Letter

Safety of probiotics used for hospital environmental sanitation.

Elisabetta Caselli[a,b*], Paola Antonioli[c], Sante Mazzacane[b]

[a]*Section of Microbiology and Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy;* [b]*CIAS Interdepartmental Research Centre for pollution control in high sterility rooms, University of Ferrara, Ferrara, Italy;* [c]*Department of Infection Prevention Control and Risk Management, S. Anna University Hospital, Ferrara, Italy.*

*Corresponding author: Elisabetta Caselli, Section of Microbiology and Medical Genetics, Department of Medical Sciences, University of Ferrara, via Luigi Borsari 46, 44121 Ferrara; e-mail: elisabetta.caselli@unife.it; telephone: +39 0532 455387

Running title: Safety of probiotics for sanitation.

Keywords: probiotic; hospital sanitation; infectious risk; safety.

Sir,

There is consensus about the need for efficient control of microbial contamination on hospital surfaces, as they represent significant pathogen reservoirs and can contribute to the transmission of healthcare associated infections (HAIs), most of which are sustained by multiresistant pathogens, representing a global concern.[1]

Control of surface bioburden is routinely addressed by conventional chemicals-based detergents/disinfectants, which however are ineffective in preventing recontamination and can select resistant strains.

Recently, cleaning agents containing probiotics of the spore-forming *Bacillus* genus have been proposed for hospital sanitation (PCHS, Copma srl, Italy), as they were shown to stably decrease surface pathogens up to 90% more than conventional disinfectants, to promote disappearance of resistant pathogen strains, and to be genetically stable even after years of continuous contact with surface pathogens.[2,3]

The rationale for the use of probiotic as sanitizing agents lies on the consideration that a healthy microbiota might provide a protective function against colonization/expansion of pathogens, not only in the human body, but also in the environment, as outlined in the so-called 'bidirectional' hygiene.[4]

Except for *B. anthracis* and *B. cereus*, all the other *Bacillus* species, included *B. subtilis, B. pumilus* and *B. megaterium* (contained in PCHS-detergents), are considered non-pathogenic for humans.[5] Nevertheless, a theoretical risk of infection exists, and a few anecdotic cases of infection were reported in surgical patients.[5] Indeed, systematic assessment of adverse events in probiotic intervention studies is lacking, whereas it was recently indicated that the most appropriate way to explore the question 'are probiotics safe' should be based on the 'totality of evidence' rather than on single case reports,[6,7] promoting active surveillance for cases of probiotic-associated infection in all probiotic-based trials.[8]

Thus, to assess any potential risk of infection associated with the environmental use of probiotics for sanitation purposes, we analysed whether the apathogenic *Bacillus* strains currently included in cleaning products might be themselves a source of HAI, performing a four-year study to detect any *Bacillus*-sustained infection in seven healthcare structures, located in the province of Ferrara (Italy), continuously using PCHS.

During the study, all the clinical samples collected from patients admitted to the enrolled hospitals were systematically analysed for the presence of *Bacillus* strains as spy organisms. A quote of samples was also analysed by a *Bacillus*-specific real time quantitative PCR (qPCR), as previously described.[2]

The number of analysed samples from each healthcare structure, as well as the period of environmental sanitation by PCHS and the molecularly assayed samples, are shown in Table I.

A total of 32,139 clinical samples were analysed, corresponding to about 90,000 patients and 800,000 hospitalization days.

Both microbiological and molecular results showed the total absence of PCHS-derived *Bacilli* in any clinical sample, for the entire period of the survey.

The absence of any HAI attributable to probiotic *Bacilli* during the entire study suggests that they apparently do not have the ability to cause infections, even in the subjects at higher risk for adverse events, such as hospitalized patients.

We think that this surveillance model might represent an essential part of the infection control policy associated to the use of probiotics, as it can assure efficient safety monitoring.

Accordingly, we are now undertaking a multicentre study to evaluate a higher number of healthcare facilities for a prolonged period, evaluating also any eventual variation in type and number of HAIs, their decrease being the final goal to achieve.

Aknowledgements

We thank the Microbiology Laboratory of S. Anna University Hospital, for the excellent technical assistance.

Conflicts of interest statement

None declared.

Funding sources

None.

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Table I. Analyses performed in the years 2011-2015 in the healthcare structures (HS) continuously using the *Bacillus*-based (PCHS) sanitation system.

Healthcare Structures	**Analyses per year (with PCHS sanitation system)**					**Total analyses (per HS)**
	2011	**2012**	**2013**	**2014**	**2015**	
HS-1	429	-	-	-	-	429
HS-2	103	704	701	613	765	2,886
HS-3	-	-	6,346	7,290	7,593	21,229
HS-4	-	76	1,025	969	1,154	3,224
HS-5	-	72	631	713	750	2,166
HS-6	-	240	403	498	554	1,695
HS-7	-	-	-	-	510	510[§]
TOTAL*	**532**	**1,092**	**9,106**	**10,083**	**11,326**	**32,139**

HS-1, Old S. Anna Hospital (Ferrara), PCHS application March 16th - August 28th 2011;

HS-2, S. Giorgio Hospital (Ferrara), PCHS application since November 1st 2011;

HS-3, New S. Anna Hospital (Cona, Ferrara), PCHS application since January 1st 2013;

HS-4, Delta Hospital (Lagosanto, Ferrara), PCHS application since June 1st 2012;

HS-5, Cento Hospital (Cento, Ferrara), PCHS application since July 1st 2012;

HS-6, Argenta Hospital (Argenta, Ferrara), PCHS application since July 1st 2012;

HS-7, Quisisana Hospital (Ferrara), PCHS application since January 1st 2015.

*A unique central Microbiology Laboratory (S. Anna University Hospital, Ferrara) performed the analyses by conventional microbiological assays.

[§] A quote of these samples were simultaneously analysed by molecular assays (qPCR), at the Section of Microbiology of the University of Ferrara.