; however, indications of their potential clinical applicability are increasing.[15]

To evaluate the potential role of environmental probiotic type of micro-organisms or their biosurfactants in the reduction of colonisation of inanimate surfaces within healthcare facilities or of medical equipment by pathogenic bacteria, we performed a search of the literature for relevant evidence. We focused on three questions: (1) whether probiotic micro-organisms are able to survive on non-biological surfaces; (2) whether probiotics or biosurfactants could antagonise the growth of potentially pathogenic micro-organisms with clinical application; and (3) whether probiotics and their products are safe for use in the hospital environment.

We identified 10 studies involving in-vitro experiments on the potential role of probiotics or their products in the inhibition of bacterial or fungal colonisation of artificial surfaces outside the human body (Table I).[16–25] Most of these studies investigated the potential role of probiotics in patients with laryngectomy who receive artificial voice prostheses. These in-vitro studies were performed in order to investigate decreased colonisation with harmful microbes that might extend the life expectancy of the prosthesis.[16–18,21,22,24] The remaining studies focused on the potential inhibition of biofilm formation in materials used in the urogenital tract or the oral cavity by preconditioning these materials with probiotic products.[19,20,23,25] All of the in-vitro experiments were performed upon hydrophobic (glass) or hydrophilic (silicon rubber) material, within different buffering solutions. The derived data (Table I), in combination with experimental evidence in favour of the ability of certain types of probiotics to form biofilms on non-biological surfaces, supports a positive answer to the first question.[26]

The critical review of the methodology and findings of relevant studies suggests that several types of probiotics under various conditions decrease the adhesion of microbial pathogens to different extents. The majority of the investigators have examined the preconditioning of the studied

Table I In-vitro studies regarding the effect of probiotic bacteria or biosurfactant use on the rate of colonisation of artificial surfaces by common microbial pathogens

Study	Surface/preparation	Probiotic organism and strain designation	Antagonistic organisms and strain designation	Outcome
Rodrigues *et al.* (2006)[16]	Silicone rubber/ preconditioning with biosurfactant	*Streptococcus thermophilus* A	*Staphylococcus epidermidis*, *Streptococcus salivarius*, *Staphylococcus aureus*, *Rothia dentocariosa*, *Candida albicans*, *Candida tropicalis*	Adhering bacterial cells were reduced by up to 97%. The two *Candida* spp. showed reduction in adhesion by up to 70%.
Rodrigues *et al.* (2004)[17]	Silicone-rubber voice prosthesis/immersion to biosurfactant	*Lactococcus lactis* 53	*Staphylococcus epidermidis*, *Streptococcus salivarius*, *Staphylococcus aureus*, *Rothia dentocariosa*, *Candida albicans*, *Candida tropicalis*	Bacterial adhesion downsized compared to adhesion to bare silicone rubber. Adhesion of yeasts also decreased in the presence of biosurfactant, but to lesser extent.
Rodrigues *et al.* (2004)[18]	Silicone rubber voice prostheses/ preconditioning with biosurfactants	*Lactococcus lactis* 53 (biosurfactant 1), *Streptococcus thermophilus* A (biosurfactant 2)	*Staphylococcus epidermidis*, *Streptococcus salivarius*, *Staphylococcus aureus*, *Rothia dentocariosa*, *Candida albicans*, *Candida tropicalis*	Biosurfactants 1 and 2 decreased significantly the number of bacteria in the biofilm, to 4% and 13% of the control, respectively. The number of fungal organisms reduced to 15% of the control by biosurfactant 1, and to 26% of the control by biosurfactant 2.
Hoogmoed *et al.* (2000)[19]	Glass with and without a salivary conditioning film/presence of probiotic or coating with its biosurfactant only	*Streptococcus mitis* strains (BA and BMS)	*Streptococcus mutans* NS	Biosurfactant-releasing *S. mitis* BA and BMS decreased the number of *S. mutans* NS cells. Preadsorption of isolated *S. mitis* biosurfactants to glass drastically reduced the adhesion of *S. mutans* NS cells and the strength of their bonds to glass.
Velraeds *et al.* (2000)[20]	Silicon rubber discs in non-pooled specimens of male and female urine samples	*Lactobacillus* spp.	Naturally developing uropathogens	The inhibition of growth of naturally developing uropathogens depended on the strain of lactobacillus used and the type of urine media.
Van der Mei *et al.* (2000)[21]	Silicon rubber voice prosthesis/suspension of probiotics	Lactobacillus spp., *Streptococcus thermophilus* B	*Candida* spp., *Streptococcus* spp., *Staphylococcus* spp.	The number of yeasts in the biofilm decreased.
Busscher *et al.* (1998)[22]	Silicone rubber voice prostheses/perfusion with buttermilk	Probiotics found in buttermilk	*Candida albicans*, *Candida tropicalis*, *Streptococcus* spp., *Staphylococcus* spp.	Biofilm formation almost fully prevented.

(*continued on next page*)

Table I (*continued*)

Study	Surface/preparation	Probiotic organism and strain designation	Antagonistic organisms and strain designation	Outcome
Velraeds *et al.* (1997)[23]	Glass or silicone rubber substratum/ preconditioning with surlactin biosurfactant	*Lactobacillus acidophilus* RC 14	*Enterococcus faecalis* 1131	Inhibition of the deposition rate of *E. faecalis* and the number of adherent bacteria after 4 h on both hydrophilic glass and hydrophobic silicone rubber, although this effect was stronger in buffer than in urine.
Busscher *et al.* (1997)[24]	Silicone rubber with and without a salivary conditioning film/ presence of probiotic or coating with its biosurfactant only	*Streptococcus thermophilus* B	2 *Candida albicans* and 2 *Candida tropicalis* strains	Significant reduction in yeast adhesion regardless of the presence of a conditioning film. Using preadsorpted surfactant only had different effectiveness in inhibiting microbial adhesion of different spp.
Velraeds *et al.* (1996)[24]	Glass substratum/ adsorption of biosurfactant	*Lactobacillus* spp.	*Enterococcus faecalis* 1131	The deposition rate of *E. faecalis* significantly decreased by up to 77%.

materials with biosurfactant products of probiotics, while others used biosurfactant-releasing strains of probiotics. In two studies that compared these two methods, no major differences in effectiveness were reported.[19,24] Data from all relevant studies suggest that the answer to the second question — on whether probiotics could antagonise the growth of potential pathogens is that regardless of underlying mechanisms, probiotics can challenge the presence of human pathogens on inanimate surfaces.

The third and probably most important question is whether environmental probiotics and their products could potentially be hazardous to humans. The available evidence suggests that probiotics (specifically lactobacilli) are considered to be safe for use in humans, a position also supported by a European Union workshop.[27] Although several species of *Lactobacillus* have been used as probiotics in humans, it is noteworthy that *Lactobacillus* bacteraemia is very rare, observed mainly in seriously ill patients.[28] A total of 180 cases of lactobacilli from blood cultures and 69 cases of endocarditis due to lactobacilli have been reported during the past 30 years.[29] However, only in a few of these cases was a connection found between lactobacilli isolated from an infection site and those consumed as a probiotic product.[30,31] In view of this, the use of envir onmental probiotics may be considered unlikely to pose serious risks to human health; however, possible relevant studies should pay attention to this issue.

Our hypothesis for the use of environmental probiotics, or their products, for infection control purposes, could be tested using an experimental model similar to those applied in the above studies. The variable to be considered in these potential experiments would be the use of more relevant environmental materials, such as those usually found within a hospital unit, and cocultures of various probiotics with common nosocomial pathogens in conditions, particularly of temperature and humidity, similar to those in hospitals. The results of such experiments could provide us with specific answers regarding the growth and inhibitory capacities of environmental probiotics for use in infection control.

Apart from technical restrictions, there are certain limitations which might obstruct the application of probiotics in practice. The majority of the studies reviewed simulated the use of probiotics on artificial surfaces within human tissues using appropriate culture materials. Thus, there might be technical limitations on the survival of probiotic organisms on non-biological surfaces without such materials. In addition, only two studies compared the application of an entire probiotic organism rather than the biosurfactant-substance released only.[19,24] It is clear that more in-vitro studies are warranted to gain a deeper insight regarding the mechanisms involved in the inhibitory activity of probiotic organisms.

Probiotics or their products may not be sufficient to restrain all microbial species and the epidemiology of hospital infections may shift towards micro-organisms not affected by probiotics. Thus, the selection of the appropriate probiotics and/or their products with a broad spectrum of pathogenic microbial inhibition would be imperative. Other issues that might play a part are the interference of regular disinfecting measures on probiotic survival on inanimate hospital and medical equipment/device surfaces along with the appropriate frequency of their potential application.

In conclusion, our hypothesis is in keeping with the principle Aristotle expressed more than two thousand years ago, 'nature abhors a vacuum'. It seems that since we cannot keep a hospital envir onmental surface or medical equipment/device absolutely free from microbes, perhaps we can utilise probiotics or their products — or even other non-pathogenic micro-organisms — as our allies against nosocomial pathogens, especially in the current era of multidrug-resistant infections. We believe that the current evidence on the use of probiotic organisms, and especially biosurfactants, for the protection of artificial prostheses from colonisation by microbial pathogens is encouraging. There are also experimental data for medical applications of artificially produced surfactants which need more in-depth research.[32] We also believe that, as our knowledge on probiotics advances and progress in biotechnology continues, perhaps the time has arrived to evaluate the potential role of environmental probiotics or their biosurfactant products in infection control.

Conflict of interest

MEF conceived the idea described in this article and formulated the hypothesis.

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