Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and β-hemolytic streptococci)*1–3*

Ulrich Glück and Jan-Olaf Gebbers

**ABSTRACT**

**Background:** As a bacterial reservoir, the nose may harbor potentially pathogenic bacteria (PPB: *Staphylococcus aureus*, *Streptococcus pneumoniae*, β-hemolytic streptococci, and *Haemophilus influenzae*). In patients carrying PPB, antiseptic regimens could be crucial for infection control after major operations on or injuries of the head, nasal sinuses, or lungs. Such regimens may also be important for diabetic patients and persons receiving hemodialysis, in intensive care units, or with impaired immunity due to various other causes.

**Objective:** We tested a possible effect of the ingestion of probiotics on the bacterial flora of the nose.

**Design:** In an open, prospective trial, 209 volunteers were randomly assigned to consume either a probiotic, fermented milk drink (65 mL with *Lactobacillus* GG (ATCC 53103), *Bifidobacterium* sp B420, *Lactobacillus acidophilus* 145, and *Streptococcus thermophilus*; n = 108) or standard yogurt (180 g; n = 101) daily for 3 wk. Nasal microbial flora were analyzed on days 1, 21, and 28. The microbial examination was blinded to the source of the samples.

**Results:** We found a significant reduction (19%; P < 0.001) in the occurrence of nasal PPB in the group who consumed the probiotic drink but not in the group who consumed yogurt. The effect was mainly on gram-positive bacteria, which decreased significantly (P < 0.05).

**Conclusions:** The results indicate that regular intake of probiotics can reduce PPB in the upper respiratory tract. The results also indicate a link of the lymphoid tissue between the gut and the upper respiratory tract.


**INTRODUCTION**

The upper airways of humans, the nose and the pharynx, harbor a flora that consists of various strains of aerobic (e.g., *Staphylococcus*, *Corynebacterium*, *Stomatococcus*, *Micrococcus*, and *Mycoplasma*) and anaerobic (e.g., *Veillonella*, *Peptostreptococcus*, *Fusobacterium*, *Porphyromonas*, *Bacteroides*, *Prevotella*, *Actinomyces*, *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium*) microorganisms. Within these genera there are several strains that have a pathogenic potential. *Staphylococcus aureus* is one of the most important, but *Streptococcus pneumoniae*, β-hemolytic streptococci, and *Haemophilus influenzae* are also members of this group of potentially pathogenic bacteria (PPB).

These bacteria can cause infectious diseases, such as sinusitis, pneumonia, or otitis. *Sta. aureus* was found in 20–25% of healthy American adults (1, 2), and ≈500 million healthy people worldwide are estimated to be colonized with *Neisseria meningitidis*, another common nasal bacterium that is potentially pathogenic (3). The clinical relevance of the nose as a reservoir of PPB is often underestimated (4).

The host defense against PPB is mainly the task of the immune system. In mammals, the mucosal surface area represents an extensive interface with the external environment through which pathogens mainly initiate infections. The mucosal surfaces of the upper airways are well equipped with an immune system that reacts to some extent independently of the systemic immune apparatus, and these surfaces are functionally linked to other mucosal surfaces, eg, the lachrymal, salivary, and mammary glands of the common mucosal immune system (mucosa-associated lymphoid tissue (MALT)) (5). It is known that the commensal microflora activates the mobilization of the defensive mechanisms of the host against exogenous pathogens. These mechanisms imply both bacterial-bacterial antagonism and modulation of the host’s reactivity against the infectious agents by commensal microflora. The positive benefit of the commensal microflora may be improved by the intake of exogenous bacteria from food. Several studies showed therapeutic benefits with the intake of probiotics, particularly for gastrointestinal disorders (6–9).

The fact that certain lactic acid bacteria activate and modulate the immune system (10, 11) opens a promising perspective concerning the use of such microorganisms as immune modulators.
(12). When these microorganisms are ingested, the gut-associated immune system (GALT) is particularly involved in activating and modulating the immune system (13, 14). The search for effects of immune system modulation in other parts of the MALT, such as that of the upper respiratory tract, presents an interesting challenge.

We previously observed that subjects who were free of nasal PPB were consumers of probiotic food (U Glück and J-O Gebbers, unpublished observation, 2000). To substantiate this observation, we conducted an open, prospective study to confirm the effect of probiotics on PPB in the nasal flora.

SUBJECTS AND METHODS

Design

In an open, prospective trial, 209 volunteers (148 men and 61 women) were randomly assigned to consume either a fermented milk product with probiotic bacteria [the test group; n = 75 men and 33 women; mean (± SD) age: 41 ± 8 y] or standard yogurt (the control group; n = 72 men and 29 women aged 39 ± 9 y) daily for 3 wk. The bacterial microflora in microbiological swabs of the nasal cavities was examined on days 1, 21, and 28. The study lasted from April to July 2000.

Subject eligibility

Of 300 volunteers (218 men with a mean age of 38 y and 82 women with a mean age of 42 y), 209 subjects were included in the study. The status of the ears, nose, and throat of each volunteer was determined. In addition to inspecting the nasal cavities by using a headlamp and nasal speculum, an otoscopy was performed to assess the eardrums. We also inspected the mouth, epipharynx, and larynx of each volunteer. Finally, the neck was palpated for pathologically enlarged lymph nodes. Any pathologic finding in the status of the ear, nose, or throat was a criterion for exclusion.

In addition, we conducted a standard skin-prick test (21 solutions; Allergomed, Reinbeck, Germany) on each volunteer. Those with ≥ 1 positive skin test, indicating seasonal or perennial sensitivity, were also excluded from our investigation.

Additional exclusion criteria were pathologic findings (acute or chronic inflammatory conditions of the upper respiratory tract, epistaxis, and nasal polyps), elevated temperature (> 37°C), or the use of any medication including antibiotics or of nasal sprays in the previous 6 mo. The test and control groups had a comparable social status in terms of income, education, and standard of living because all subjects were office workers at Suva, Swiss National Accident Insurance Institute, Lucerne, Switzerland. The study was performed in accord with the Helsinki Declaration of 1975 as revised in 1983, and the subjects provided written informed consent.

Administration of the products

The subjects in the test group consumed one vial of a probiotic drink each day at breakfast from day 1 to day 21 and were asked to renounce the consumption of other probiotic products. The subjects in the control group were supplied with standard yogurt and asked to abstain from consumption of any probiotic food. No further restrictions in dietary intakes were required for either group.

Compositions of the probiotic product and the yogurt

The probiotic, fermented milk drink (65 mL Aktifitplus; Emmi Schweiz AG, Lucerne, Switzerland) contained Lactobacillus GG, Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacterium sp. According to the manufacturer, the mean (± SD) contents of Lactobacillus GG (ATCC 53103), Str. thermophilus, L. acidophilus 145, and Bifidobacterium sp B420 in this fermented milk drink during the study period were 8.04 ± 0.16, 8.63 ± 0.2, 7.7 ± 0.7, and 8.12 ± 0.5 log colony-forming units/mL, respectively (7.1 × 109, 27 × 109, 3.2 × 109, and 8.4 × 109 colony-forming units/d, respectively). The standard yogurt (180 g) consumed by the control group contained the conventional lactic acid bacteria Str. thermophilus and Lactobacillus delbrueckii subsp bulgaricus at a combined concentration of ≥ 107 colony-forming units/g.

Microbiological examinations

Swabs (sterile cotton carriers; TRANSWAB Medical Wire & Equipment Co Ltd, Corsham, Wilts, United Kingdom) from both nasal cavities of each subject were taken by a single investigator (UG) and were analyzed as one for each patient. Swabs from both middle meati were taken with the use of a head mirror and a Killian nasal speculum. Because of the long leaves of the Killian speculum, any contamination of the cotton carriers in the vestibule was prevented during removal from the cavity (15).

The microbiological investigations were conducted at the Institute of Microbiology, Kantonsspital Luzern, Lucerne, Switzerland. The microbiologists were blinded to the source of the samples. The microbiological assays were performed according to instructions in the American Society of Microbiology’s Manual of Clinical Microbiology (16). The nasal swabs were inoculated onto a series of culture media (chocolate agar, MacConkey agar, sheep-blood agar, Columbia-CAN agar) and investigated according to the guidelines of the American Society of Microbiology’s laboratory standards (16). Bacterial species of the nasal cavity were regarded as potentially pathogenic on the basis of the definition given by this manual (Sta. aureus, Str. pneumoniae, β-hemolytic streptococci, and H. influenzae).

The result “pathogen-free” meant that none of the above mentioned PPB was detectable in the cultures after 72 h of cultivation. The microorganisms contained in the probiotic product were not found in cultures of the nasal swabs.

Data analysis

Statistical analysis was done by using the 2 × 2 contingency table for the chi-square test.

RESULTS

In 40 of the 108 subjects who consumed the probiotic drink daily for 3 wk, PPB were not detected in the nasal cavity throughout the study. Of the other 68 subjects in this group who had PPB in their nasal cavity at day 1, 60 subjects had 1 type of PPB, 3 subjects had 2 types, and 5 subjects had 3 types. At day 21, PPB were found in the nasal swabs of 55 subjects. Thus, the occurrence of PPB in the nasal flora decreased 19% from day 1 to day 21 (P < 0.001). All of the subjects whose PPB were eliminated had been carriers of only a single type of potentially pathogenic microorganism on day 1. After a week of follow up (day 28), no further change in the number of occurrences of PPB was detected (Table 1).

Of the 13 subjects from whom PPB were eliminated during the study period, the gram-positive organisms Sta. aureus, Str. pneumoniae, and group A β-hemolytic streptococci were eliminated in 5, 4, and 2 subjects, respectively, and the gram-negative bacte-
In a recent Finnish trial (25), the long-term consumption of a probiotic milk drink with *Lactobacillus* GG decreased the number of children in day care centers who suffered from respiratory tract infections by 17%. The effects of *Lactobacillus* GG include improvement of colonization resistance against harmful bacteria, lowering of oxidative enzyme activity, reinforcement of the mucosal barrier, and stimulation of immunologic memory (24).

Because no direct interaction of *Lactobacillus* GG with the nasal flora was found in our study, a possible mechanism of enhancing the immune system is postulated. There still remain significant gaps in our understanding of the normal interactions between a host and its intestinal microflora (26). As yet, the effect of exogenous ingested bacteria, such as probiotics, on sustained activation at the germinal centers of MALT is not known. There is evidence that the circulation of MALT lymphocytes provides immunologic information to all mucosal surfaces. Precursors of polyimmunoglobulin A (IgA)—producing plasma cells are indeed able to migrate from Peyser’s patches to secretory sites of the upper digestive and respiratory tracts (5). T and B lymphocytes primed specifically in the gut are distributed to all parts of the MALT, where B cells differentiate into immunoglobulin-producing plasma cells after local antigenic exposure. They preferentially produce IgA, which is secreted by the epithelial cells onto the mucosal surfaces as secretary IgA (SIgA). These mucosal defense mechanisms discriminate accurately between commensal organisms, symbiotic microflora, and exogenous pathogens. The precise mechanisms of discrimination are not currently understood. By contributing to such activation, probiotics may promote an IgA response that is specific not only against bacterial antigens but also against bystander antigens sampled through the follicular-associated epithelium of the GALT (27).

IgA exists as 2 subclasses: IgA1, which is preferentially produced in nasal and bronchial mucosa, and IgA2, which is produced predominately in the gut (28). This is intriguing in view of the frequent synthesis of IgA1-specific proteases by *H. influenzae, Str. pneumoniae*, and *N. meningitides*—3 bacterial species that produce infectious diseases of the upper respiratory tract. A relation has been proposed between proneness to infection by these organisms and a deterioration of regional SIgA1-dependent immunity caused by enzymes of these species (29). Interestingly, children with atopic allergies have

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<th>Bacterium</th>
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<td>Day 1</td>
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<td>Gram-positive</td>
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<td><em>Staphylococcus aureus</em></td>
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<td><em>Streptococcus pneumoniae</em></td>
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<td>β-Hemolytic streptococci</td>
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<td>Gram-negative</td>
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<td><em>Haemophilus influenzae</em></td>
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<td><em>Moraxella catarrhalis</em></td>
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<td><em>Proteus mirabilis</em></td>
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<td><em>Citrobacter spp</em></td>
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¹Number of subjects with potentially pathogenic bacteria in brackets.

²Significantly lower than day 1, *P* < 0.001.
increased amounts of IgA1 split products in their nasopharyngeal secretions (30), and their nasopharynx may be colonized by IgA1-specific protease-producing bacteria in an early, vulnerable period (31).

After oral immunization with microparticles of ovalbumin, antigen-specific SlgA was found in saliva, nasal lavage, and the vagina (32). The clinical effect of the luminal antigenic stimulation of the GALT was observed in patients who showed a marked reduction of SlgA in the nasal lavage and an increase in upper respiratory tract infections a few days after exclusive parenteral nutrition (33).

Studies in animals indicate that probiotics, *e.g.* *Lactobacillus casei*, only cause resting B lymphocytes to enter the IgA cycle, increasing the number of plasma cells that produce IgA in the lamina propria of the MALT, without stimulating other mechanisms such as immunologic memory (34). Stimulation of resistant IgA2 antibodies by enteric vaccination or stimulation, as may occur following the ingestion of probiotics, may enhance the immune barrier of the upper respiratory tract and therefore constitute a clinically practical preventive therapy (35).

In conclusion, the present study showed that an orally administered fermented milk product containing the probiotic bacterium *Lactobacillus GG* significantly reduces the occurrence of nasal colonization with PPB. The mechanisms underlying this result may have involved stimulation of the B lymphocytes of the GALT, which may have migrated to the upper respiratory immune system and led to the production of the more effective SlgA2, which may have contributed to the elimination of PPB, particularly *Sta. aureus*, *Str. pneumoniae*, and β-hemolytic streptococci. To assess this reaction in humans, a study is planned in which the concentrations of SlgA1 and SlgA2 in nasal lavage samples are measured before and after the ingestion of probiotics.

Both U Glück (specialist in diseases of the ear, nose, and throat) and J-O Gebbers (pathologist) designed the study, evaluated the results, and prepared the report. U Glück performed the clinical examinations, acquired the microbiological samples, and collected the data. We herewith declare that there is no conflict of interest, either personal or financial.

REFERENCES