An Innovative Approach to Hospital Sanitization Using Probiotics: In Vitro and Field Trials

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Abstract

Background: The nosocomial infections continue to be a problem, even in hospitals where meticulous sanitization procedures are in place. The most commonly used methods employ chemical disinfectants which carry some disadvantages.

Objective: To investigate the effectiveness of an innovative sanitization procedure using probiotic bacteria based on the principle of biological competition: Probiotic Cleaning Hygiene System (PCHS).

Methods: The study included survival tests and in vitro and field trials. The in vitro trials tested three surfaces (washbasin, floor and desk) in the absence of recontamination. Field trials were carried out in order to evaluate the efficacy of probiotics in the presence of contaminants and to study whether probiotics are able to contain pathogens over time. Samples were taken from the floor in a corridor and an inpatient room and the dispensary washbasin twice daily (pre-sanitization and post-sanitization).

Results: The in vitro tests on three surfaces, not subject to recontamination, resulted in an average reduction ranging from 92.2% to 99.9% after 24 h. From field trials it emerged that the bacterial count was totally eliminated for Enterococcus faecalis and Candida albicans and almost 100% elimination of Pseudomonas aeruginosa, Acinetobacter baumannii e Klebsiella pneumoniae on all three surfaces after only six hours even when recontaminated. However, less satisfactory results were attained for Staphylococcus aureus.

Conclusion: PCHS acts constantly and is durable over time due to the stabilization of a biofilm which is able to reduce and contain the proliferation of pathogenic microorganisms. Probiotics are therefore effective innovative products to sanitize the hospital environment.

Keywords: Hospital sanitization; Probiotics; Nosocomial infections

Introduction

Hospital infections continue to be a huge healthcare problem worldwide to which no facility, public or private, is immune. The importance of inanimate surfaces as sources of nosocomial pathogens has long been recognized/acknowledged [1-3]. Environmental sanitization is an essential and effective part of programs to prevent and control hospital infections [4]. Sanitization procedures in hospitals, combined with antibiotic prophylaxis for patients, are designed to reduce and prevent the proliferation of microorganisms. Nevertheless, nosocomial infections continue to be a problem, even in hospitals where meticulous sanitization procedures are in place. The most common environmental sanitization methods involve the use of chemical disinfectants. However, these are not without disadvantages: 1) the limited effectiveness of biocides over time (normally 20-30 minutes after application, after which microorganisms multiply exponentially); 2) the ability of microorganisms to mutate thereby annihilating the biocidal effects; 3) increased pollution of the natural environment arising from the massive use of chemicals that may accumulate and persist over time. The seriousness of these problems prompted us to conduct trials using an innovative sanitization technique using probiotic bacteria adopting an approach based on the principle of biological competition in which the aim is no longer to destroy the microorganisms on surfaces, but to form a biofilm to counteract the proliferation of pathogens. Probiotic studies have attracted considerable interest in recent literature, particularly in view of increased bacterial resistance [3,5-7]. These products have long been used to reduce the occurrence and/or duration of diarrhoea attacks linked to antibiotics [8,9]; and in vitro trials are now underway to assess the possibility of using probiotics outside the human body on surfaces. Some studies have focused on the potential ability of a biofilm to inhibit bacterial growth on silicone materials used in the urogenital tract [10,11,13,15] oral cavity [12,14] and/or other matrices [10,16]. Recent trials have shown that probiotic bacteria may also be used to sanitize hospital environments in order to combat the increase in nosocomial pathogens [17,18]. Probiotic bacteria (Probiotics in progress/PiPs) are spores of Bacillus spp, and considered to be innocuous microorganisms as, unlike disinfectants, they do not act as biocides. They are able to colonize surfaces to which they are applied, thereby effectively counteracting the proliferation and survival of other types of bacteria, including germs, by means of “competitive exclusion”.

Materials and Methods

The aim of this study, carried out in the year 2013 at the University Hospital “G. Martino” in Messina (Italy), was to measure the reduction and elimination of pathogenic microorganisms using probiotics and thereby assess the effectiveness of this sanitization method. The Probiotic Cleaning Hygiene System (PCHS) was adopted for this study

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conducted in the UOC laboratories for Hospital Hygiene where both in vitro and field trials were conducted.

Sanitizing solution

The probiotic-based solution contained 1% of spores (30 × 10^6 CFU/ml) of Bacillus subtilis, Bacillus pumilus and Bacillus megaterium, in addition to ionic surfactants (0.6%), anionic surfactants (0.8%) and enzymes (amyloses 0.02%) [18].

Microorganisms and growth media used

Strains of S. aureus, P. aeruginosa, K. pneumoniae, A. baumannii, E. faecalis and C. albicans, isolated from cases of nosocomial infections in hospitalized patients, were used for the in vitro trials. These strains were cultivated on the following respective media: Baird-Parker Agar, Cetrimide Agar, MacConkey Agar, Enterococcosel Agar and Sabouraud Dextrose Contact Agar.

Tryptic Soy Agar Contact (TSA) was used for the total microbial count (TMC) in field trials, in addition to the specific cultures for the same microorganisms tested in vitro. All bacterial strains were cultivated by incubation at 37ºC for 24-48 h.

Identification of microorganisms

Pathogenic strains were identified using API 20 NE for Pseudomonas spp, API 20 E for Enterobacteriaceae microorganisms including K. pneumoniae and A. baumannii, API Staph for S. aureus, and API AUX C for Candida spp.

Sanitization procedures

Sanitization was carried out using microfibre cloths, cleaned after each use following the manufacturer’s instructions. Dry surfaces were first dusted followed by wet cleaning using the probiotic liquid. The microfibre cloths were soaked in the solution and stored in clean containers until use. The sanitization steps were all carried out by the same trained staff member in order to minimize any variations in the procedure adopted. The solution used to treat surfaces was prepared in accordance with the manufacturer’s instructions for application of 1.5 × 10^6 spore/m².

Survival tests

The above microbial strains were used in survival tests to contaminate the surfaces of a wash-basin, a floor and a desk in order to assess their survival in the external environment. For this purpose, we used solutions of the microbial strains with an initial count of about 1.5 × 10^5 CFU/m² to study whether probiotics are able to contain pathogens over time. Samples were taken from the floor in a corridor and an inpatient room and the dispensary washbasin twice daily, at 8:00 (pre-sanitization) and 14:00 (post-sanitization). As control group, at the same time, we carried out a comparable microbiological monitoring in similar surfaces situated in the opposite part of the ward, subjected to the same type of recontamination and sanitized using normal chemical products.

Results

Survival tests

The tests conducted to assess the survival of the same strains used for in vitro trials, showed microorganisms were still alive after 24 or 48 h. E. faecalis and S. aureus were particularly robust, as they continued to survive in the external environment even after four and eight days respectively. Moreover, the latter showed a continuous and progressive growth until the day 4 after which it decreased to the level at zero time (Table 1).

In Vitro trials

The in vitro tests on three surfaces, not subject to recontamination, resulted in an average reduction ranging from 92.2% to 99.9% after 24 h (Table 2).

| Strains         | Survival CFU/m² x 10^5 / % | Zero Time | 24 h CFU | 48 h CFU | 96 h CFU | 192 h CFU |
|-----------------|-----------------------------|-----------|----------|----------|----------|----------|
| Staphylococcus aureus |                             | 1516      | 1562     | 103 %    | 185      | 122%     | 2500     | 165%    | 1395     | 92%      |
| Pseudomonas aeruginosa |                          | 1500      | 75       | 5 %      | 0        | 0        | 0        | 0        | 0        | 0        |
| Candida albicans  |                             | 1583      | 7        | 0.44 %   | 0        | 0        | 0        | 0        | 0        | 0        |
| Enterococcus faecalis  |                           | 1480      | 1156     | 78 %     | 474      | 32%      | 89       | 6 %      | 0        | 0        |
| Acinetobacter baumannii |                          | 1550      | 8.4      | 0.54 %   | 0        | 0        | 0        | 0        | 0        | 0        |
| Klebsiella pneumoniae |                             | 1586      | 7.5      | 0.47 %   | 0        | 0        | 0        | 0        | 0        | 0        |

Table 1: Survival ability in external environment of bacterial strains, isolated from cases of nosocomial infections in hospitalized patients, used for in vitro trials.

Field trials

Field trials were carried out in the Thoracic and Vascular Surgical Ward over a three-month period (May–July 2013) in order to evaluate the efficacy of probiotics in the presence of a contamination related to the daily hospital activity of healthcare workers, inpatients and relatives and to study whether probiotics are able to contain pathogens over time.
Analysis of the individual results for each contaminated surface and each pathogenic microorganism tested showed similar results for two of the materials (wooden desk and porcelain washbasin), since after three hours only *P. aeruginosa* survived on both surfaces while *E. faecalis* survived only on the wooden desk. After six hours they disappeared from both the desk and washbasin. In contrast, all microorganisms were still alive after six hours on the linoleum floor. These differences arising from the different materials (wood, porcelain, and linoleum) were eradicated after 24/30 hours when all microorganisms were destroyed on all three surfaces. Comparison of the individual microorganisms demonstrated the following percentage reductions: *S. aureus*, 99.4% after 3 h, 99.5% after 6 h, 99.7% at 24 h and total destruction at 30 h; *P. aeruginosa*, 70.2% after 3 h, 90.0% after 6 h and 100.0% at 24 h; *A. baumannii* and *K. pneumonia*, 98.6% and 96.5% respectively after 3 h, 99.5% and 99.0% at 6 h, and 99.9% and 99.5% at 24 h with total destruction after 27 h; *E. faecalis* 96.7% after 3 h and total destruction after 6 h; with similar results for *C. albicans*.

**Field trials**

Before the sanitation with PIPs, we evaluated the contamination rate of the ward by microbiological sampling using specific contact plates (not only floor, desk and washbasin but even beds, bedside tables and door handles). In these surfaces we found a contamination by *S. aureus*, *E. faecalis*, *P. aeruginosa* and *C. albicans* in the amount of $4 \times 10^2$ CFU/m², $2.5 \times 10^2$ CFU/m², $2 \times 10^2$ CFU/m² and $0.5 \times 10^2$ CFU/m² respectively. After that, destruction and/or reduction of each pathogenic microorganism was achieved by sanitization using the PCHS system over the trial period (from 6 May to 30 July). While in the survival test all microorganisms were still alive after 24 or 48 h, for *E. faecalis* and *C. albicans* the microbial count was totally eliminated (Figure 1-2). It also disappeared almost completely for *P. aeruginosa* on all three surfaces after only 6 h, even when recontaminated (Figure 3). The probiotic system was equally as effective against *A. baumannii* and *K. pneumoniae*, for the first two months, while in the third month of trials six hours of contact were no longer enough to reduce bacterial contamination.
count significantly, although growth continued to be contained particularly for the washbasin surface (Figure 4). The efficacy of PIPs was far less evident for S. aureus (Figure 5). Indeed, sanitization using PIP following recontamination was unable to act continuously to reduce bacterial growth even three months after treatment. This was most evident on ward floor surfaces. In the control group, we observed a constant and remarkable presence of S. aureus, P. aeruginosa and E. faecalis, in variable concentration, for all the period of the monitoring.

Discussion

Our study confirms that probiotics are able to reduce the growth of specific pathogenic microbial species namely: S. aureus, P. aeruginosa, K. pneumoniae, E. faecalis, A. baumannii and C. albicans. The in vitro tests allowed the reduction in microbial count of the pathogens to be verified under controlled conditions, thus verifying the efficacy of PIP to combat bacteria where there is no risk of recontamination. The field trials demonstrated that the bacterial count remained low over time following sanitization despite the surfaces treated being constantly exposed to the risk of recontamination by patients, healthcare workers and visitors. The results achieved improved as time progressed but this improvement was linked to the type of material treated. It was found that sanitization was more effective on the porcelain washbasin than the linoleum flooring. This confirms that the continuous and constant action of the PCHS system over time is the result of the stabilization of the biofilm, which is able to reduce and contain the proliferation of microorganisms.

Both the in vitro and field trials demonstrated the efficacy of these products in containing the total microbial count; and this positive effect was found to persist throughout the trial albeit with some adverse variances for A. baumannii, K. pneumoniae and S. aureus. The results obtained for these pathogens differed from the others tested as...
The probiotic biofilm was unable to compete effectively for the entire duration of the trials undertaken. In the case of S. aureus this finding is probably linked to its greater resistance and vigour in the environment; as shown by the survival tests.

Probiotics are ecological, easy to use and biodegradable. They render the environment hygienically stable and are able to survive on and colonize non biological surfaces, combatting the proliferation of other bacteria. In this study they were also found to perform well on surfaces in the hospital environment that are subject to regular recontamination. Probiotics are therefore effective innovative products for sanitizing the hospital environment and constitute a valid "green" alternative to the chemical disinfectants used up to now. However, further trials are necessary to test the product on surfaces which expose hospitalized patients to the greatest risks of infection.

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