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The Sanitation of Hospital Stays: New Strategies For The Reduction of HAIs

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Key Points

- This article addresses the issue of sanitation of hospital stays and criticalities inherent in the techniques commonly used for cleaning surfaces and furnishings
- Experimental research proposes a new intervention protocol which involves the use of a sanitising probiotic product containing *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus megaterium* in the vegetative and spore form
- Compared to customary cleaning techniques, tested surfaces showed an 80% reduction of pathogenic agents with the use of the new

cleaning protocol
- This P.C.H.S. (Probiotic Cleaning Hygiene System) also offers direct economic benefits, with savings of about 5-15% compared to traditional techniques of chemical disinfection

Reseach findings are based on in vitro and in situ trials

Introduction

Sanitising procedures have the primary purpose of reducing and containing the proliferation of microorganisms existing in hospital environments.

Healthcare-associated infections (HAI) are one of the most frequent complications that can occur in healthcare facilities. 5% -15% of all hospitalised patients may develop at least one HAI during hospitalisation [1].

Three studies conducted in Italy have shown a frequency of 6.7% of the HAI [2], with a prevalence of infections of the lower respiratory tract followed by urinary tract infections. In 1998, the Italian National Health Plan identified the reduction of healthcare-associated infections as a priority [3].

One of the most controversial and debated issue is the quality and quantity role of the environmental context in the process of patient contamination, in particular the role of the confining surfaces and furniture. Indeed, it is known that these surfaces act as *reservoirs* [4] for the microorganisms, increasing the risk of cross-contamination through direct contact and / or indirect contact with the patient.

For this reason, sanitation procedures are carried out on all the furniture and objects that may interact with people.

Commonly, these techniques use chemical disinfectants, with the consequent risks for the environment and for the safety of users, and with considerable critical outcome [5].

There are several factors that determine the biocide effectiveness of a chemical disinfectant: the contact time, concentration, temperature, pH, the presence of organic material and the type of microorganism, and this is emphasised to dispel the myth that any disinfectant can be used to sanitise any surface.

Therefore, sanitation procedures carried out by the use of chemical disinfectants have several disadvantages, due to:

- The limited biocidal efficacy over time, which normally runs out within 20-30 minutes after application, with subsequent exponential growth of microbiological agents; this is also due to the fact that the action of the disinfectant determines the production of organic material decomposition, that is nutritional material, which promotes the proliferation of the microorganisms;
- The different effectiveness of the disinfectant on the basis of physical - chemical characteristics of the treated surface;
- The capacity, by microorganisms themselves, to develop continuous genetic mutation and defenses of a different kind, with the purpose to make the chemical biocidal action ineffective, with the consequent phenomena of biocide resistance, well described in literature;
- The problems of allergens and of natural environmental pollution generated by massive chemicals use that can accumulate in a persistent way in the large natural reservoirs (soil, water, air).

All this has also resulted in a process of natural selection of the microbial pathogenic strains, increasingly resistant to common disinfection techniques.

Recent experimental studies have identified the possibility of using new methods of sanitisation [6] [7], which exploit the "*principle of biological competition*," using probiotic products (PIP) - consisting of *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus pumilus* vegetative form and spore - with non-pathogenic microbial load, able to colonise the surfaces on which they are applied, counteracting the proliferation of other bacterial species based on the principle of competitive exclusion (law of Gause, 1934).

This principle lies in the fact that two different species (bacterial and / or fungal), insisting on the same ecological microcosm, cannot coexist in stable equilibrium if they refer to the same nutrient substrates, but one of them, usually the less demanding nutritional factors become dominant over the other, being able to also cause extinction.

From a microbiological point of view, for the surfaces treated with probiotic products, the existing biofilm is in fact replaced by a new type of biofilm, mainly formed by the novel microorganisms artificially placed with the cleaning products.

These procedures can then be connoted as " *bio-stabilisation techniques* " of one species over another, therefore implying not a biocidal

generalised action, if not as a final effect against specific microbial species.

The recent availability of these biostabilising products, hence used for sanitising/ sanitation of surfaces and for the resident microbial load control, suggested to conduct an extensive experimental research aimed to a qualitative and quantitative verification, both "*in vitro*" and "*on the field*", of their effectiveness over the use of traditional treatments based on chemical disinfectants.

Research Classification

The effectiveness of the procedures used was assessed by comparing the value of the potentially pathogenic bacteria load, detected on the surfaces of nosocomial environments treated with PIP products, compared to the similar charge obtained with traditional products and by calculating the consequent percentage difference.

The microorganisms investigated were those considered most attractive in terms of hospital infections: *Staphylococcus aureus*, *Pseudomonas species*, coliforms (including *Escherichia Coli*), *Candida albicans* and *Acinetobacter spp.*. Currently, further experimental investigations relating to *Clostridium spp* ., are ongoing.

The study was conducted both with *in vitro and with in situ* tests in different hospital structures.

In Vitro Trials

The purpose of the *"in vitro"* trials (UNI ISO 13697:2001) was to assess the effectiveness of competitive products PIP compared to other bacterial species in the absence of any external noise (in the laboratory), i.e. those recontamination processes of treated surfaces which naturally occur in the environment occupied by human.

In-vitro experiments were conducted by treating samples of materials found in hospital areas (i.e., ceramic, PVC, rubber, vitreous-china) with the probiotic-based solution. A solution containing a known concentration (30×10^6 cells/ml, 15 ml/m2) of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains was used for the contamination of sampling surfaces. The bacterial load was measured by the determination of colony count on RODAC plates (BD), containing TSA medium added with lecithin, histidine and Tween-20, in order to neutralise the action of disinfectants. The number of colonies was determined as total microbial count (TMC), or as specific colony count by exploiting strain-specific medium. The control plates for sterility (1 plate/lot) have been used.

After 1 hour from the application of the products PIP on sample surfaces previously polluted with various microbial strains, the reduction of the concentration of the pathogens was found to be 7 logarithms (99.999%) compared to the initial count.

In Situ Trials

Tests on real field concerned various Italian hospitals; the Sant' Anna Hospital of Ferrara and the "Arcispedale Sant'Anna" of Cona (Ferrara), the S. Giorgio Hospital of Ferrara, the Delta Hospital of Lagosanto, and the Hospital of Lokeren (Belgium).

The first experiments were conducted in 2011 in some areas of the plant care of the "Arcispedale Sant' Anna" Hospital, with the aim of , verifying the action exerted by PIP in nosocomial real conditions and then under continuous phenomena of recontamination of the treated surfaces.

That study was intentionally conducted in not newly built hospital environments and free from a filtration and air mechanical ventilation plant, in order to make the most critical processes of pollution.

Two different care areas of the S. Anna Hospital of Ferrara were then identified, the first of which consists of an area of inpatient general medicine and the second one of a clinic area.

Since both are divided into two divisions each (Hall S and Hall T in the first case and Ophthalmology/Cardiology and Orthopedics in the second case), it was possible to conduct a parallel experiment, applying the protocol involving the use of probiotics in one of the two departments and the protocol with traditional products in the other department of the same area.

The products used in the protocol were traditional chlorine-based, while the probiotic product used was the product marketed by Chrysal (Lommel, Belgium).

This allowed result comaprison of different methods of sanitation in areas (of the same area) with the same intended use, type of use and contamination characteristics.

At predetermined time intervals, the values of the bacterial pathogen of interest obtainable by the two different cleaning systems, were detected.

To verify the reproducibility of the results, it was decided to reverse the type of cleaning process across each area departments after 1 month, (as shown in Table 1), then continuing the experiments for another month.

The monitoring campaigns were conducted at regular intervals (almost every 2-3 days) both at 7:00 am, immediately after the intervention of sanitation, and at 2:00 pm.

Each sampling was performed three times, using Rodac contact plates. The samplings were conducted at different points of the concerned departments, as schematised:

- Beginning of the floor of the corridor leading to the Department;
- End of the corridor floor;
- Toilet Floor;
- Toilet Sink.

The first two points remained the same during the whole experiment, while those on the floor and the sink of the hygienic service were chosen randomly from time to time, in order to faithfully represent the average state of contamination of the entire department.

Microbiological samples for the evaluation not only of the total microbial load but also the existing initial microbial load of potential pathogens were performed in advance. This time it was referred to as time zero (T0 at 2:00 p.m.).

The trial continued with a third phase, which began the 22nd July 2011, approximately 1 month after the end of the second phase. In the latter period, which lasted up to 23rd August 2011, probiotic products PIP were employed, in both departments of inpatient medicine, in order to verify a possible further reduction of the pathogenic charge after prolonged periods of application of the PIPs.

Overall, within this first research referring to the Sant' Anna Hospital, a total of 12,528 samples were performed.

The sampling procedure of the surfaces and the microbiological analysis were performed according to the " CONTARP-INAIL Guidelines ", 2005, to the "UNI EN ISO 19698:2004" protocol and according to the practices codified in the literature [12].

The use of probiotics based protocols, called PCHS, has determined a generalised compression and stabilisation of the pathogenic charge compared to the case of the traditional procedures.

Once the values of the microbial load for each sample and for each pathogen were obtained, it was possible to calculate the average value for each phase and for each protocol sanitisation, and then the percentage reduction of the same charge in the case of use of the probiotic-based protocol with respect to the chlorine-based products (Table 2).

Experimentally, it was observed that a prolonged action of probiotics protocols (over 2 months) allows a substantial reduction/ containment/ stabilisation of potentially pathogenic microbial load compared to the case in which the environments are treated with traditional products. In many occasions the reduction values of the microorganisms of interest are close to 90%, as in the case of the sink, which represents a critical surface for the patient, for the possibility of contact with the hands and other parts of the body.

Further Development of Research Activities

Since the positive results obtained in the first phase of the research, the hypothesis of a possible relationship existing between infectious events (HAI) and environmental microbiology was tested. A second experimental research [3], based on an integrated approach between the sanitation methodology (PCHS system - Probiotic Cleaning Hygiene System) and the good hygienic practices (compliance of the hands), has shown a downward trend with a reduction of more than 60% of infectious events (HAI) over 14 months of sampling in the San Giorgio Hospital of Ferrara.

Having to logically implement an all-out policy to manage the risk of infection, the cleaning protocol was not limited to the use of a particular sanitiser (the one based on probiotics), but it has been integrated with a set of coordinate operations. These included, among other things, an adequate staff training, the use of equipment, mops and materials with high technological content, as well as a program of checks and controls to ensure the achievement of an appropriate level of hygiene of the environments.

The analysis of the above data and the availability of the results of a large number of sampling (25.748) overall conducted in different hospital facilities, actually allows a more systematic and conscious approach of the sanitation practices of hospital stays.

It was observed that, when using the PCHS system (with probiotic products) the following results are obtained:

- A compression of the charge of potentially pathogenic microorganisms by over 80% compared to the use of traditional techniques based on chemical products;
- The stabilisation of this same charge, both throughout the day, with much smaller oscillations between two successive sanitisation, and in the following months after the first application (in particular from the third month).

The trends were obtained by applying the Poisson analysis and the related confidence intervals. The top confidence interval represents the 95th percentile higher (95% of the collected data has a value that stands below this limit), while the bottom confidence interval represents the 95th percentile lower (5 % of the data has a lower value than the one indicated).

It may be noted that at month 0, corresponding to the beginning of the first application of the PCHS system, and therefore to the value of the contamination obtainable though the traditional chemical products, the charge of the microorganisms is significantly higher than in the rest of the year, with a gradual decrease that becomes quite stable from the third month, in which, evidently, the colonization of the *Bacillus spp*. becomes predominant.

The Evaluation of the Microbiological Contamination

Microbial contamination is commonly assessed using methods based on the analysis of Rodac or Petri plates (by counting the CFUs per unit area) containing selective solid medium or on the non-specific growth.

In literature, the Pitzurra I.M.S. index is a consolidated method (index of microbial surface), which represents the value of the total contamination (CFU/cm2) for the operating rooms [12].

This index is representative, however, of the state of contamination of a surface in the instants immediately following the sanitising treatment (30 minutes later), this being considered as (chemical) disinfection of the surfaces of interest, or as an indistinct reduction of the microbiological charge, referring to all microorganisms, and not only to those potentially pathogenic.

This parameter, however, does not lend itself to the evaluation of the results outlined above.
First of all, while the operating rooms are considered areas with high risk of infection, within which almost the total absence of bacteria is expected, the same cannot be said for a ward or for a Polyclinic.

Secondly, once the surfaces of an operating room have been sanitised, the room is partitioned and air-conditioned with absolute filtration. The processes of re-contamination that occur are attributable solely to the natural growth of microorganisms which may survive disinfection.

Conversely, in hospitalisations the increase of the microbial load is mainly due to the recontamination phenomena caused by the passage of people and materials and to the phenomena of gravitational sedimentation of atmospheric dust.

Thirdly, always talking about hospital stays, it is not useful to establish a maximum threshold value of contamination in the time intervals immediately following the time of cleaning, since the growth processes of micro-organisms have dynamic nature and involve an exponential increase in bacterial counts even within a few hours.

The evaluation of surface contamination using the method of the total count of microorganisms (UFC) is therefore not at all descriptive of the actual risk of acquiring infections for the patient. In the case of the use of probiotics, the microbial population that is consolidated on the surfaces sanitised with probiotic products is largely constituted by *Bacillus spp.* considered safe for human health, and only a small percentage is constituted by other bacterial species.

Therefore, it is always convenient to use the method of counting UFC/m2, per unit area (UFC/m2), but deriving it for single potentially pathogenic microorganism.

Since the bacterial load varies during time, it is also convenient that the microbiological samples may be carried out at 2:00 p.m., that is about 7 hours later from the sanitation made in the morning, and before the daily refresher process.

In fact, surveys carried out by comparing the different results for different times of the day, lead the results shown in Table 3.

In the chemical protocol, the UFC/m2 roughly double from the first moments after disinfection to about 7 hours later; with regards to treatment with the probiotic the UFC/m2 double or triple (as effect of the recontamination phenomena) from 7 hours to 24 hours after the treatment,

therefore with a kinetic significantly lower compared to the previous case.

24 hours after the probiotic cleaning intervention, the contamination with the probiotic protocol is even half or a third of the one obtained with the chemical disinfection in the instant immediately following the disinfection.
Furthermore, the amplitude of oscillation of the values in the case of probiotics is much more reduced compared to the alternative protocol, therefore this produces an effect, on the field, of *bio-stabilisation* of potential pathogens.

Percentage reduction of pathogens obtained with the use of the PCHS protocol in comparison with the use of chemical disinfectants

Sampling point	Microorganism	Reduction %
Corridor	Staphylococcus aureus	81 %
	Coliforms	79,7 %
	Pseudomonas spp.	88,4 %
	Candida spp.	68,4 %
	Acinetobacter spp.	44,7 %
Bathroom floor	Staphylococcus aureus	85,8 %
	Coliforms	78,3 %
	Pseudomonas spp.	78,5 %
	Candida spp.	71,7 %
	Acinetobacter spp.	74,2 %
Bathroom sink	Staphylococcus aureus	95,6 %
	Coliforms	85,1 %
	Pseudomonas spp.	95,1 %
	Candida spp.	94,8 %
	Acinetobacter spp.	75,6 %

Table 1.
The two main drivers, time and economy, The results obtained (25.748 microbiological samples) with the use of PCHS system are exhibited in Figures 1-5.

Proposal of New Indicators of Environmental Hygiene

It is clear that, regardless the manner with which it is carried out, hospital sanitisation is an industrial type process. Therefore it must be combined with a methodology for verification, on the field, of the results obtained, with the consequent identification of a scale of values and criteria for the acceptability of the final *outcomes*.

It is therefore considered methodologically correct to propose the introduction of a Microbiological Quality Index (IQM) for the measurement of the level of hygiene of the hospital wards, with the exception of the areas classified as "high risk" and the operating rooms.

The characteristics of the scale of measurement are as follows:

- The UFC/m2 represent the measure unit assumed as specimen;
- The detection of surface contamination is carried out through Rodac plates added with the following selective media : Baird Parker Agar (BD); Cetrimide Agar ; Sabouraud Dextrose Agar+CFL (SDA) e MacConkey Agar ;
- Plates should be leaned on the surface to be sampled ; a light pressure must be carried out for 30 sec;
- The sampling must be performed at least twice (possibly three times) and the plates , once incubated and read, must be photographed and stored prior to disposal;
- The areas sampled are patient contact areas in particular (nightstand, headboard , etc. ..), and those areas subject to treatment (inpatient floor , floor corridor of the departments in the maximum transition zones, bathroom floor and sanitary appliances);
- The UFC/m2 of the single pathogens as *Stafilococcus aureus*, P*seudomonas species*, coliforms (including *Escherichia coli*), *Candida albicans, Acinetobacter spp.*, *Clostridium spp.* should be monitored;
- The UFC/m2 of the total charge must be monitored too (this in order to identify , in case of use of the protocol with probiotics, the presence of *Bacillus spp*, with the purpose of checking the correct application of the product);
- The samples should be performed 7 hours after sanitation (2:00 p.m.), before the daily revision;
- The number of samples should allow the achievement of a statistically significant result.
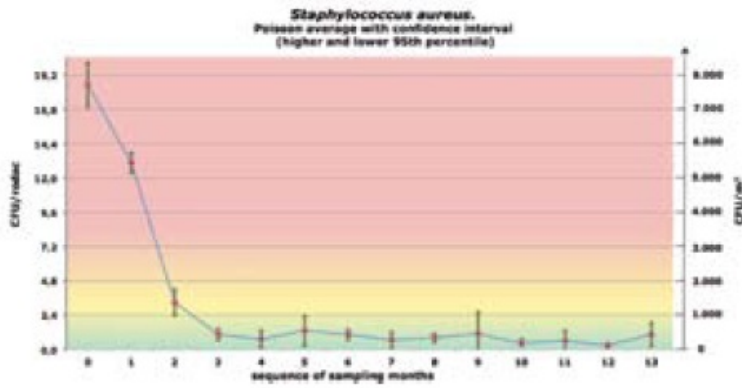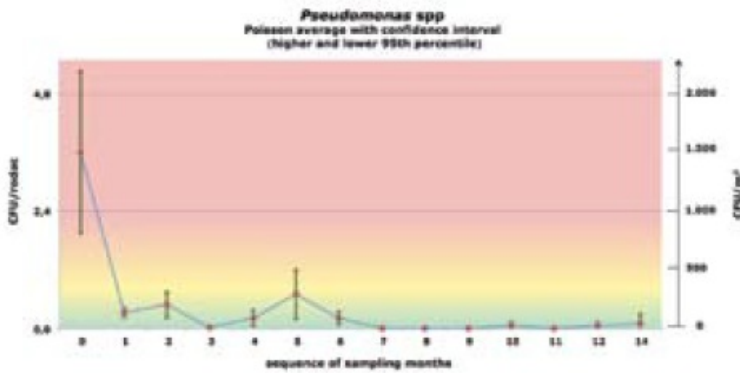
Figure 1.
Staphylococcus aureus load trend.

Figure 2.
Pseudomonas spp load trend.

Based on the results shown in the previous Figures, it was possible to identify a scale of values for the acceptability of sanitation procedures, reported in Table 4 in the case of use of probiotics.

Nothing obviously forbids the use of any alternative products to those based on probiotics, as long as they comply with the threshold values, above which the result of the sanitising treatment is judged negative.

The basic principle proposed consists in the fact that, regardless of the type of protocol chosen, a unique, shared and objective method should be used, in order to evaluate the effectiveness of the treatment, and introduce a method of measurement of the results obtained.

2 p.m.	MQI - Microbial Quality Index
S.aureus	< 1.000 UFC/m2
Pseudomonas spp.	< 500 UFC/m2
Total Coliforms	< 500 UFC/m2
Candida spp.	< 1.000 UFC/m2
Clostridium difficile	< 2.000 UFC/m2

Table 2.
IQM Acceptability scale of the Microbial Quality Index (MQI)

Safety of the Probiotics Products

The genus *Bacillus* includes gram-positive bacteria, which occur in nature in the vegetative form of spore (for this reason are defined spore-forming bacilli); they are saprophytes, widely distributed in nature (ubiquitous) and they are commonly isolated from environments such as water, soil (4), air, and decomposing plant residues.

Among the probiotic bacteria of the genus *Bacillus*, the most studied species, also found in some probiotic supplements, is the *Bacillus subtilis* [8].

Its genome was completely sequenced a decade ago, and three research studies prove its safety as a probiotic published [9-12].

Its vegetative form, with aerobic and facultative anaerobic metabolism and with low nutritional needs, is able to multiply and colonise the environment by competing with other potentially pathogenic bacteria.

The spore (Figure 6) instead allows the permanence of the microorganism in the environment during adverse conditions, maintaining the ability to germinate when favorable conditions are renewed for the vegetative form.

The beneficial effects of *B. subtilis* spores, as a probiotic preparation, are related to the balance of the intestinal microflora for the treatment or the prevention of intestinal disorders [10].

The data on infections caused by *B. subtilis* are poor, and the statistics of the World Health Organisation concerning the cause of death, do not include any.

The potential pathogenic *B. subtilis* is generally described as low or absent [9,10].

Also in the *Bacillus subtilis* genome no genes responsible for the production of toxins or other harmful substances, such as hemolysin and lecithinase, were found. In one experiment (6) these were administered for a long time to guinea pigs without side effects.

All strains of *Bacillus spp.* tested are susceptible to antibiotics [13].

The phenomenon of microbial resistance to antibiotics derives mainly from the potential gene transfer by certain bacteria, which possess these resistance genes, to pathogenic bacteria, which in turn are able to acquire or develop resistance of multiple antibiotic resistance.

In 2008, a study on antibiotic resistance of the genus Bacillus was conducted; all strains were found sensitive to all antibiotics frequently used in the medical field, as shown by the report of the European Food Safety Authority (EFSA) [14].

Several tests of acute and subchronic toxicity were carried out in animals; studies *"in vitro"* were performed on a number of species, including *B. subtilis* var. natto (5), *B. indicus* [9], *B. coagulans* (19) and *B. subtilis 2335* [10], without detecting any side effects.

Bacillus subtilis is used safely in the production of food type enzymes and, in the last decade, recombinant strains of *Bacillus subtilis* were used safely in the manufacture of a variety of edible bio-industrial products, such as enzymes, vitamins, antibiotics, biopolymers, additives for the production of certain foods such as miso in Japan (from *B. subtilis* var. natto).

The enzymes derived from *B. subtilis* are alpha-acetolactate decarboxylase, alpha-amylase, beta-glucanase, glutaminase, maltogenic amylase, pullulanase, protease and xylanase.

The B. subtilis is classified as Class 1 (no risk) from the National Institute of Health (NIH - U.S.). [15]; it is not toxigenic according to the criteria of the U.S. Environmental Protection Agency (EPA) and it is one of 10 host organisms in Tier I that qualify for an exemption under the EPA regulations concerning the classification of risk .

In addition, the *B. subtilis* is used as a soil inoculant in agriculture and horticulture.

Some enzymes produced by *B. subtilis* are widely used as additives with biological activity of soak cleaning in laundry detergents.

A remarkable range of fermented foods are also obtained from the proteolytic and enzymatic activity of the *Bacillus subtilis*.

The *B. subtilis strain QST 713* (marketed as QST 713 or *Serenade*) has natural fungicidal activity, and is used as a biological control agent [19].

The *Bacillus spp.* based products were popular all over the world before the introduction of antibiotics as subtilic vaccines, that is as immunostimulating agents to help the treatment of diseases of the gastrointestinal tract and the urinary tract.

In conclusion it can be stated that the bacteria of the genus *Bacillus*, as considered safe, are used in agriculture, [19, 20], horticulture, in human nutrition [21] and in veterinary medicine [22, 24].
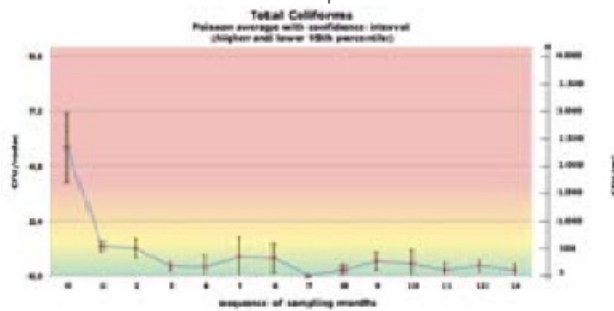
Several *Bacillus* species have been classified GRAS (Generally Regarded As Safe), as used in food processes or in pharmaceutical preparations, and therefore recognised by the FDA (Food and Drug Administration) as treatments for human purposes without side effects [16, 25, 27].

They do not induce the formation of pathogenic bacteria, they are biodegradable and environmentally safe.[h1]

Conclusion

The use of the PCHS system, based on probiotics in sanitising procedures of hospital stays , was found to be a technique of great interest, able to reduce by approximately 80% and beyond the levels of potentially pathogenic bacterial load , regardless of the surfaces sanitised. However, a proper cleaning system of hospital stays is not only centered on the specific agent or product used , but on an integrated set of operations and cross-checks which can ensure health departments in terms of effectiveness of the overall result and the valorisation and quantification of the

result.

Figure 3.
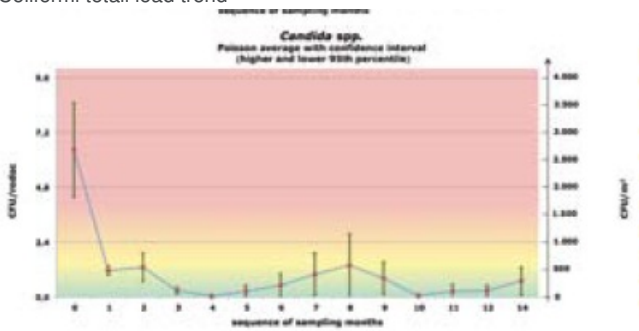Coliformi totali load trend

Figure 4.
Candida spp. load trend. From the use of traditional chemical products (month 0) to the use of the PCHS system, the microorganism load progressively decreases, with a consequent decrease of the risk of infection.
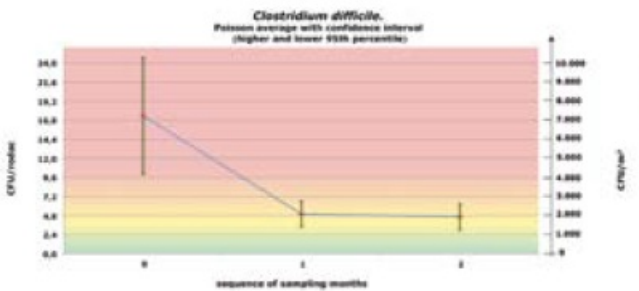

Figure5.
Clostridium difficile load trend.

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