Hard Surface Biocontrol in Hospitals Using Microbial-Based Cleaning Products

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Abstract

Background

Healthcare-Associated Infections (HAIs) are one of the most frequent complications occurring in healthcare facilities. Contaminated environmental surfaces provide an important potential source for transmission of many healthcare-associated pathogens, thus indicating the need for new and sustainable strategies.

Aim

This study aims to evaluate the effect of a novel cleaning procedure based on the mechanism of biocontrol, on the presence and survival of several microorganisms responsible for HAIs (i.e. coliforms, Staphylococcus aureus, Clostridium difficile, and Candida albicans) on hard surfaces in a hospital setting.

Methods

The effect of microbial cleaning, containing spores of food grade Bacillus subtilis, Bacillus pumilus and Bacillus megaterium, in comparison with conventional cleaning protocols, was evaluated for 24 weeks in three independent hospitals (one in Belgium and two in Italy) and approximately 20,000 microbial surface samples were collected.

Results

Microbial cleaning, as part of the daily cleaning protocol, resulted in a reduction of HAI-related pathogens by 50 to 80%. This effect was achieved after 3–4 weeks and the reduction in the pathogen load was stable over time. Moreover, by using microbial cleaning as a conventional cleaning alternative, we found that this effect was directly related to the new procedure, as indicated by the raise in conventional cleaning. In contrast, microbial cleaning was replaced by the conventional procedure. Although every question remained concerning the actual mechanisms involved, this study demonstrates that microbial cleaning is a more effective and sustainable alternative to chemical cleaning and non-specific disinfection in hospital facilities.

Conclusions

This study indicates microbial cleaning as an effective strategy in continuously lowering the number of HAI-related microorganisms on surfaces. The first indications on the actual level of HAIs in the trial hospitals monitored on a continuous basis are very promising, and may pave the way for a novel and cost-effective strategy to counteract or (bio)control healthcare-associated pathogens.

Introduction

Healthcare-Associated Infections (HAIs) are one of the most frequent complications occurring in healthcare facilities and represent a problematic concern regarding the safety and quality of healthcare worldwide[9] as also stated in a recent report by the World Health Organization estimating hospital-wide prevalence in high-income countries at 8%.[10] The European Center for Disease Control[3] points out that healthcare-associated infections are a major public health problem in Europe with a prevalence of 5.7% (4.9–6.4%) which means 81,093 (64,325–105,893) patients with one HAI for each day in European acute care hospitals.[10] In particular, the European survey reported a similar estimation of nosocomial infections in Italy and Belgium, where the percentage of patients with HAIs has been calculated as 6.9% (5.4–7.7%) and 7.1% (6.2–8.5%), respectively.[10] Based on this study, the estimated total annual number of patients with an HAI in European acute care hospitals in 2011–2012 was 3.2 million, albeit with a wide confidence interval from 1.9 to 5.2 million patients. Similar incidences were measured in the United States[11] besides human suffering, also impressive economic costs are related to HAIs management. Indeed, as reported by the Centers for Disease Control and Prevention (CDC), it has been estimated that the overall annual direct medical costs for healthcare-associated infections in hospitals ranges from 25.7 to 45 billion dollars in the United States[12]. In addition, the management, prevention and monitoring of HAIs nowadays still represents a challenge for healthcare facilities.[13]

The microorganisms most frequently isolated from HAIs are, in decreasing order, Escherichia coli (15.9%), Staphylococcus aureus (12.3%), Enterococcus spp. (9.9%), Pseudomonas aeruginosa (9.9%) Klebsiella spp. (8.7%), coagulase-negative staphylococci (7.6%), Candida spp. (6.1%), Clostridium difficile (5.4%), Enterobacter spp. (4.2%), Proteus spp. (3.8%) and Acinetobacter spp. (3.6%).[13]

A very controversial and debated question is the qualitative and quantitative role of the environment in the patient contamination process, particularly the role of confinement and furnishing surfaces. It is well known that surfaces act as reservoirs for microorganisms and could contribute to the transmission of hospital pathogens, increasing the risk of cross-contamination through indirect contact with the patient.[14,15] To reduce such risks, sanitation procedures are applied to every surface that directly or indirectly comes into contact with people. Despite experimental evidence suggesting that a reasonable use of disinfectants is recommended, their routine use is still controversial.[16] Nevertheless, a proper surface disinfection is recommended by all institutions when microbial cleaning is important for the prevention of infections. Although more plausibly reasonable regarding the benefits of hospital cleanliness towards reducing HAIs,[16] indeed, failure to ensure proper cleaning and disinfection may lead to patient-to-patient transmission of pathogens.[17]

However, the widespread use of chemical disinfectants presents risks towards the environment and the safety of personnel. It is clear that microorganisms can adapt to a variety of environmental physical and chemical conditions, and it is therefore not surprising that resistance to extensively used antiseptics and disinfectants has been reported.[18] For these reasons, the importance of cleaning procedures that are aimed to control the load of pathogenic bacteria indicates that a new and sustainable strategy is necessary.

A very promising approach, as suggested by Falagas & Makris in 2009, is the use of non-pathogenic microorganisms, namely probiotics and defined as living microorganisms able to confer a health benefit on the host, to colonize hard surfaces in order to counteract the proliferation of other bacterial species[19] according to the competitive exclusion principle (Gause’s law)[20,21]. This concept has been designated as biocontrol when the application is antagonistic towards a certain pathogen[22] and has already successfully been applied to the abatement of Legionella in water systems[23]. Several investigators have pointed to evidence that probiotic type microorganisms and their bioficiants may antagonize the growth of nosocomial pathogens on inanimate surfaces.[24–26] However, the actual application of probiotic type microorganisms on hard surfaces as a cleaning procedure has never been tested. Therefore, this study aims to provide sufficient data to conclude whether the technique of biocontrol of hospital surfaces can act as a sustainable alternative to chemical disinfectants.


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Methods

Preliminary trials

Prior to the actual field trials in the hospitals, a number of preliminary trials were done at Ghent University, Ferrara University and AZ Lokeren hospital in order to determine the most suitable formulation of the microbial cleaning products to be used in the hospital. The identity and concentration of the micro-organisms used in the preliminary trials were chosen in view of the average microbiological load on hard surfaces, pH, temperature and humidity. The microbial cleaning products used in the field trials comprised spores of Bacillus subtilis, Bacillus pumilus and Bacillus megaterium, with a fixed quantity of $10^5$ CFU per ml of product concentrate. All products were manufactured by Chrisal (Lommel, Belgium) and supplied to AZ Lokeren by Chrisal and the two Italian hospitals by Copma scrl. (Ferrara, Italy).

In order to prevent bias in the eventual hospital trials due to the detergents in the products used, several field trials in the AZ Lokeren hospital were performed to compare the effect between the microbial and non-microbial version of the products to be used in the hospital trials (data not shown).

Ethics Statement

The study protocol was reviewed and approved by the local Ethics Committees. The trials in the two Hospitals residing in Ferrara (Sant’Anna University Hospital) were approved by the Ethics Committee, named Cardinalino Unico della Provincia di Ferrare (Ethics Committee of the Ferrara province), of the Azienda Ospedaliero-Universitaria of Ferrara (Ferrara, Italy). For the AZ Lokeren setting, the study was reviewed and approved by the AZ Lokeren Ethics Comittee (Lokeren, Belgium). The two Ethics Committees waived that a formal authorization was not necessary because the probiotic products would not be directly administered to patients but exploited for cleaning of hospital surfaces only. For this reason, the Committees waived the need for written informed consent from participants because of the observational nature of the study.

Hospital trial setup

Three independent hospital trials, separated in time and location were performed. In each trial setting, comparison was made between cleaning with microbial cleaning products and the conventional hygiene protocols (using chemical cleaning products and disinfectants). Control cleaning products in AZ Lokeren consisted of chemical detergents (EcoNat, Groot-Bijgaarden, Belgium) and in both Italian hospitals chlorine based detergents (Askulor for all washable surfaces, Disinigy S.p.A., Italy) were applied. The microbial cleaning products in all three hospitals comprised a floor cleaner, interior cleaner and bathroom cleaner (Chrisal, Lommel, Belgium). Comparison between control and microbial cleaning was made both over time and on units with identical characteristics of cleaning within the hospital (e.g. two floors of geriatrics in AZ Lokeren). Except for the products, all other parameters related to the cleaning procedure (e.g. frequency, equipment) were the same between control and microbial cleaning. Cleaning in AZ Lokeren was performed according to the existing hygiene protocol of the hospital, and cleaning in the two Italian hospitals was performed according to the Probio Cleaning Hygiene System (PCHS) by Copma scrl. Cleaning staff was not aware whether or not they were operating with microbial cleaning products.

Description of hospital wards

San Giorgio Rehabilitation Hospital

The San Giorgio hospital (total surface 12,300 m²) is a centre for the rehabilitation of acquired severe brain-damaged disorders. The wards involved in this study were distributed on a total of three floors, with each floor (4,100 m²) consisting of two identical areas, namely the Severely Brain-Damaged Unit and Rehabilitation Medicine Unit. These two wards are separated by a central corridor and each consists of 22 recovery rooms (28 m²) with two beds and a toilet (4.2 m²). In these settings, treated surfaces consisted of corridor, room, floor and toilets. In addition, cleaning procedures and samplings were also performed in the six gymnasiums used for patient rehabilitation.

Sant’Anna University Hospital

The wards involved in the study within the Sant’Anna University Hospital are referred to as an in-patients general medicine ward, consisting of two identical areas (named S- and T-Area, each with a surface of 550 m²), and an out-patient ward containing the Ophthalmology/Cardiology and Orthopaedics departments (286 m² each, with rooms of 22 m²). Recovery rooms (a total of 20, with a surface of 38 m² each) and toilets (10 m²) were monitored during microbiological samplings.

AZ Lokeren Hospital

In the Lokeren hospital setting, two structurally identical (500 m² per unit) geriatrics units were involved in the study. The wards host elderly people suffering from a wide range of diseases and several chronic conditions. At the same time, elderly people are visited by surgeons, short-stay care physicians or general practitioners. In addition, the wards also host elderly people under the influence of psychotropic drugs.

Microbiological trials

The effect of the microbial and conventional cleaning products on HAi related microorganisms on surfaces was assessed by means of surface sampling and culture-based microbiology tests. The microbiological tests were performed on all three hospital settings. Two HAi-related microorganisms were monitored: Staphylococcus aureus and Escherichia coli in all three hospitals; Clostridium spp. in AZ Lokeren Hospital; Candida albicans in Sant’Anna and San Giorgio hospital. During six weeks in Sant’Anna University Hospital, 24 weeks in AZ Lokeren hospital and 66 weeks in San Giorgio Hospital, a total of 25000 microbiological samplings were collected. From these, six and 24-hour collections were made from a broad variety of surfaces such as floors, doors, showers, window sills, sinks, tiles, walls, stone, wood, glass, plastic or metal.

All surface samples were performed in triplicate by contact RODAC (Replicate Organism Detection and Counting) plates, used for microbiological monitoring surface equivalent to 24 cm² of the surface. The following growth media were used: M-COACGY agar (BB, MacConkey Agar BD) as selective and differential medium for the detection and enumeration of Enterobacteriaceae (especially the group of coliform bacteria); Baird Parker Agar (Merck Millipore Baird-Parker Agar) as moderately selective and differential medium for the detection and enumeration of coagulase-positive staphylococci, and used for detecting Staphylococcus aureus; Clostridium difficile agar (BB, Clostridium difficile agar) for the selective detection of Clostridium difficile; and Sarcochromasamic Fast Contact agar added with chloramphenicol (Merck Millipore) as selective medium for the detection and enumeration of Candida albicans. Incubation was done aerobically at 37°C (6–72 h) for MacConkey, Baird Parker, Catalase Agar (BB, Catalase Agar BD) and aerobically on anaerobic jar (GasPak, BD) at 37°C (72–96 h) for the detection of Clostridium difficile agar. Colony Forming Units (CFU) on all agar plates were manually counted after their respective incubation period.

Occasionally isolates from the above agar plates were identified to check the selectivity of the used media. Identification of isolates was assessed by API 20 E (BioMérieux, Inc, Durham, NC, USA); BBL Enterotube II (BD Diagnostic Systems) for Escherichia coli; API Staph (bioMérieux, Inc) for Staphylococcus aureus, and API 20 A-U (bioMérieux, Inc) for Candida albicans.

Antibiotic susceptibility

The protocol used was based on the Kirby-Bauer disk diffusion antimicrobial susceptibility test [32–35], following the criteria outlined by the Clinical and Laboratory Standard Institute (CLSI) [36]. Briefly, a 90 mm Mueller-Hinton agar plate was inoculated with a suspension of Bacillus subtilis (ATCC 6633), or other strains isolates, corresponding to a 0.5 MacFarland standard. After 15 minutes, a paper disk (Oxoid Ltd., Thermo Scientific, Basingstoke, Hampshire, United Kingdom) impregnated with 5 µl of an antimicrobial compound was added, and plates incubated at 37°C for 24–48 hours. The zones of inhibition (expressed in mm) were measured, and the interpretation of results was based on CLSI reference criteria [36,37].

Results

Prior to hospital trials, a broad range of in vitro and small scale field trials were performed in order to determine both the best possible formulations and on maintenance product formulations for the actual study, as well as to provide relevant information to the Ethics Committees. The focus of the study was on microbiological counts and no evaluations of actual dirt removal were assessed. The results of the initial field trials showed that a higher microbiological influence on hard surfaces could be obtained by using Bacillus subtilis, Bacillus pumilus and Bacillus megaterium, with a fixed quantity of $10^5$ CFU per ml of product concentrate. All products were manufactured by Chrisal (Lommel, Belgium) and supplied to AZ Lokeren by Chrisal and the two Italian hospitals by Copma scrl. (Ferrara, Italy).

In order to prevent bias in the eventual hospital trials due to the detergents in the products used, several field trials in the AZ Lokeren hospital were performed to compare the effect between the microbial and non-microbial version of the products to be used in the hospital trials (data not shown).
surfaces upon cleaning should be of the same magnitude as the average total count observed before the microbial cleaning application. Finally, a number of laboratory scale tests demonstrated that the same product formulations, with or without the addition of the Bacillus spores, produced significantly different effects on pathogen load on the treated surfaces.

The study was conducted in three different hospitals, and approximately 20000 microbial surface samples were collected. The microbial analyses focused on Staphylococcus aureus, coliform bacteria, Clostridium difficile, and Candida albicans as representatives or indicators for proper hygiene and HAI-related microorganisms. In order to allow proper comparison among the different hospital settings, results are presented as relative values to the control, which is referred to the value of microbial surface contamination (CFU count) at the beginning of the trials (week 0), as shown in Tables S2, S3, S4.

Notably, the effect of microbial cleaning on the different HAI-related pathogens displayed similar trends among the three hospitals under investigation. In the Sant’Anna University hospital, the microbial sampling lasted six weeks, whereas the other two hospital sites were monitored until week 24 (AZ Lokeren) and week 66 (San Giorgio Hospital). However, because no significant differences in counts were observed between week 24 and 66 at the San Giorgio Hospital, data presented here are limited to 24 weeks. In addition, during trials the total microbial count was also measured, but no significant changes were observed during the entire period spanning the hospital trials (data not shown).

**Coliforms and E. coli**

Figure 1 shows the mean relative values for coliform bacteria load as a general indicator for hygiene. The reference values measured at week 0 for coliforms, with E. coli representing about one half of the count, are reported in Table S2. However, individual surface counts could fluctuate strongly, especially in specific areas such as bathrooms or geriatric departments. Noticeably, the beginning of microbial cleaning resulted in a fast reduction of coliform and E. coli counts, achieving a maximum effect approximately after two weeks of daily cleaning. The average reduction over time was 74±21% for the coliforms and 89±18% for E. coli. The observed reduction was statistically significant for all the monitored hospital settings (Table S2).

**S. aureus**

The average levels of S. aureus on tested surfaces and the corresponding percentage reduction over time are reported in Table S3 and Figure 2, respectively. For the sake of clarity, no distinction was made between antibiotic-resistant or antibiotic-sensitive S. aureus. After 10 days of microbial cleaning within the San Giorgio Hospital setting, the S. aureus counts on surfaces dropped, on average, by 48±12% and 73±15% after six weeks, with a reduction that was highly significant in all the hospital settings (Table S3). In both conventional and microbial cleaning, S. aureus counts on treated surfaces showed a good stability with no large exceptions throughout the study (data not shown), suggesting that this organism is not very susceptible to different environmental conditions within a hospital and might therefore survive most common conditions.

**C. difficile**

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0108598
A less abundant but still common HAI-related organism is Clostridium difficile, with average counts of about 500 CFU/m² (Table S4), which is near the detection limit of the test protocols used. These microorganisms were monitored on a regular basis only in the AZ Lokeren setting. C. difficile showed much larger specific counts on different surfaces and time points, which, in combination with the overall lower mean counts, resulted in larger standard deviations, thus making the effect of microbial cleaning less significant when compared to control. The overall average reduction in C. difficile load was achieved very quickly after three days of microbial cleaning, with a reduction corresponding to 69.4% (Figure 3). Drop-out measurements in the next 24 weeks indicated that C. difficile levels on microbial cleaned surfaces dropped below the detection limit of the analysis method. The observed reduction was significant for weeks 4 (p=0.048) and 12 (p=0.007), and particularly for weeks 18 (p=0.0005) and 24 (p=0.004), indicating the long-term effect on C. difficile load reduction.

Figure 3. Effect of microbial cleaning on C. difficile surface counts.
Surface counts are reported as relative percentage of reduction compared to the control, which was cleaned with conventional (disinfecting) cleaning products. The statistical analysis is reported in Table S4.
https://doi.org/10.1371/journal.pone.0108598.g003

Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>Count Reduction (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. difficile</td>
<td>69.4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C. albicans</td>
<td>91.2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. aureus</td>
<td>89.1%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The susceptibility profile of the other antibiotics tested (cefpodoxime, ceftiraxone and gentamicin) was comparable to that of the ATCC reference strain, with the only exception of ciprofloxacin, for which intermediate values were observed (Table S2).

Figure 4. Effect of microbial cleaning on Candida albicans surface counts.
Surface counts are reported as relative percentage of reduction compared to the control, which was cleaned with conventional (disinfecting) cleaning products. The statistical analysis indicated that results observed were statistically significant (Table S4).
https://doi.org/10.1371/journal.pone.0108598.g004

Time trial with conventional and microbial cleaning
A separate experiment was conducted within the geriatrics department in the AZ Lokeren setting, where the conventional and microbial cleaning procedures were alternatively applied and the bacterial load was monitored for 10 weeks. Microbial cleaning (from week 0 to week 2) was applied after two weeks of conventional cleaning (from week 2 to week 4), which was then applied for a second period spanning the last 8 weeks (from week 6 to week 10). Consistently with previous observations, the microbial cleaning strongly reduced both coliforms and S. aureus load, whose counts dropped from 9250±1750 CFU/m² to 3200±1200 CFU/m² and from 4200±1200 CFU/m² to 950±450 CFU/m² after 2 weeks, respectively. Conversely, when the microbial cleaning was replaced by the conventional procedure, both loads raised to colony counts comparable to those observed before the application of the microbial cleaning (between weeks -2 and 0) (Figure 5). The application of the microbial cleaning led to a significant decrease in the pathogenic load of coliforms (p=0.001), from week 0 to week 3 and S. aureus (p=0.003), from week 1 to week 3 in comparison with that measured at week 0), whereas no significant differences were observed after replacement of the microbial cleaning with the conventional procedure.

Figure 5. Time course of coliforms and S. aureus surface counts.
A time-trial of coliforms (black circles) and S. aureus (white circles) counts was performed at the geriatrics department of the AZ Lokeren hospital. Surface counts, indicated as CFU/m², were measured after application of conventional (from week -2 to 0) and microbial (from week 0 to 2) cleaning, followed by a subsequent period of conventional cleaning (from week 2 to 10). The application of the probiotics-based products led to a significant decrease in the pathogenic load of coliforms (p=0.001) and S. aureus (p=0.003). Black arrow: beginning of the microbial cleaning. Black dotted arrow: conventional cleaning.
https://doi.org/10.1371/journal.pone.0108598.g005

Overall, our data on microbial cleaning indicated that the strong reduction of the pathogen load was stably maintained over time, and the observed effect was directly related to its application, as indicated by comparison with conventional cleaning.
To increase the number of antimicrobial resistance factors analyzed, 20 Bacillus isolates, which were collected from 6 to 12 months after the beginning of the trial in the hospital that were also analyzed by a qPCR assay that detected 87 different genes, were tested for antibiotic resistance. Prior to testing Bacillus isolates, the method was set up on known Bacillus spp. DNA samples, which indicated this technique as a reliable tool for subsequent analysis. The analysis of the probiotic-based cleaning products revealed the existence of constitutive resistance genes in the Bacillus species contained in the original product formulation, confirming previous literature reports (Figure 6).

![Figure 6](image1)

Table 1. Antibiograms on Bacillus spp. from the cleaning products and isolates.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spa</td>
<td>1.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Spa</td>
<td>2.5</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Spa</td>
<td>3.5</td>
<td>3.0</td>
<td>2.5</td>
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<tr>
<td>Spa</td>
<td>4.5</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Spa</td>
<td>5.5</td>
<td>5.0</td>
<td>4.5</td>
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</table>

Noticeably, new or acquired resistance genes were completely absent in all the Bacillus isolates tested, indicating that these bacteria did not undergo mutagenicity or gene transfer events, even after 12 months after application, thus confirming the results observed in the antibiogram assays.

Discussion

Given the latest official reports on the prevalence of HAIs and the evolution of microbial resistance to antibiotics and disinfectants, it is clear that new and sustainable strategies to address contaminated surfaces in hospital settings are of great interest. Besides measures that deal with person-to-person transmission of pathogens, contaminated environmental surfaces provide an important potential source for transmission of many healthcare-associated pathogenic bacteria due to their persistence on such inanimate surfaces. This highlights the importance of cleaning procedures aimed to control the load of pathogenic bacteria that reside on hard surfaces and, as a consequence, of HAIs, as indicated by considerable evidence about the benefits of hospital cleanliness towards reducing HAIs.

This study exploited the hypothesis proposed by Faiagas & Maia (20) to use non-pathogenic microorganisms, namely probiotics, as part of daily cleaning products to lower the incidence of HAIs in healthcare facilities. Evidence on the efficacy of probiotics for the prevention and treatment of infections have been observed both in vitro and in vivo. A variety of HAIs-related microorganisms exist and for this study the selection was mostly determined by the reliability and relative abundance of the microorganisms in the hospitals willing to participate to the present study. Coliforms were selected as an indicator of overall hygiene and cleaning assessment. Although some HAIs are associated with outbreaks and others show rather constant prevalence, the related microorganisms can be found on hard surfaces at any time as presented in this study. This implies that surfaces act as a reservoir that might initiate an outbreak when people get contaminated and start spreading the pathogens amongst each other.

The aim of this study was to collect a substantial amount of data from different independent healthcare facilities and the 20000 microbial surface samples contribute to the significance of the results. However, the amount of analyses makes it impossible to present all specific data and observations in this study. Therefore, given the rather comparable mean microbial counts between the several hospitals, the witnessed trends in surface microbiology upon microbial cleaning was presented.

In general, it was observed that microbial cleaning of hard surfaces resulted in a clear change in the microbiology within two weeks. With the exception of Clostridium difficile, a very significant decrease in HAIs-related microorganisms, compared to control cleaning and disinfection, was observed. As of two weeks of microbial cleaning, the reduced HAIs-related microorganisms count remained constant and mostly just below detection limit of the test protocol. The standard deviation of some values was high (likely due to large differences in the type of tested surfaces), but still the difference was clearly significant to demonstrate the effects exerted by the microbial cleaning compared to the control. Our results indicate that microbial cleaning clearly lowered the prevalence of HAIs-associated microorganisms on hard surfaces compared to conventional cleaning procedures, without significantly lowering the total microbial counts. In addition, our data show that when microbial cleaning was switched to the conventional procedures, the HAIs-related microorganism counts raised to the original (higher) levels observed before the beginning of microbial cleaning. This observation indicates that microbial cleaning has a time-limited effect on the counts of HAIs-related microorganisms and that microbial cleaning needs to be continuously applied on regular basis in order to stably maintain the reduced levels of these microorganisms. Microbial cleaning therefore resulted in the partial non-permanent replacement of HAIs-related microorganisms by the Bacillus strains used in the microbial cleaning products.

Although this study did not aim to investigate the actual mechanisms involved in the observed changes in surface microbiota, some assumptions can be made based on existing literature. The most relevant mechanism underlying the observed effect is likely due to competitive exclusion: Bacillus strains applied on the surfaces within the cleaning solution may compete, in terms of nutrients and space, with the microflora already present on the surfaces (44). Who & Ahe have suggested that competitive exclusion can also destabilize certain biofilms (48), which was observed in the present study as removal of growth on certain hard surfaces after several weeks of microbial cleaning. Such biofilm removal on hard surfaces by means of microbial cleaning deserves further research.

Another mechanism that is likely to contribute to the changes in microbiota upon microbial cleaning, and also destabilizes biofilm formation, is quorum sensing and quorum quenching (49). The constant artificial introduction of dominant counts of Bacillus spp. through the cleaning products could destabilize the bacterial population dynamics on surfaces and in biofilm (e.g. growth). Given the observed general effect of microbial cleaning on several HAIs-related microorganisms, it is likely that non-selective effects such as competitive exclusion and quorum sensing are the most important mechanisms involved. Besides these general mechanisms, other specific interactions could contribute to the reduction effect on one or more HAIs-related microorganisms. For instance, the production of either bacteriocins or some enzymes known to be produced by Bacillus spp. might affect other organisms to a certain extent. Besides those general mechanisms, more specific interactions could contribute to the reduction effect on some or more HAIs-related microorganisms. For instance, the production of either bacteriocins or some enzymes known to be produced by Bacillus spp. might affect other organisms to a certain extent.
However, it is clear that microbial cleaning alone will not solve the emerging HAIs problem. There is strong evidence indicating that a correct hand hygiene program in healthcare settings represents an effective behavior for the purpose of reducing HAIs [64, 65].

Therefore, microbial cleaning needs to be part of a global hygiene protocol, such as the proposed microbial cleaning system [PCL2(2)], used in this study. Such protocol outlines in which specific areas or events a proper disinfection is required over microbial cleaning, as well as the instructions to proper hand hygiene or isolation of contaminated persons. Also, sporadic outbreaks such as viral infections might ask for exceptional measures that do not follow the routine cleaning or disinfection procedures. Despite the reductive effect on several HAII-related microorganisms, microbial cleaning products are not to be used or considered as disinfectants. Indeed, when a surface is actively contaminated, disinfection is needed, in particular for those surfaces that are located in high risk areas. Cleaning with probiotic-based products can overcome the limited action of traditional disinfectants by decreasing the re-occurrence of pathogenic loads on surfaces and by removing biofilms that can act as a shelter for other pathogens. The probiotic-based products used in this study are suitable only for cleaning, thus indicating that when a disinfection is really necessary, a disinfectant must be used, but the combination with probiotic-based cleaning will allow optimal long-term maintenance of low levels of contamination.

In order to evaluate the susceptibility or resistance of the Bacillus strains to antibiotics, we recently implemented our research by exploiting antibiotic assays, which have been performed on colonies of Bacillus spp. coming from the same sample surfaces (e.g. floor) monitored in the hospital settings. Our first results indicate that the isolated Bacillus spp. strains are susceptible to the antibiotics tested, with the exception of those towards which the Bacillus spp. is naturally resistant, as also indicated by antibigrams performed on the standard ATCC strain.

We are aware that the study was focused on a limited number of microorganisms, and that culture-dependent techniques display some limitations due to the fact that single colonies might be not fully representative of the bacterial genus analyzed. As to safety concerns, in order to increase the representativeness of the results, 20 Bacillus isolates were also tested by a PCR assay, which was designed to simultaneously assess the presence of 87 different resistance genes. Despite the high number of resistance factors analyzed, this method takes advantage of a high sensitivity, which permits the detection of very few copies of the target gene, thus allowing the identification of antibiotic resistance genes even when only a very low number of bacterial cells within the samples are positive to antibiotic resistance. The reliability of this assay for the analysis of microbial strains is supported by the detection, as expected, of those resistance genes known to be present in the Bacillus spp. contained in the formulation of the cleaning product used [67]. Although these assays were performed on only 20 Bacillus spp. isolates, these preliminary results show that they did not display acquisition of new resistance genes, even in a period of 12 months, thus strengthening the hypothesis that the use of Bacillus spores in cleaning products might be considered as safe. On the other hand, results also show that this method can be reliable for the evaluation of antimicrobial resistance factors in Bacillus, and designate this assay as an important implementation for future studies also focused on safety concerns.

Conclusions

Given the recent and fast evolution of multi-resistant pathogens in healthcare facilities there is a need for sustainable and effective alternativaives to the cleaning and disinfection chemicals used today. This study demonstrates that a microbial (probiotic-based) cleaning is more effective in the long-term lowering of the number of HAII-related microorganisms on surfaces, when compared to conventional cleaning products, even those containing disinfectant molecules such as chlorine. The first indications of the anti-reproductive effect on several HAI-related microorganisms, microbial cleaning products are not to be used or considered as disinfectants and, therefore, a novel and cost-effective strategy to counteract or (bio)control healthcare-associated pathogens.

When it comes down to risk management, one has to decide whether a patient should stay in an environment dominated by food grade microorganisms or in an environment harboring an elevated level of increasingly resistant pathogens.

Supporting Information

Table S1. Reference criteria for interpretation of the zones of inhibition in the disk diffusion antimicrobial susceptibility test. The table shows the disk diffusion interpretative criteria for the correlation of the diameter (expressed in mm) of the zones of inhibition with the corresponding interpretation, referred as Susceptible (S), Intermediate (I) and Resistant (R), according to the CLSI reference criteria [18].

Table S2. Statistical analysis on the reduction of coliforms load. Results are expressed as CFU/m².

Table S3. Statistical analysis on the reduction of Staphylococcus aureus load. Results are expressed as CFU/m².

Table S4. Statistical analysis on the reduction of Clostridium difficile load. Results are expressed as CFU/m².

Table S5. Statistical analysis on the reduction of Candida albicans load. Results are expressed as CFU/m².

Author Contributions

Conceived and designed the experiments: RT PA PGB EC SM. Performed the experiments: AV RT AF EC. Analyzed the data: SM. Contributed reagents/materials/analysis tools: SM. Wrote the paper: RT AB DP EC SM.

References


