Sabouraud Dextrose Agar (SDA) - CAF + Neutralizing contact plates:

selective medium for yeasts (*Candida species)* and moulds (e.g. *Aspergillus species*) isolation with inactivation of disinfectants.

Composition:

Ingredients	Grams/Litre
Glucose / Dextrose	40.0
Meat Extract	10.0
Cloramfenicolo (CAF)	0.05
Agar	15.0
Final pH 5.6 ± 0.2	

Sabouraud Dextrose Agar è impiegato per l'isolamento dei lieviti patogeni, *Candida albicans*, e dei funghi patogeni opportunisti (*Aspergillus species*).

I lieviti possono essere identificati con test biochimici.

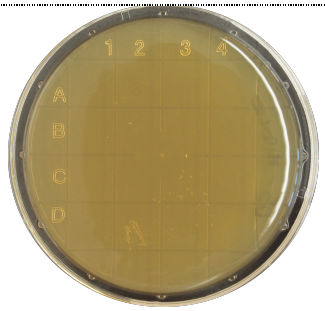


Figure 46:

SDA before sampling

Figure 47: SDA: *Candida species* colonies after sampling and incubation.

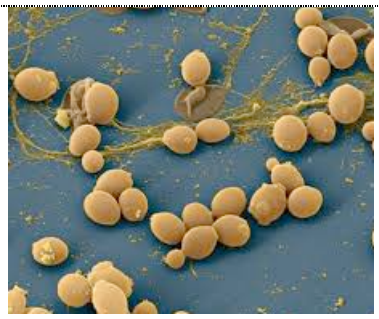


Figure 48: Coloured scanning electron micrograph (SEM). (Source: http://sulamed.info/article/candidosi-acido-caprilico)

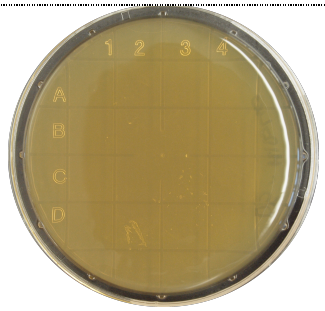


Figure 49:

SDA before sampling

Figure 50: SDA: *Aspergillus species* colonies after sampling and incubation.

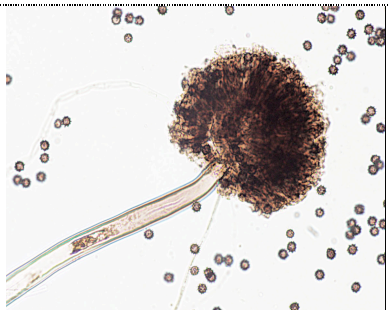


Figure 51: Coloured scanning electron micrograph (SEM). (Source: https://en.wikipedia.org/wiki/Aspergillus_awamori)

Clostridium difficile Selective Agar:

selective medium for *Clostridium difficile*

Composition:

Ingredients	Grams/Litre
Proteose Peptone	40.0
Disodium hydrogen phosphate	5.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Fructose	6.0
D-cycloserine	250.0 mg/ litre
Cefoxitin	8.0 mg/ litre
Agar	15.0
Final pH 7.4 ± 0.2	

Anaerobic Equipment:

anaerobe atmosphere generation bags, anaerobe indicator test and anaerobic jar.

For the detection of Clostridium difficile is used Latex test (Liofilchem).

Clostridium are relatively large, Gram-positive , rod-shaped bacteria that can undergo only anaerobic metabolism. Most Clostridium cannot grow aerobic conditions and can even be killed by exposure to O_2; however, they form endospores that are able to survive long periods of exposure to air and other adverse environmental conditions.

Figure 51: Clostridium difficile Agar before sampling

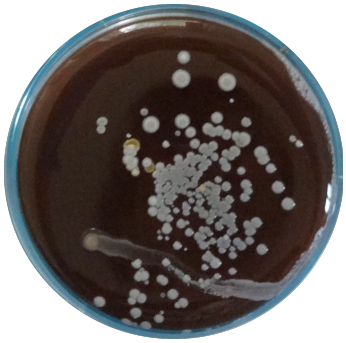


Figure 52: Clostridium difficile Agar: *Clostridium species* colonies after sampling and anaerobic incubation.
For the generation of a CO_2 rich atmosfere Carbon dioxide generating system (Oxoid)

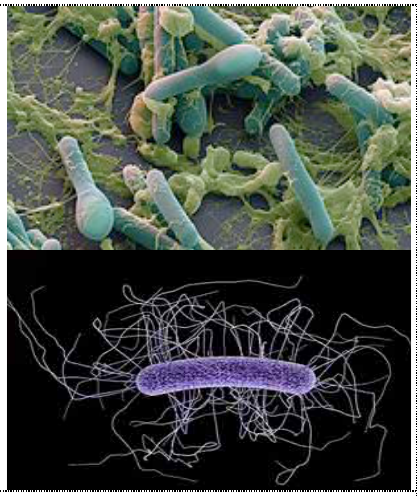


Figure 53: Coloured scanning electron micrograph (SEM).
(Source: Clostridium spore capsulated (up)– vegetative form motile anaerobic (down) Oxidase negative Catalase negative Hemlysis negative)

Figure 54: Reagents Latex test (Source: http://www.liofilchem.net/it/)

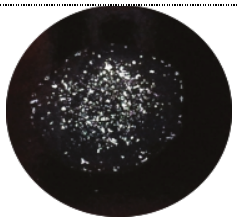


Figure 55: Result positive Latex Agglutination Test for conforming *Clostridium*

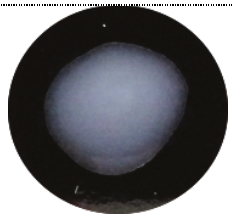


Figure 56: Result negative Latex Agglutination Test

Herellea Agar Contact Plate (Lickson):

Selective medium for gram-negatives isolation Acinetobacter species.

Composition:

Ingredients	Grams/Litre
Triptone	15.0
Peptone di soia	5.0
Sodio cloruro	5.0
Lattosio	10.0
Maltosio	10.0
Sali biliari n.3	1.25
Porpora di bromocresolo	0.02
Agar	15.0
Final pH 6.8 ± 0.2	

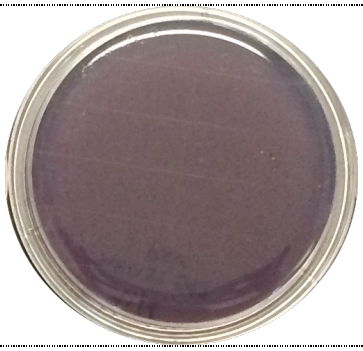


Figure 57: Herella Agar before sampling

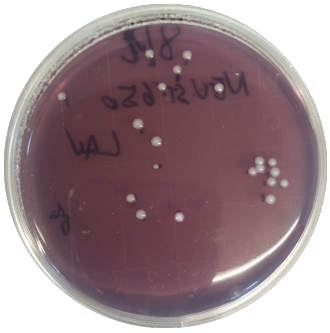


Figure 58: Herella Agar: *Acinetobacter species* colonies after sampling and incubation.

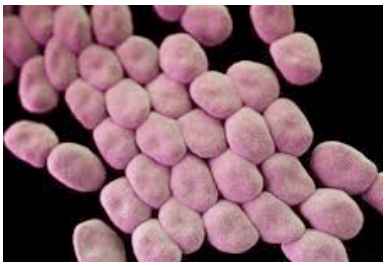


Figure 59: Acinetobacter Gram negative coccobacilli strictly aerobic Nonmotile Catalase + Oxidase (Source: http://www.cidrap.umn.edu/news-perspective/2016/11/detection-multidrug-resistant-pathogens-europe)

Herellea Agar according to the formula of Mandel, Wright and Mc Kinnon, is a differential medium suitable for the isolation of negative Grams bacteria and for the differentiation of fermenters from non fermenters. It is indicated for the isolation of *Acinetobacter species*: two carbohydrates (lactose and maltose) and a pH indicator (purple cresol bromide) are present in the formula, which is yellow when there is acidification of the medium.

Acinetobacter is not fermenting and gives colonies of the same color like medium, sometimes leaking towards a more intense color of the medium, while fermenting enterobacteria give yellow colonies surrounded by a yellow sun. Gram positive bacteria are inhibited by bile salts n. 3.

Microbial Typing

Identification system is a standardized identification system which uses 23 miniaturized biochemical tests (strip) and a data base for the microbial code.
The microbial identification uses this strip, that consists of a microbute containing 23 dehydrated substrates. These tests are inoculated with a bacterial suspension which reconstitutes the media. During incubation at 36°C, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents.
The reaction are read and the identification is obtained by referring to interpretation table, the Analytical Profile Index.

For *Enterobacteriacea* API 20 E (bioMérieux)
For *Enterobacteriacea* ENTEROTUBE II (Liofilchem)
For *Pseudomonas species* OSI/FERM TUBE (Liofilchem)
For *Staphylococcus species* API STAPH (bioMérieux)
For *Candida species* API 20 AUX (bioMérieux)

Test Antibiogram (ABG)

Kirby –Bauer Method is used to check the antibiotical susceptibility (defined Antimicrobial Susceptibility Testing o AST) of probiotic-cleaning's *Bacillus* isolates from test surfaces.
Kirby –Bauer Method is based on agar-diffusion technique [135].
The effectiveness of an antibiotic in sensitivity testing is based on the size of the zone of inhibition that surrounds a disk that has been impregnated with a specific concentration of the antibiotic.
The zone of inhibition varies with the diffusibility of the antibiotic and the value of the diameter is evaluated by Clinical and Laboratory Standard Institute (CLSI) reference criteria [136].

The disk diffusion susceptibility method is performed by applying a bacterial inoculum of approximately 1.0×10^6 CFU/mL to the surface of a large (90 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared antibiotic disks with known antibiotic concentration are placed on the inoculated agar surface (Figure 60). Plates are incubated for 24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the antibiotic through the agar medium. The zone diameters of each antibiotic are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS).

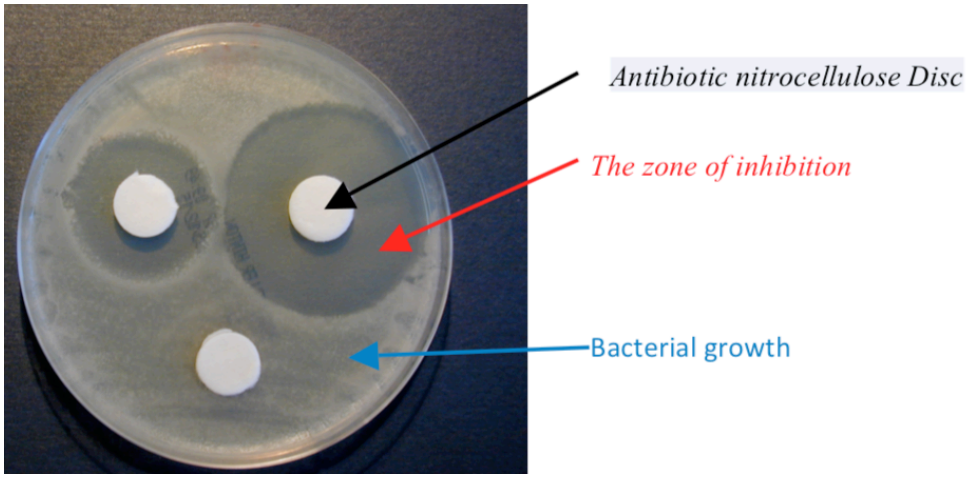


Figure 60: Results after incubation on Mueller-Hinton agar plate.
It is evident the two zones of inhibition related to the diffusion rate of the antibiotic.

The AST is defined internationally by the National Committee for Clinical Laboratory Standards (NCCLS) and the interpretation of results includes three levels: resistance (R), susceptibility (S), or intermediate susceptibility (I) of the test microorganism against the each antibiotic.

The nitrocellulose disks imbibed with a known concentration of antibiotics (Oxoid) shown in the following table:

Table 5: Interpretation: According to performance standards for antimicrobial susceptibility testing the zone diameters in mm:

Antimicrobial agent	Potency	Resistent (R)	Intermediate (I)	Susceptible (S)
Penicillin	P 10U	≤28		≥29
Cephalothin	CF 30µg	≤14	15÷17	≥18
Cefoperazone	CFP 30µg	≤15	16÷20	≥21
Netilmicin	NET 10µg	≤12	13÷14	≥15
Gentamicin	G 10µg	≤12	13÷14	≥15
Clindamycin	CC 2µg	≤14	15÷20	≥21
Erythromycin	E 15µg	≤13	14÷22	≥23
Nalidoxic Acid	NA 30µg	≤13	14÷18	≥19
Chloramphenicol	C 30µg	≤12	13÷17	≥18

References: The parzial table is obtained from document M100-S20 (M02-A10) Clinical and laboratory Standard Institute. (Source: Oxoid http://www.oxoid.com/pdf/uk/2013-CLSIFDA-table-update.pdf)

''in vitro'' trials

The bacterial reduction *''in vitro''* effectiveness of probiotic cleaning is estimated with following preliminary test:

according to European Standard EN 13697 [XI] to test, 0,5 ml of bacterial suspension was added in 4,5 ml of 1% probiotic cleaning solution (1/100) at room temperature. Later, 200 µl of this solution are captured at different intervals (7 hours), and added in 2 ml of a diluent, made up of Lecithin, Histidine and Tween. At last, 1 ml of these last solutions was transferred on a Petri dishes with TSA agar in order to record the minimum time of exposure after which no growth occurred.

The inoculated plates are incubated at 36° C for 24 hours.

The test is repeated with 3% Bovine Fetal Serum and once with 0.3% of Albumin added to the probiotic solution. Total Vital Count (TVC) was simultaneously measured with inoculums of 1 ml broth at 36° C.

Microorganisms for antibacterial testing:

- Bacillus species spore –forming isolated from probiotic cleaning products;
- Strains pathogens as follow:

Table 6: ATCC strains

ATCC microbial strain		Microbial group	Batch / Expiration date	Concentration:
Staphylococcus aureus	ATCC* 6538	G + bacteria	Lotto 485-307-1 Exp. 02/2018	$6.4x10^6$ cfu/g
Enterococcus hirae	ATCC* 8043	G + bacteria	Lotto 491-403-1 Exp. 02/2018	$5.0x10^6$ cfu/g
Pseudomonas aeruginosa	ATCC* 9027	G - bacteria	Lotto 484-715-1 Exp. 04/2018	$6.6x10^6$ cfu/g
Escherichia coli	ATCC* 8739	G - bacteria	Lotto 483-582-1 Exp. 07/2018	$4.7x10^6$ cfu/g
Candida albicans	ATCC*10231	Lievito / Yeast	Lotto 392-505-1 Exp. 01/2018	$6.9x10^5$ cfu/g
Aspergillus brasiliensis	ATCC*16404	Muffa/ *Molds*	Lotto 392-505-1 Exp. 02/2018	$6.0x10^5$ cfu/g

**ATCC (American Type Collection Control)* (Microbiologics:Biolife):

They are represented the test specimens ‘*‘in vitro”* in order to evaluation the effectiveness of inhibition by competition exclusion of probiotic cleaning

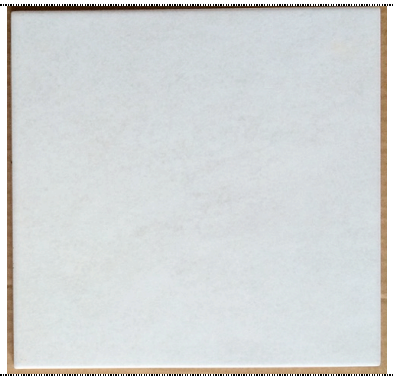


Figure 61: Porcelain stoneware tile specimen (e.g. floor)

Figure 62: Stoneware tile specimen (e.g. floor)

Figure 63: rubber specimen specimen (e.g. floor)

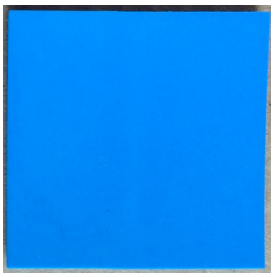


Figure 64: Plastic (ABS) specimen (e.g. headboard and footboard bed)

Figure 65: PVC (e.g. bedside table)

Test specimens *‘in vitro’*.

Calculation microbial reduction:

Red= (N x 10^{-1}) / N_a

Where:

Red = reduction of vitality
N = bacterial test suspension count
N_a = test bacterial count after the contact time

Field trials: Microbiological surfaces sampling in patient rooms

The microbiological sampling of the surfaces are performed for a period of 30 mouths in several occupied patient rooms (see Table 3 expecially Medicine Department and Table 4 for critical point) with the hygienic services (bathroom). The rooms are identical as far as the layout and the furnishings are concerned. A specific protocol of cleansing was associated to one room for each Unit.

Sanitation procedures are carried out by using microfiber mops and cleaning cloth colour-coded according to the type of target surface. The wet cleaning is a phase with aqueous solutions of either the probiotic-based or the chemical-based solutions. Both solutions are fresh prepared before each use. The microfiber cloths are soaked into the solution and stored inside clean containers until use. Mopping phases are performed by the same trained operator in order to exclude or minimize the introduction of potential variables in the implementation of procedures. Floors are treated with a concentration range of 14.44 to 17.33 g of solution per square meter (ppm/m^2). Hand/body-touched surfaces such as doorknobs, bed frames, tables and chairs or sink, toilet and other bathroom fixtures, are treated with 2 g of solution per room or per bathroom (ppm/m^2), respectively. Each measure is done in duplicate or in triplicate.

The probiotic-based sanitation solution contained 1% spores (30 x 10^6 CFU/mL) of probiotic bacteria (ATCC *Bacillus species*) added with ionic surfactants (0.6%), anionic surfactants (0.8%) and enzyme (0.02%) (FloorCleaner PIP-60160; InteriorCleaner PIP-60140 and SanitaryCleaner PIP-60150).

In order to estimate the ability of disinfectants to remove microrganisms compared to probiotic cleaning from the surfaces, the superficial microbiological load was measured at:

- After 7 hours the chemical cleaning procedures, *Microbial Level* (T_0);
- After 7 hours the probiotic cleaning procedure, *Microbial Level* ($T_{1;}\ T_{2;}\ T_{3;}\ T_{1+n}$).

18232 samples (microbiological samplings) are performed following MEM methodology, tested using Rodac plates (55mm ϕ) containing TSA medium added with Lecitina, Istidina and Tween and for detection of pathogenic strains (BPA, MAC, Cetrimide, Herella, SDA and Clostridium Agar). The plates are placed against the surface to sample, applying a light pressure for 30 seconds. All the sample plates and the control plate are incubated at 36°C for 48 h and, then, 25 °C for another 24 h.

The TMC was recorded in cfu/100cm^2. Subsequently, the percentage of microbial load reduction is calculated [--].

Only 13003 are samples obtained under the protocol H7, that they have been subjected to this elaboration with the application of the protocol 7/7 days.

Finally, the microbiological identification is carried out. The microrganisms are isolated after the growing on the plates and are initially identified through the Gram stain.

Then the microrganisms are coated with a selective medium, and identified using biochemical tests (API – bioMerieux and Enterotube –Liofilchem). 270 identifications are carried out.

The surfaces points that are controlled in this study are are given in Table 4.

Ethics Statement

The trials in the all Hospitals and RSAs are approved by the Ethics Committee which has given its consent and stated 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.

This experimental study initially included the use of probiotics skin-care formulations, which for the non-consensus of the Ethics Committee were not used by the patients.
The aim was that probiotic cosmetics could restore the skin microbioma, counteracting any potential pathogens [109].
Indeed many potentially pathogenic bacteria living as commensals in the human skin microbiome.

Patient-to-patient transmission of MRSA within healthcare settings primarily occurs via carriage on the hands of healthcare workers [137].

The hygiene of the skin and body with probiotic cosmetics can be able to reduce the pathogenic microbial load on contaminated hand skin, as a strategy for preventing the spread of methicillin-resistant Staphylococcus aureus (MRSA) from hands skin of the patients [138]

5. Results:

'in vitro' trials

Table 7: The effectiveness of laboratory probiotic cleaning /sanitation procedure in removing microbial loads on inanimate surfaces (product Floor probiotic cleaning) :

Organisms tests	***Staphylococcus aureus Enterococcus hirae***		***Escherichia Coli Pseudomonas aeruginosa***		***Candida albicans Aspergillus brasiliensis***		***Mean % reduction (pre-postcleaning)***
	cfu/100cm^2						
Materials kind	**T_0** Bacterial inoculum	**T_1**	**T_0** Bacterial inoculum	**T_1**	**T_0** Bacterial inoculum	**T_1**	
1. Porcelain stoneware	1,0x10^6	8,0x10^4	1,2x10^6	7,0x10^4	5,5x10^5	2,5x10^4	93,87%
2. Stoneware	1,0x10^6	1,8x10^4	1,2x10^6	2,5x10^4	5,5x10^5	2,8x10^4	97,01%
3. Rubber	1,0x10^6	1,0x10^5	1,2x10^6	9,8x10^3	5,5x10^5	1,0x10^5	90,33%
4. Plastic	1,0x10^6	5,0x10^3	1,2x10^6	5,6x10^3	5,5x10^5	1,0x10^4	99,07%
5. PVC	1,0x10^6	3,0x10^4	1,2x10^6	2,9x10^4	5,5x10^5	7,0x10^4	93,95%

Table 8: The effectiveness of laboratory probiotic cleaning /sanitation procedure in removing microbial loads on inanimate surfaces (product Floor probiotic cleaning) :

Materials kind	***Strain G+ Staphylococcus***	***Strain G- Enterobatteri***	***Fungi Group Candida / Aspergillus***
1. Porcelain stoneware	92,00%	94,17%	95,45%
2. Stoneware	98,20%	97,92%	94,91%
3. Rubber	90,00%	99,18%	81,82%
4. Plastic	99,50%	99,53%	98,18%
5. PVC	97,00%	97,58%	87,27%

Explanation of results:

The results obtained *'in vitro'* indicated that the probiotic-based product results in a significant lowering of specific bacterial load in a contaminated-controlled conditions. The different types of material did not show differences in efficacy against ATCC pathogens.

The percentage of inhibition of microbial patogens growth is between 81,8% and 95,45%.

Field trials:

Interpretation of environmental surface monitoring

Elaboration esperimental data: Elaboration microbiological data by mathematic method.

The Elaboration of the esperimental data includes the anlisys of 32058 samples on Excel file.

Each sample included:

- Time and date of the sample
- Hospital or nursing home
- Sampling point
- Sanitized material (surface)
- Department of the sampling point
- Microorganism that has been sampled
- Colony forming units - CFUs (if in the same sample more than one microorganism was considered, these may appear on different lines or on different columns in the same line)

Each measure for sample has been repeated 2 or 3 times (respectively contact plate in duplicate or triplicate) depending on the case, with no specific rule.

The main objectives of this analysis are:

1. Extracting the temporal series that represents the evolution of the number of CFUs of each single sampling point in time
2. Designing a classification algorithm for a single series (classified YES/NO – that is, classified as witnessing the effectiveness of microbial inhibition, reduction or compression, or not)
3. Performing simple descriptive statistics of the set of series (general evaluation of data, strict eclassification of 60-30-2 series and loose classification of 90-10-5 series).

Strict classification: a series is classified 'YES' if and only if we measure at least 90 percent of decrese of CFUs from initial count of at least 2 units and with no more than 10 percent of outlier observations

Loose classification: a series is classified 'YES' if and only if we measure at least 60 percent of decrese of CFUs from initial count of at least 5 units and with no more than 30 percent of outlier observations

18232 samples (microbiological samplings) are performed following MEM methodology, tested using Rodac plates, but only 13003 are samples obtained under the protocol H7, that they have been subjected to this elaboration with the application of the protocol 7/7 days.

Table 9.1: Generic number total indipendet of Hospitals and RSAs	
1(Yes)	0(No)
996	303

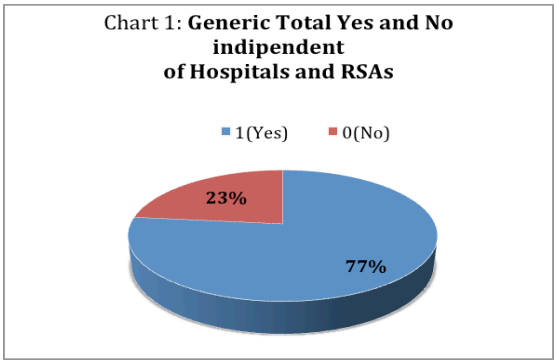


Table 9.2 (60_30_2): Generic number total indipendet of Hospitals and RSAs	
1(Yes)	0(No)
1219	80

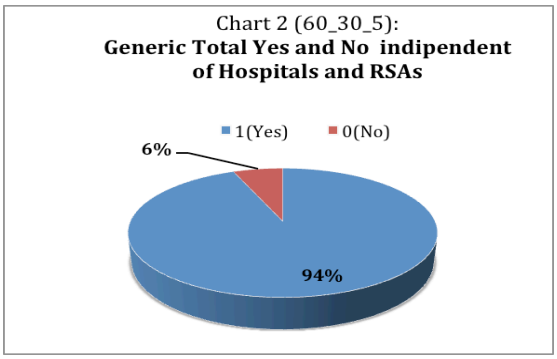
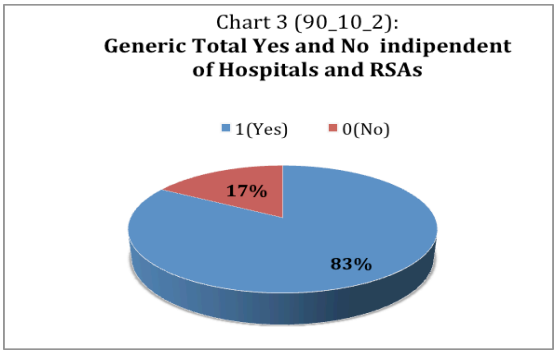


Table 9.3 (90_10_2): Generic number total indipendet of Hospitals and RSAs	
1(Yes)	0(No)
1082	217



Explanation of results of the Table 9.1, 9.2 and 9.3:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results generic total (all results examined) are independent of the structure type (Hospital or RSA).

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

Table 9.4: Total of Material kind	
308	stoneware tiles floor
146	marble floor
21	rubber floor
238	plastic (bedside table hand touch)
219	metal / steel (hand touch)
48	PVC (hand touch)
286	vitreous china (washbasin)

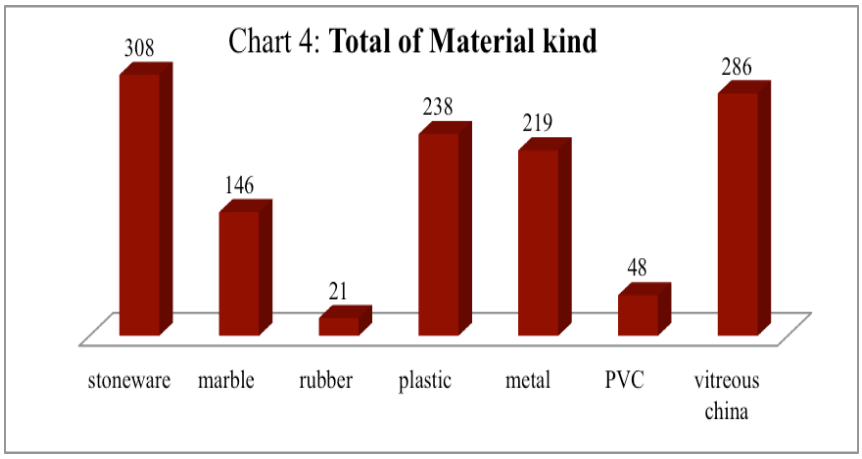


Table 9.5 (60_30_5): Total of Material kind		
280	28	stoneware tiles floor
138	8	marble floor
17	4	rubber floor
232	6	plastic (bedside table hand touch)
213	6	metal / steel (hand touch)
43	5	PVC (hand touch)
265	21	vitreous china (washbasin)

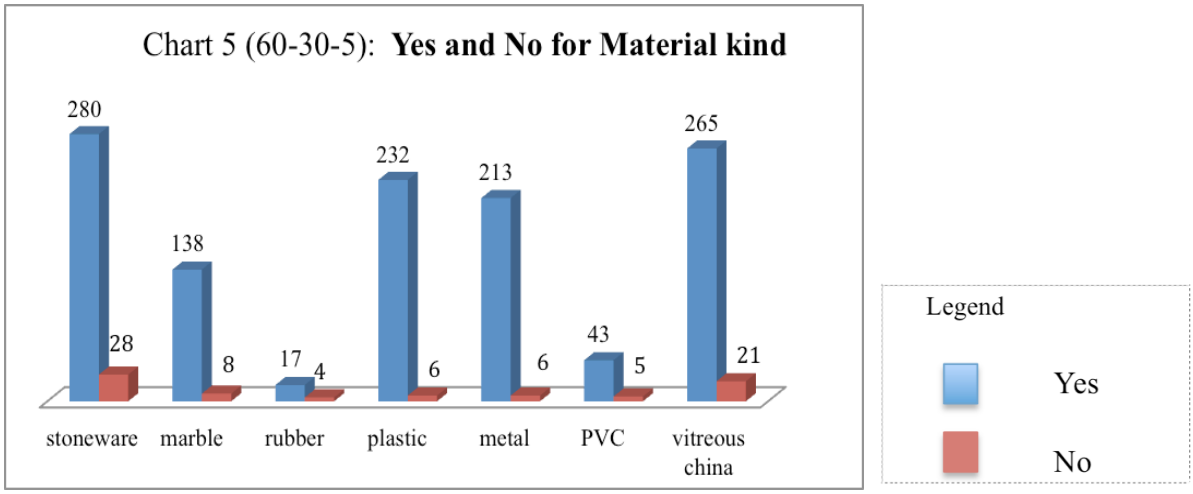
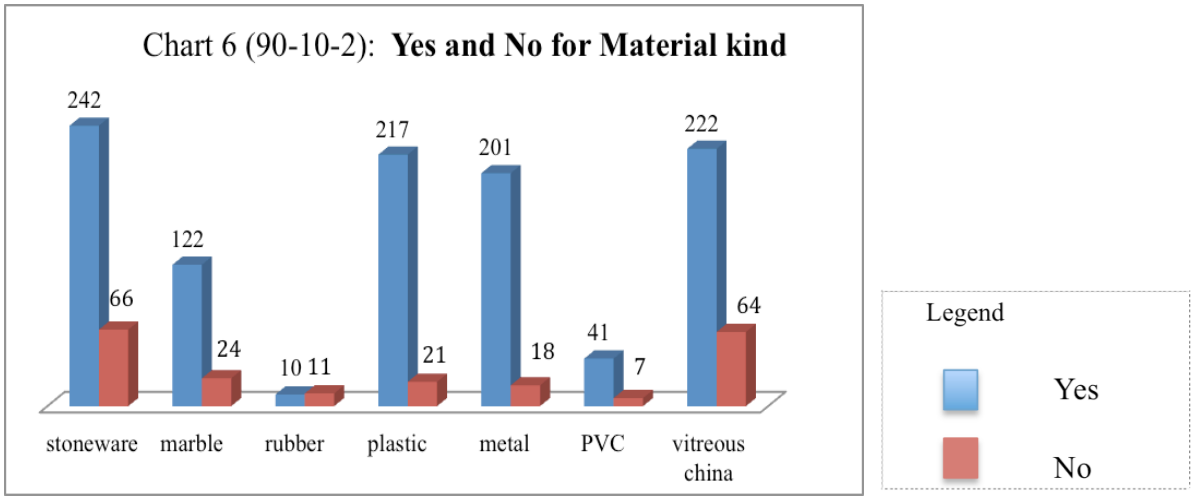


Chart 5

Table 9.6 (90_10_2): Total of Material kind		
242	66	stoneware tiles floor
122	24	marble floor
10	11	rubber floor
217	21	plastic (bedside table hand touch)
201	18	metal / steel (hand touch)
41	7	PVC (hand touch)
222	64	vitreous china (washbasin)



Explanation of results of the Table 9.4, 9.5 and 9.6:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained in relation to the type of material are independent of the test material.
Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).
The effectiveness of the rubber is low compared to other types of materials, while is evident especially high efficacy has in stoneware.

Table 9.7: Total Hospitals and RSAs	
North Hospital 1	66
North Hospital 2	20
North Hospital 3	60
North Hospital 4	90
North Hospital 5	86
North Hospital 6	12
North Hospital 7	264
North Hospital 8	76
North Hospital 9	48
North Hospital 10	64
North Hospital 11	117
North RSA 1	44
North RSA 2	56
North RSA 3	41
North RSA 4	43
North RSA 5	16
North RSA 6	75
South Hospital 1	33
South Hospital 2	72
South RSA 1	16

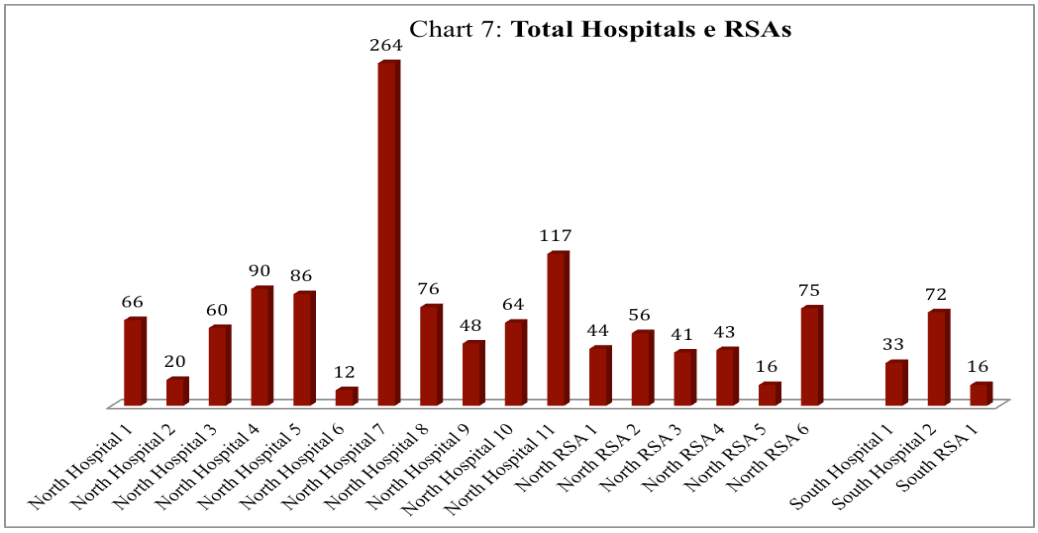


Table 9.8 (60_30_5): Total Hospitals and RSAs		
North Hospital 1	60	6
North Hospital 2	18	2
North Hospital 3	59	1
North Hospital 4	89	1
North Hospital 5	68	18
North Hospital 6	8	4
North Hospital 7	258	6
North Hospital 8	72	4
North Hospital 9	46	2
North Hospital 10	60	4
North Hospital 11	112	5
North RSA 1	43	1
North RSA 2	53	3
North RSA 3	39	2
North RSA 4	41	2
North RSA 5	12	4
North RSA 6	71	4
South Hospital 1	31	2
South Hospital 2	64	8
South RSA 1	15	1

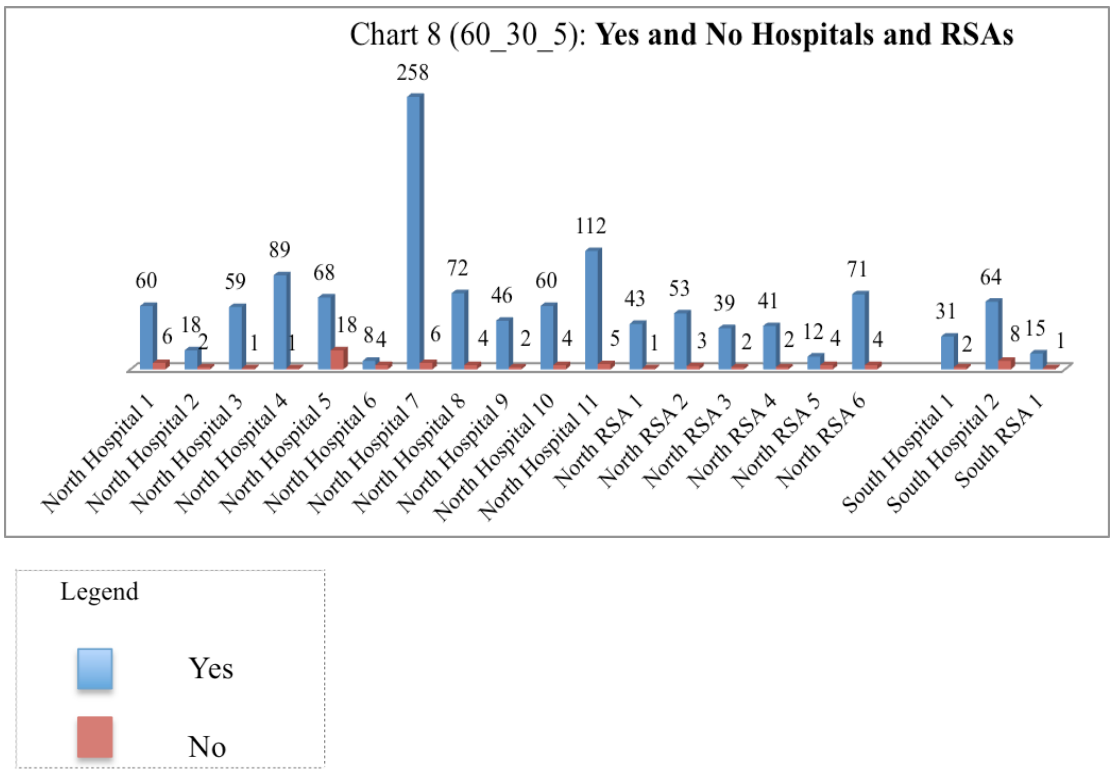


Table 9.9 (90_10_2): Total Hospitals and RSAs		
North Hospital 1	58	8
North Hospital 2	14	6
North Hospital 3	47	13
North Hospital 4	72	18
North Hospital 5	43	35
North Hospital 6	8	4
North Hospital 7	244	20
North Hospital 8	72	4
North Hospital 9	44	4
North Hospital 10	56	8
North Hospital 11	108	9
North RSA 1	37	7
North RSA 2	46	10
North RSA 3	29	12
North RSA 4	37	6
North RSA 5	12	4
North RSA 6	60	15
South Hospital 1	29	4
South Hospital 2	55	17
South RSA 1	11	5

Explanation of results of the Table 9.7, 9.8 and 9.9:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained in relation to both Hospital and RSA:

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

It is evident especially high efficacy has in North Hospital 7, because It is new hospital not yet microbiologically colonized and constructed using materials such as epoxy (e.g. epoxy flooring).

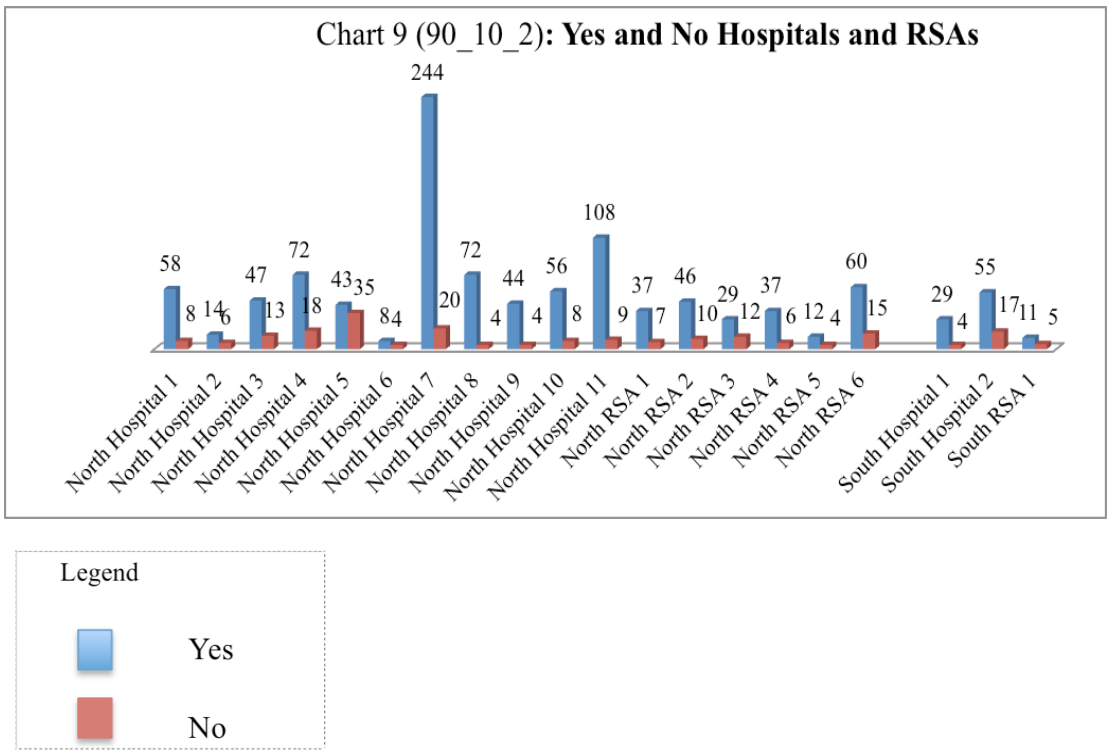


Table 9.10: Total Typing microrganisms	
75	***Staphylococcus aureus*** **(coagulase +) (Alert pathogen)**
39	*Staphylococcus coagulase* + (typing St. epidermidis)
31	*Staphylococcus epidermidis*
11	*Staphylococcus hominis*
9	*Staphylococcus xylosus*
140	*Staphylococcus spp coagulase* - (CoNS)
27	*Micrococcus spp*
209	*Enterobatteri*
54	***Escherichia coli*** **(Alert pathogen)**
3	*Enterobacter cloacae*
3	*Enterobacter gergoviae*
88	*Klebsiella spp*
3	*Klebsiella oxytoca*
18	***Klebsiella pneumoniae*** **(Alert pathogen)**
11	*Serratia spp*
3	*Serratia marcescens*
11	*Citrobacter*
12	*Proteus spp*
7	*Proteus mirabilis*
3	*Providencia stuartii*
3	*Yersinia entercolitica*
128	*Pseudomonas spp*
8	***Pseudomonas aeruginosa*** **(Alert pathogen)**
4	*Pseudomonas fluorescens*
1	*Pseudomonas putida*
27	*Pseudomonas stutzeri*
83	*Acinetobacter spp.*
121	*Candida spp*
17	***Candida albicans*** **(Alert pathogen)**
7	*Candida krusei*
3	*Candida tropicalis*
3	*Rhodotorula rubra*
6	*Saccharomyces cerevisae*
34	*Muffe not typing*
42	*Penicillium spp*
28	***Aspergillus spp*** **(Alert pathogen)**
27	***Clostridium difficile*** **(Alert pathogen)**

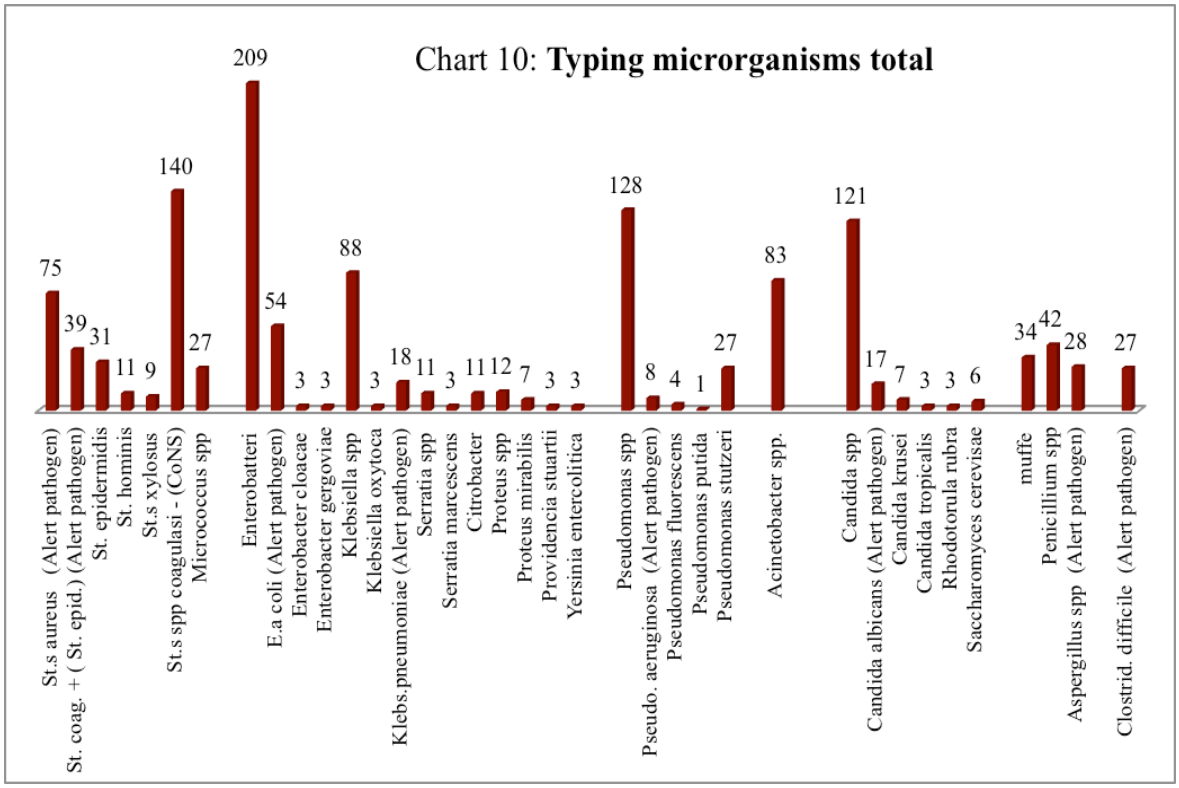


Chart 10: Typing microrganisms total
75
39
31
11
9
140
27
209
54
3
3
88
3
18
11
3
11
12
7
3
3
128
8
4
1
27
83
121
17
7
3
3
6
34
42
28
27
St.s aureus (Alert pathogen)
St. coag. + (St. epid.) (Alert pathogen)
St. epidermidis
St. hominis
St.s xylosus
St.s spp coagulasi - (CoNS)
Micrococcus spp
Enterobatteri
E.a coli (Alert pathogen)
Enterobacter cloacae
Enterobacter gergoviae
Klebsiella spp
Klebsiella oxytoca
Klebs.pneumoniae (Alert pathogen)
Serratia spp
Serratia marcescens
Citrobacter
Proteus spp
Proteus mirabilis
Providencia stuartii
Yersinia entercolitica
Pseudomonas spp
Pseudo. aeruginosa (Alert pathogen)
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Acinetobacter spp.
Candida spp
Candida albicans (Alert pathogen)
Candida krusei
Candida tropicalis
Rhodotorula rubra
Saccharomyces cerevisae
muffe
Penicillium spp
Aspergillus spp (Alert pathogen)
Clostrid. difficile (Alert pathogen)

Table 9.11 (60_30_5): Yea and No Typing microrganisms		
75	0	***Staphylococcus aureus*** **(coagulase +) (Alert pathogen)**
27	12	*Staphylococcus coagulase +* (typing St. epidermidis)
27	4	*Staphylococcus epidermidis*
11	0	*Staphylococcus hominis*
9	0	*Staphylococcus xylosus*
87	53	*Staphylococcus spp coagulase -* (CoNS)
27	0	*Micrococcus spp*
203	6	*Enterobatteri*
54	0	***Escherichia coli*** **(Alert pathogen)**
3	0	*Enterobacter cloacae*
3	0	*Enterobacter gergoviae*
88	0	*Klebsiella spp*
3	0	*Klebsiella oxytoca*
18	0	***Klebsiella pneumoniae*** **(Alert pathogen)**
11	0	*Serratia spp*
3	0	*Serratia marcescens*
11	0	*Citrobacter*
12	0	*Proteus spp*
7	0	*Proteus mirabilis*
3	0	*Providencia stuartii*
3	0	*Yersinia entercolitica*
128	0	*Pseudomonas spp*
8	0	***Pseudomonas aeruginosa*** **(Alert pathogen)**
4	0	*Pseudomonas fluorescens*
1	0	*Pseudomonas putida*
27	0	*Pseudomonas stutzeri*
83	0	*Acinetobacter spp.*
119	2	*Candida spp*
16	1	***Candida albicans*** **(Alert pathogen)**
7	0	*Candida krusei*
3	0	*Candida tropicalis*
3	0	*Rhodotorula rubra*
6	0	*Saccharomyces cerevisae*
33	1	*Muffe not typing*
42	0	*Penicillium spp*
28	0	***Aspergillus spp*** **(Alert pathogen)**
26	1	***Clostridium difficile*** **(Alert pathogen)**

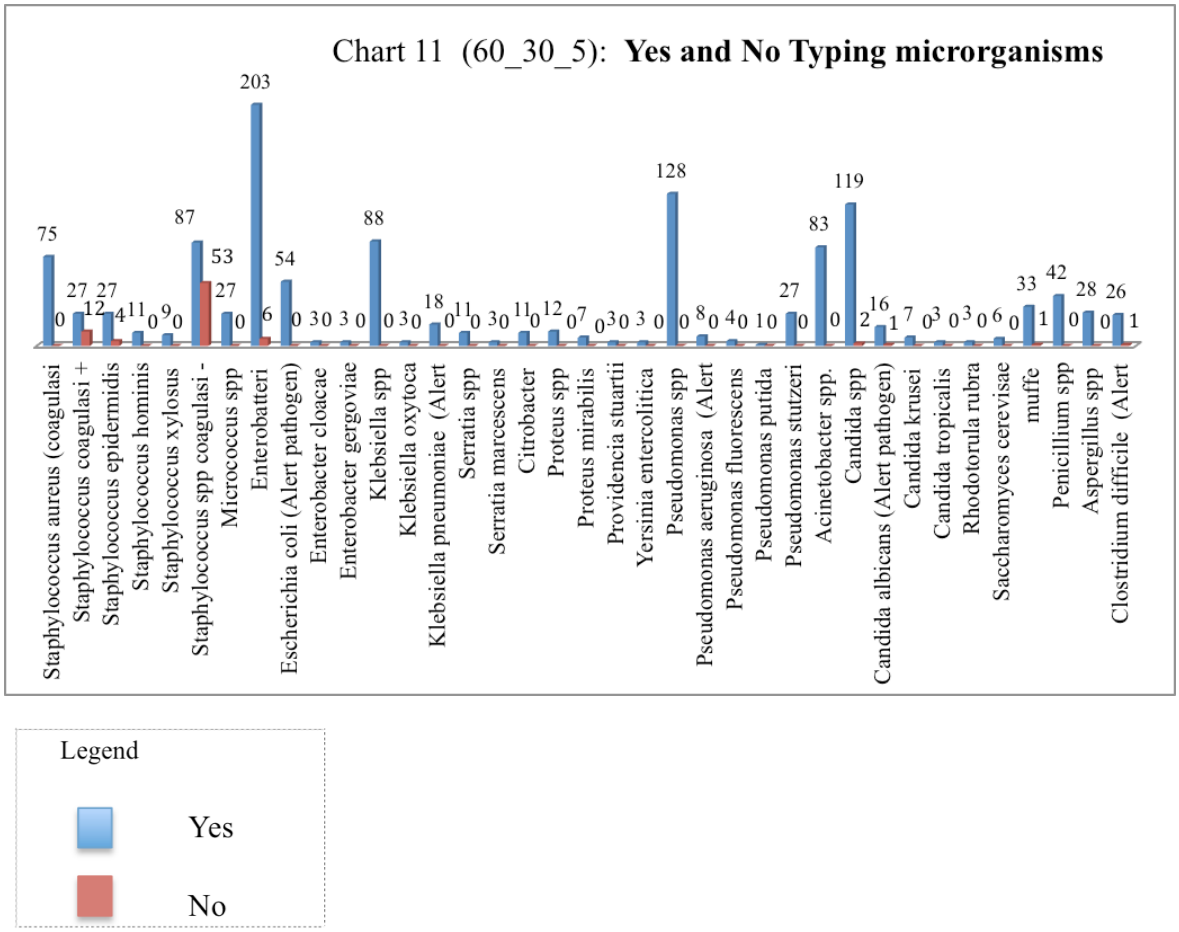
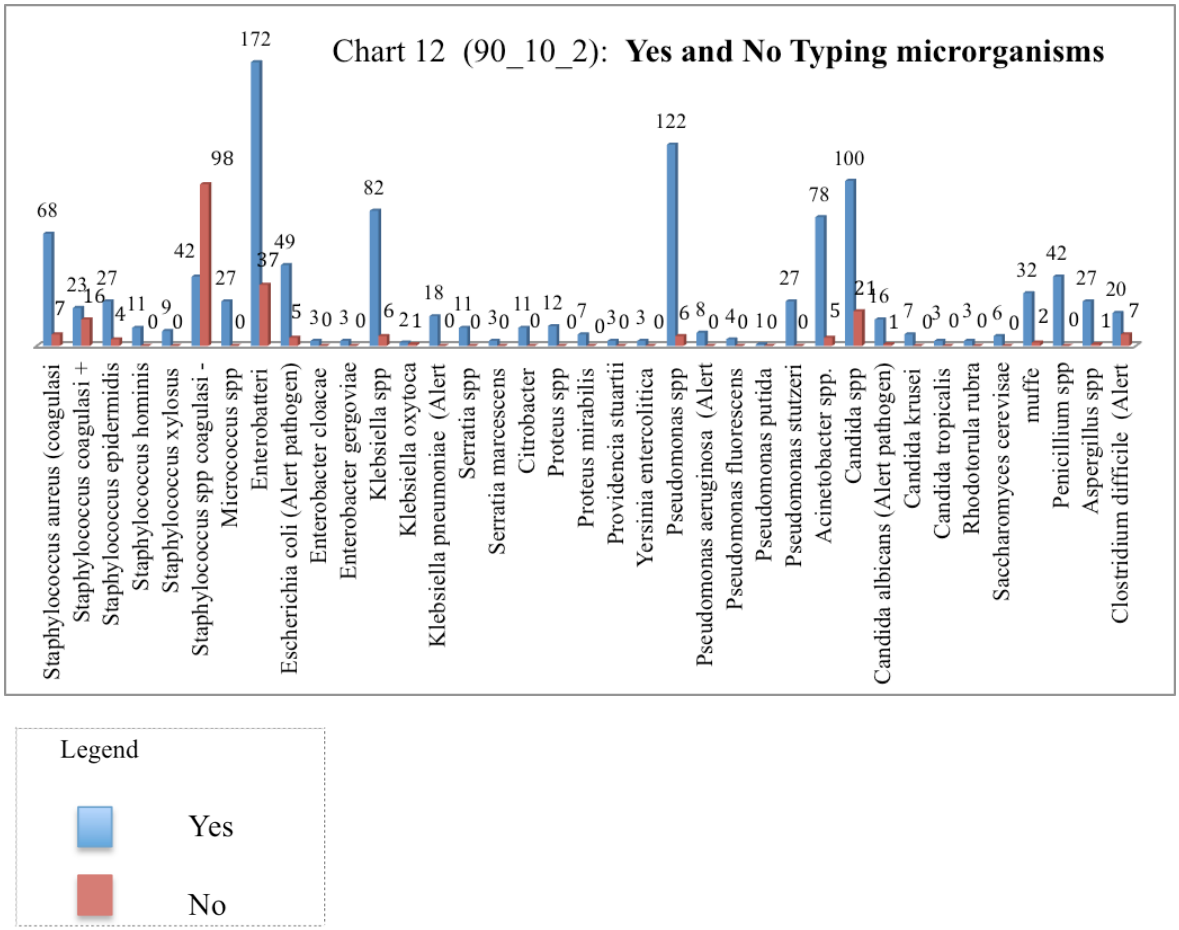


Chart 11 (60_30_5): Yes and No Typing microrganisms
Staphylococcus aureus (coagulasi
Staphylococcus coagulasi +
Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus xylosus
Staphylococcus spp coagulasi -
Micrococcus spp
Enterobatteri
Escherichia coli (Alert pathogen)
Enterobacter cloacae
Enterobacter gergoviae
Klebsiella spp
Klebsiella oxytoca
Klebsiella pneumoniae (Alert
Serratia spp
Serratia marcescens
Citrobacter
Proteus spp
Proteus mirabilis
Providencia stuartii
Yersinia entercolitica
Pseudomonas spp
Pseudomonas aeruginosa (Alert
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Acinetobacter spp.
Candida spp
Candida albicans (Alert pathogen)
Candida krusei
Candida tropicalis
Rhodotorula rubra
Saccharomyces cerevisae
mufle
Penicillium spp
Aspergillus spp
Clostridium difficile (Alert
Legend
Yes
No

Table 9.12 (90_10_2): Yea and No Typing microrganisms		
68	7	***Staphylococcus aureus* (coagulase +) (Alert pathogen)**
23	16	*Staphylococcus coagulase +* (typing St. epidermidis)
27	4	*Staphylococcus epidermidis*
11	0	*Staphylococcus hominis*
9	0	*Staphylococcus xylosus*
42	98	*Staphylococcus spp coagulase -* (CoNS)
27	0	*Micrococcus spp*
172	37	*Enterobatteri*
49	5	***Escherichia coli* (Alert pathogen)**
3	0	*Enterobacter cloacae*
3	0	*Enterobacter gergoviae*
82	6	*Klebsiella spp*
2	1	*Klebsiella oxytoca*
18	0	***Klebsiella pneumoniae* (Alert pathogen)**
11	0	*Serratia spp*
3	0	*Serratia marcescens*
11	0	*Citrobacter*
12	0	*Proteus spp*
7	0	*Proteus mirabilis*
3	0	*Providencia stuartii*
3	0	*Yersinia entercolitica*
122	6	*Pseudomonas spp*
8	0	***Pseudomonas aeruginosa* (Alert pathogen)**
4	0	*Pseudomonas fluorescens*
1	0	*Pseudomonas putida*
27	0	*Pseudomonas stutzeri*
78	5	*Acinetobacter spp.*
100	21	*Candida spp*
16	1	***Candida albicans* (Alert pathogen)**
7	0	*Candida krusei*
3	0	*Candida tropicalis*
3	0	*Rhodotorula rubra*
6	0	*Saccharomyces cerevisae*
32	2	*Muffe not typing*
42	0	*Penicillium spp*
27	1	***Aspergillus spp* (Alert pathogen)**
20	7	***Clostridium difficile* (Alert pathogen)**



Explanation of results of the Table 9.10, 9.11 and 9.12 Typing microrganisms:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained after microbial identification of the surviving species present on the surfaces both to T_0 (traditional protocol) and $T_{1\ (1+n)}$ (probiotic cleaning ptotocol).

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

In general it is evident the the activity of compression occurs to all pathogens present on the sampling points microbiologically monitored.

The probiotic Bacillus check to reduction of the pathogens, also those Alert organism, such as *S. aureus*.

It is noted that the values of *Enterobacteriaceae* are high, because it corresponds to many repeated sampling. This is to evaluate the reduction of Carbapenem Resistant Enterobacteriaceae [CRE], now an emerging problem.

Table 9.13: Total place indipendent	
North	South
1178	121

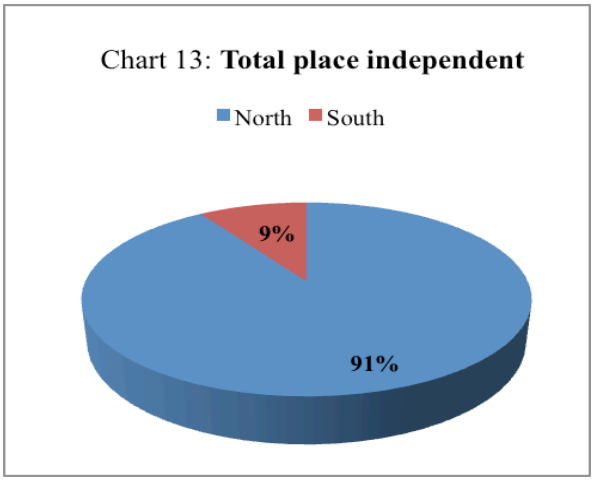


Table 9.14 (60_30_5): Total Yes and No place indipendent			
North		South	
1(Yes)	1109	1(Yes)	110
0(No)	69	0(No)	11

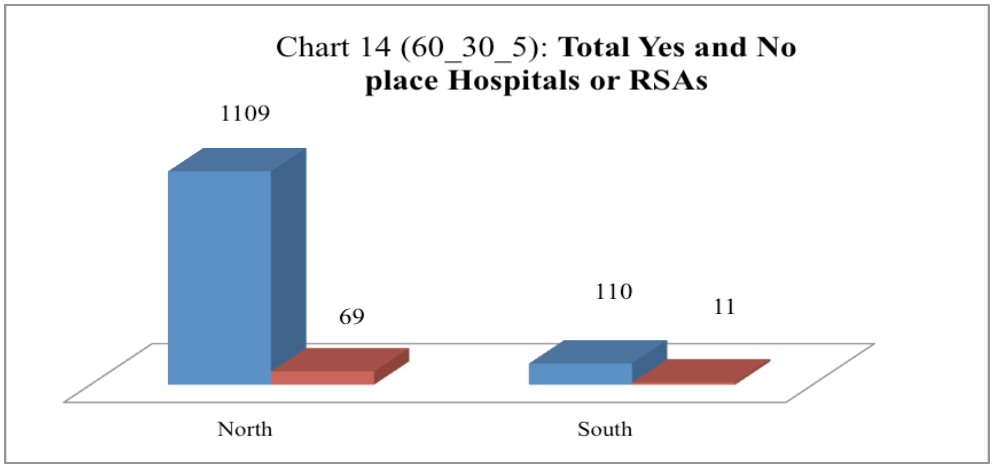
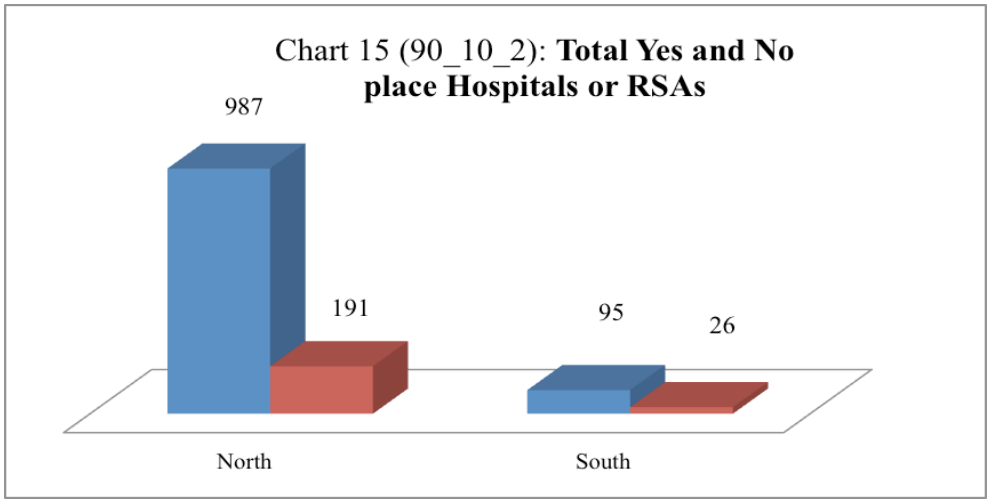


Table 9.15 (90_10_2): Total Yes and No place indipendent			
North		South	
1(Yes)	987	1(Yes)	95
0(No)	191	0(No)	26



Explanation of results of the Table 9.13, 9.14 and 9.15:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained are independent of the place where they are both hospitals or RSAs. No difference between north and south

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

Table 9.16: **Total of microganisms strains**	
305	Staphylococcus Spp.
27	Micrococcus Spp.
428	Enterobacteriacea
168	Pseudomonas Spp.
83	Acinetobacter Spp.
157	Candida Spp.
104	Muffe
27	Clostridium difficile

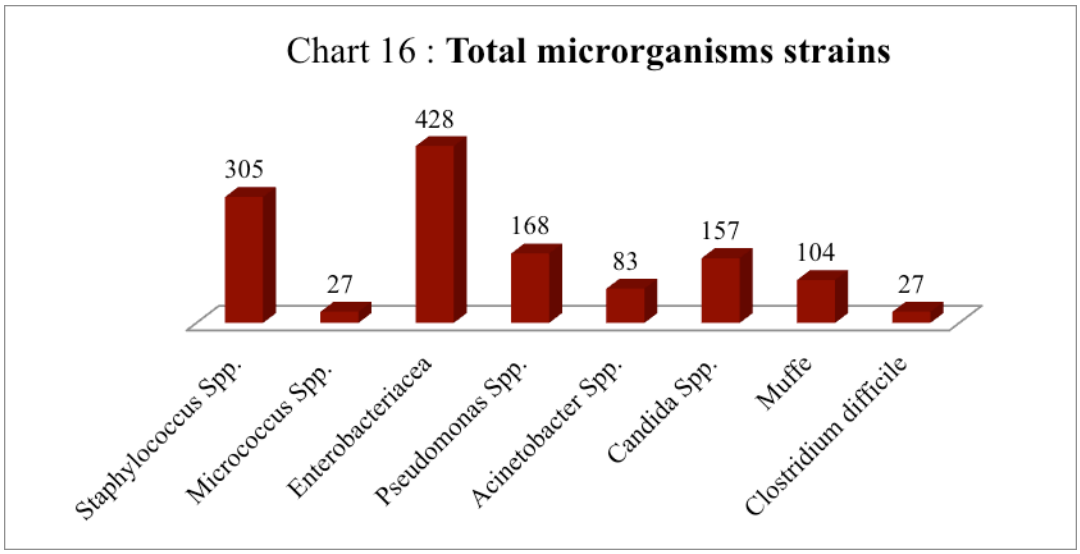


Table 9.17 (60_30_5): Yes and No for microganisms strains		
236	69	Staphylococcus Spp.
27	0	Micrococcus Spp.
422	6	Enterobacteriacea
168	0	Pseudomonas Spp.
83	0	Acinetobacter Spp.
154	3	Candida Spp.
103	1	Muffe
26	1	Clostridium difficile

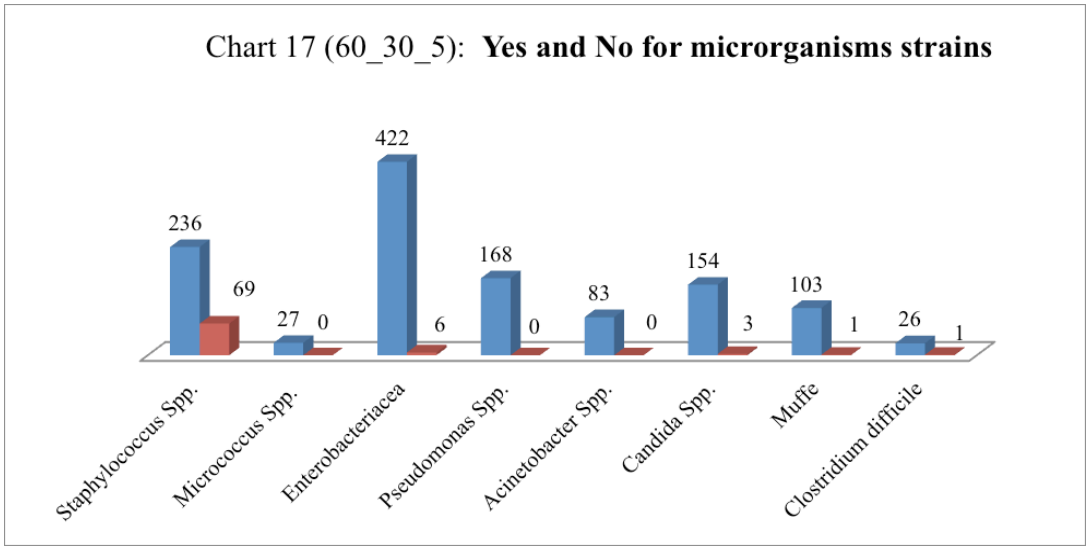
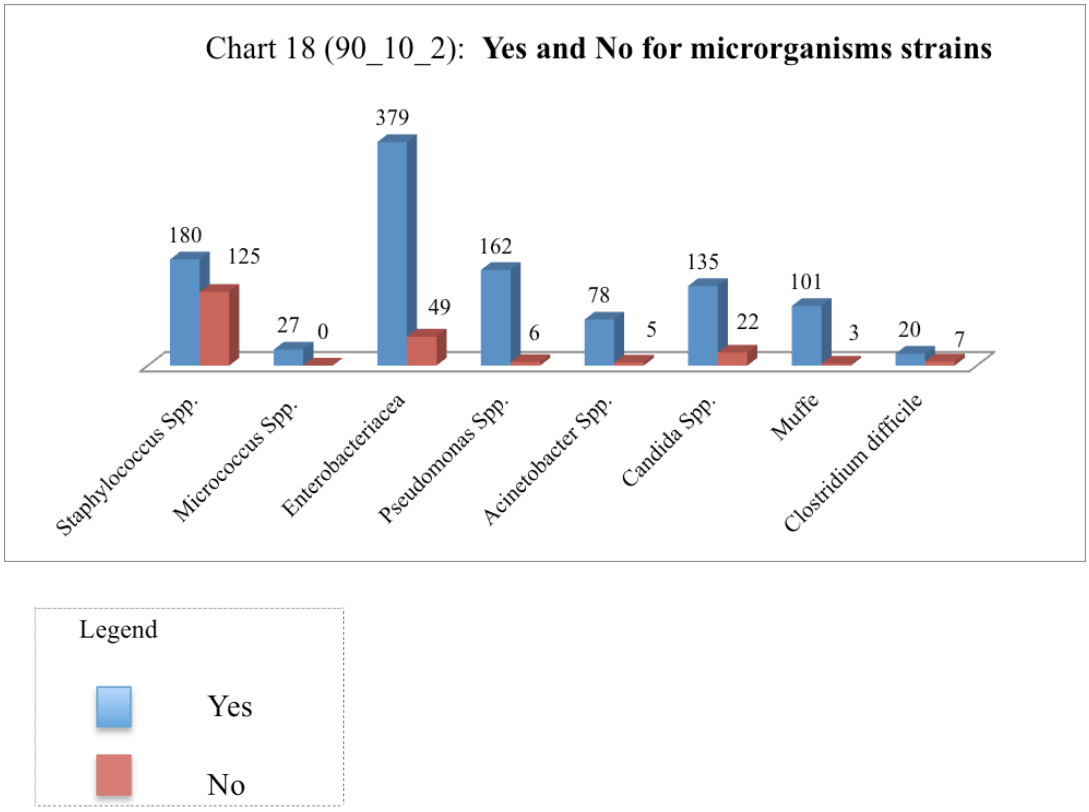


Table 9.18 (90_10_2): Yes and No for microganisms strains		
180	125	Staphylococcus Spp.
27	0	Micrococcus Spp.
379	49	Enterobacteriacea
162	6	Pseudomonas Spp.
78	5	Acinetobacter Spp.
135	22	Candida Spp.
101	3	Muffe
20	7	Clostridium difficile



Explanation of results of the Table 9.16, 9.17 and 9.18 that shown the microrganisms strains:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained by analyzing the groups of microorganisms:

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

In general it is evident the the activity of inhibition of microbial growth occurs to all pathogens present on the test surfaces.

The probiotic bacillus prove to be capable of compression in accordance with the principle of competitive exclusion.

Table 9.19 : **Total sampling points**	
178	Corridor floor
291	Hospital room floor
31	Toilet floor of the hospital room
62	Internal handle toilet
52	Hospital room washbasin
87	Bedside table
23	Headboard bed
51	Footboard bed
134	Siderail bed hospital
23	Internal open-door button
8	Room light switch
23	Worktop
21	Multiparameter monitor keyboard
21	Pulmonary Monitor Keyboard
23	Infusion pump keypad
11	Keyboard_workstation
15	Telephon_cordless

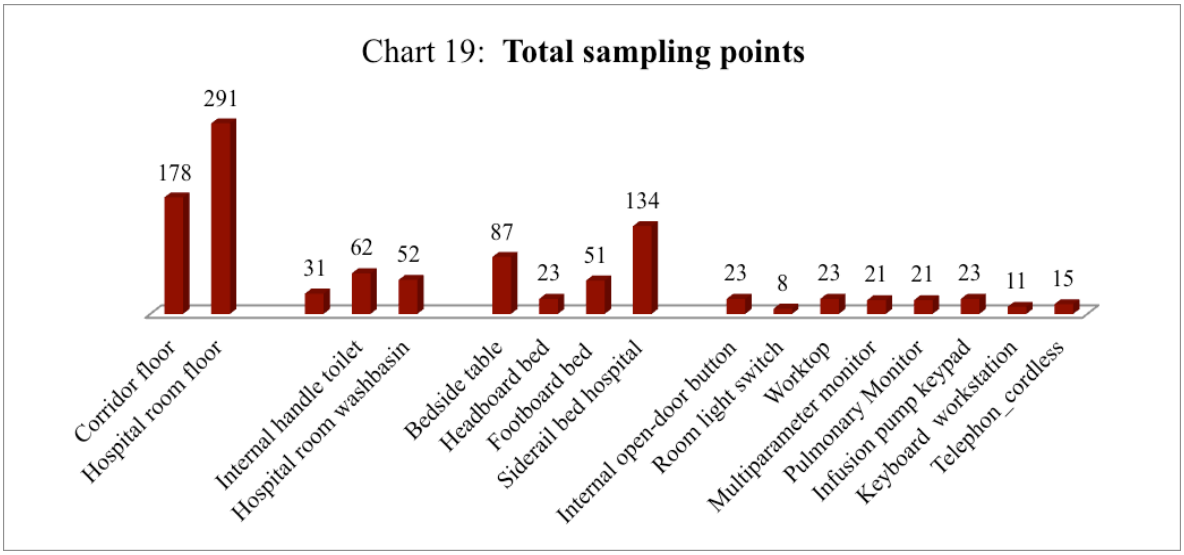


Table 9.20 (60_30_5): Yes and No for sampling points		
162	16	Corridor floor
272	19	Hospital room floor
25	8	Toilet floor of the hospital room
56	2	Internal handle toilet
48	6	Hospital room washbasin
81	6	Bedside table
23	0	Headboard bed
47	4	Footboard bed
128	6	Siderail bed hospital
22	1	Internal open-door button
7	1	Room light switch
22	1	Worktop
21	0	Multiparameter monitor keyboard
20	1	Pulmonary Monitor Keyboard
23	0	Infusion pump keypad
11	0	Keyboard workstation
13	2	Telephon_cordless

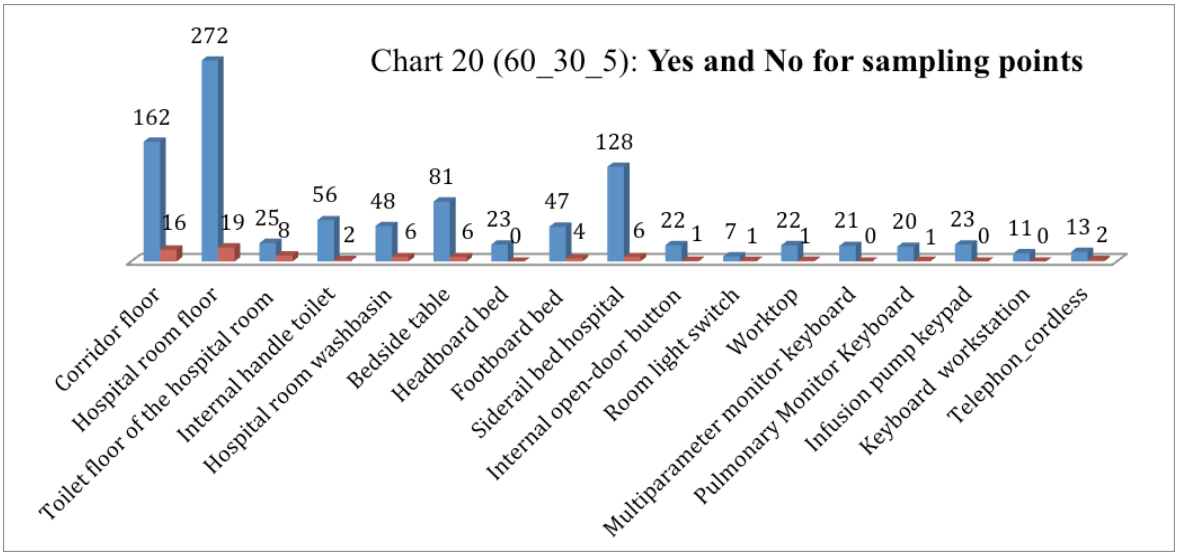
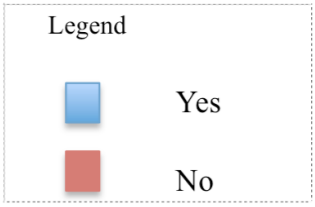
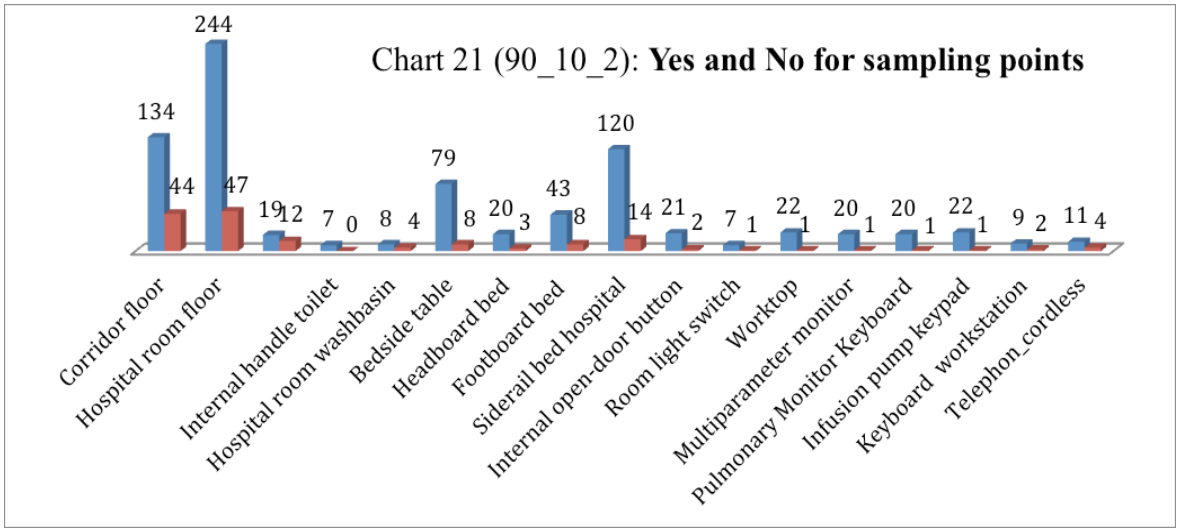


Table 9.21 (90_10_2): Yes and No for sampling points		
134	44	Corridor floor
244	47	Hospital room floor
19	12	Toilet floor of the hospital room
7	0	Internal handle toilet
8	4	Hospital room washbasin
79	8	Bedside table
20	3	Headboard bed
43	8	Footboard bed
120	14	Siderail bed hospital
21	2	Internal open-door button
7	1	Room light switch
22	1	Worktop
20	1	Multiparameter monitor keyboard
20	1	Pulmonary Monitor Keyboard
22	1	Infusion pump keypad
9	2	Keyboard workstation
11	4	Telephon_cordless





Explanation of results of the Table 9.19, 9.20 and 9.21 Sampling points:

on field after probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants the results obtained by analyzing the sampling points:

Both loose classification (60-30-5) and strict classification (90-10-2) indicated that the 'Yes' series (effectiveness of microbial inhibition, reduction or compression) is high compared to the 'No' series (ineffectiveness).

In general it is evident the the activity of inhibition of microbial growth have to all control points. The probiotic bacillus prove to be capable of compression in accordance with the principle of competitive exclusion.

Results antibiograms on Bacillus isolated on filed:

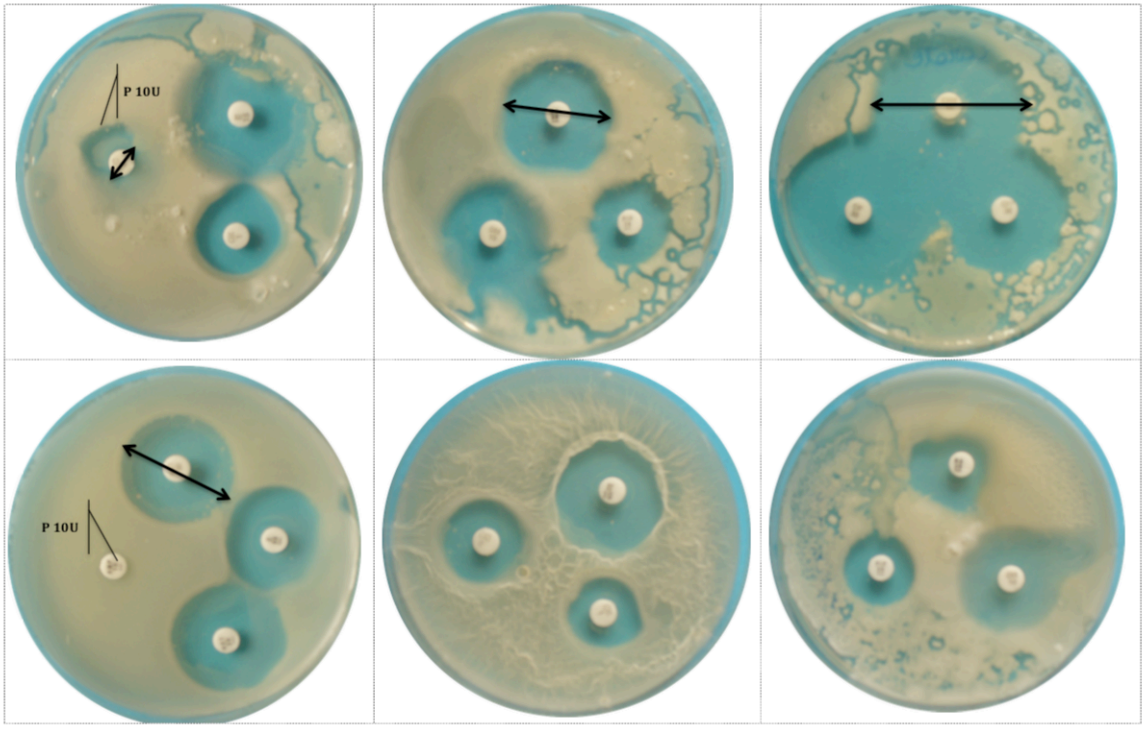


Figure 66*: Bacillus* colonies are isolated for each sampling time after probiotic-based cleaning procedure.

Bacillus isolates are tested by antibiotic susceptibility test against 12 different antibiotics.

It is shown the zones of inhibition tha are measured, to establish bacterial susceptibility or resistance according to CLSI references.

Table 9.22 *Bacillus* colony isolated from TSA medium	β-lactams			Aminoglycosides		Lincosamides
	P 10U	**CF 30µg**	**CFP 30µg**	**NET 10µg**	**G 10µg**	**CC 2µg**
Colony *Bacillus subtilis* ATCC 6633	7	30	20	28	25	25
Colony *Bacillus* wild type	12	20	19	30	25	20
Colony *Bacillus* product probiotic Interior	10	10	14	19	19	15
Colony Bacillus product probiotic Floor	14	24	19	30	24	18
Colony *Bacillus* product probiotic Washsink	20	23	20	30	28	25
Colony 1 *Bacillus* Δt 2 years	4	23	23	25	19	18
Colony 1 Bacillus Δt 18 months	15	30	20	32	25	16
Colony 1 *Bacillus* Δt 1 year	0	32	30	29	25	12
Colony 1 *Bacillus* Δt 6 months	3	28	24	30	26	18

Explanation of results: *On field* Bacillus they have developed and acquired no resistance to antibiotics except for their natural resistance (genetic) to penicillin.

The zone diameters in mm obtained demonstrate that the Bacillus bacteria are susceptible against the test antibiotic, such as CF, CFP, NET an CC.

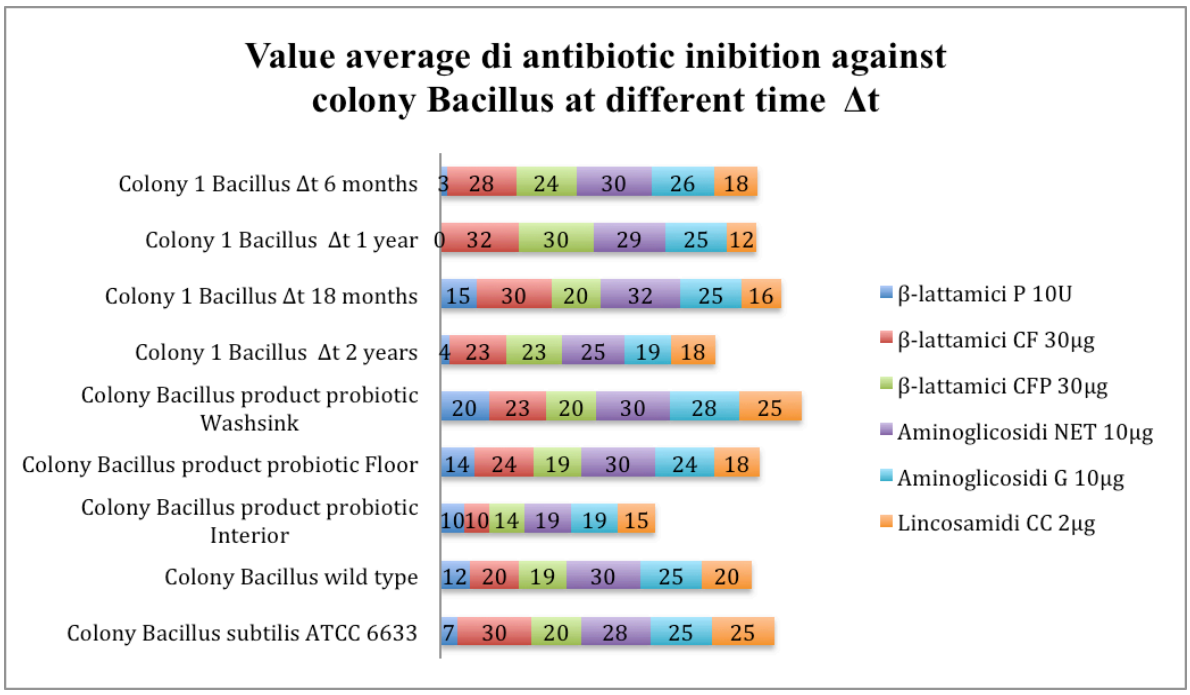


Chart 22*:* Results obtained for antibiotical inhibition of *Bacillus* colonies are isolated for each sampling time after probiotic-based cleaning procedure.

Table 5.23 Strains microoganisms	chimic sanitizer cfu/m^2	probiotic cleaning cfu/m^2	**% Total Reduction**
Staphylococcus spp	9750	1470	85%
Enterobacteriacea	2301	460	80%
Pseudomonas spp	929	121	87%
Candida spp	1513	378	75%
		Average Value Reduction (%)	**81,75%**

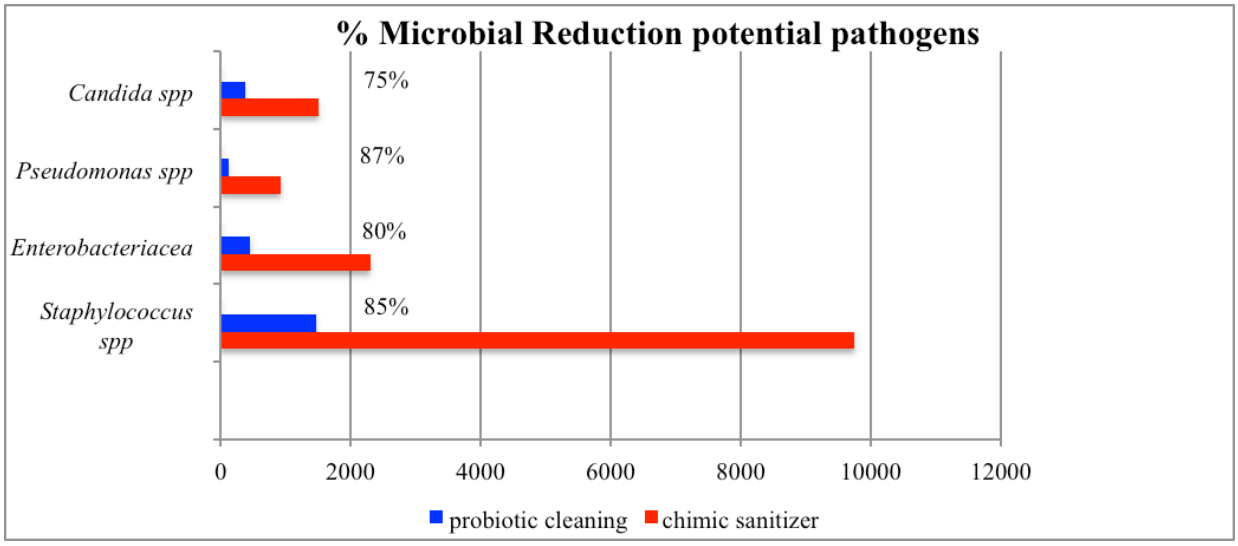


Chart 23: Percentual overall reduction of the alert organisms (pathogens) with the use of probiotic detergents compared to traditional methods of cleaning with the use of chemicals.

The charge of pathogens on average decreases of 75% ÷ 87%.

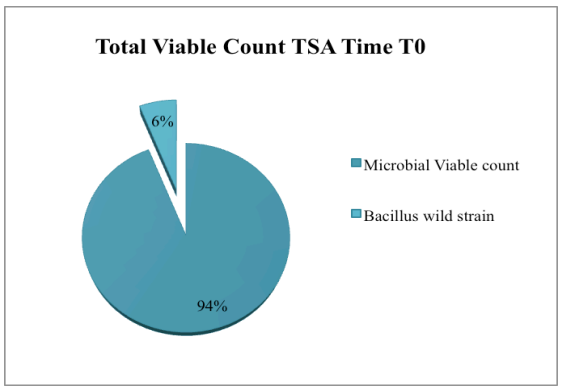


Chart 24

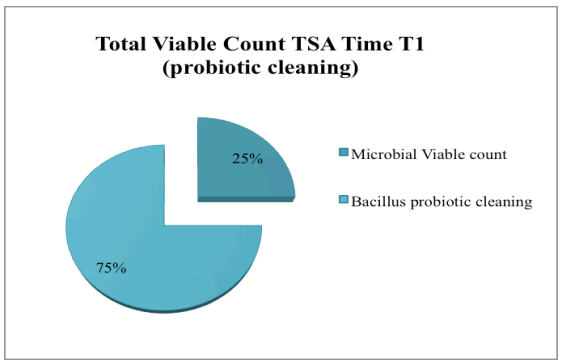


Chart 25

Explanation of results: Composition of the Microbial Viable total count:

on field before probiotic-based cleaning procedure was composed of 6% of wild Bacillus and 94% of potential hospital pathogens.

After probiotic cleaning there was a biostabilization with 25% potentially pathogenic and 75% probiotic *Bacillus* (safe bacteria).

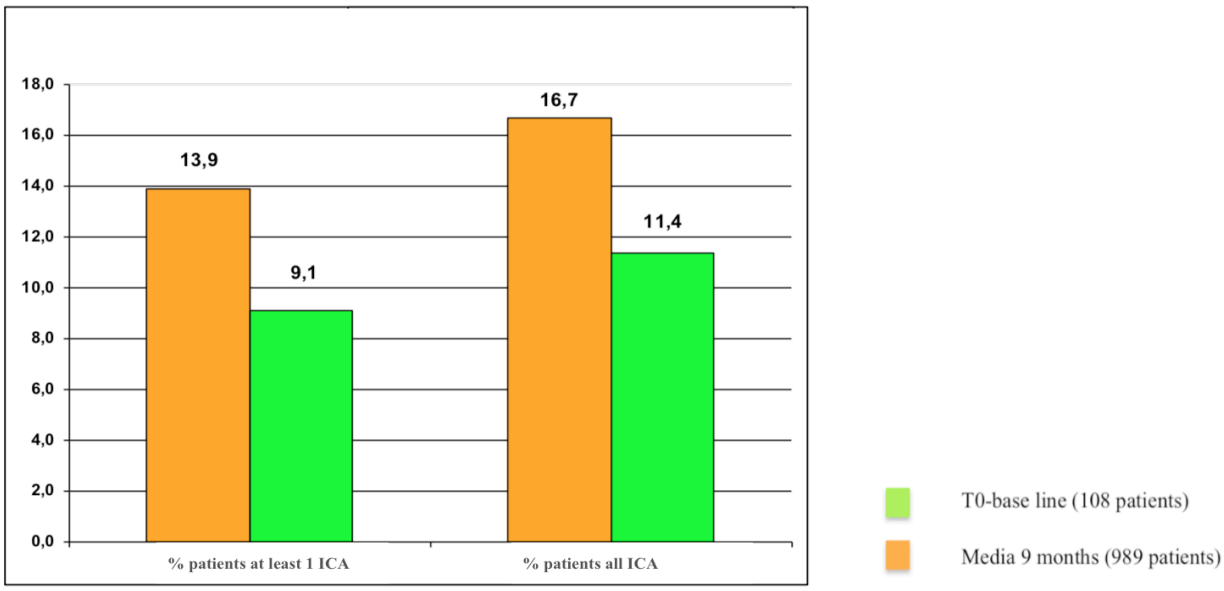


Chart 26: Reduction infection T0-base line (108 patients) compared with 989 patients observed (subjected to diagnostic examinations and hospital surveillance) to assess the presence of any infections acquired during hospitalization for a period of 9 months. The data show a possible correlation between microbiological contamination of environment and care-associated intecfions (ICAs).

The probiotics are able to reduce the growth of specific pathogenic microbial species.

6 Discussion:

The study is carried out in occupied rooms of an inpatient division of 13 public hospitals (11 North Hospitals and 2 South Hospitals) and 7 RSAs (6 North Hospitals and 1 South Hospital). Nine kinds of material (surfaces) per room are analyzed (Table 2) by plate contact before and after the probiotic-based cleaning procedure in comparison with traditional chlorine-based chemical disinfectants.

The microbiological screening targeted the most common HAI-related microorganisms known to reside on surfaces, such as *Staphylococcus species (*alert organism*: St. aureus*,) *Enterobacteriacea* (alert organism: *Escherichia coli*,) *Pseudomonas species (*alert organism: *Ps. aeruginosa), Acinetobacter species (*alert organism*: Acinetobacter baumanii)* and *Candida species (*alert organism*: Candida albicans* and alert organism *Clostridium difficile*.

The results obtained *'in vitro'* (under contamination-controlled conditions) show that their effectiveness is not influenced by the type of surface treated.

The results in field are evaluated for 30 months within patient wards in several departments (such as medicine, geriatrics, long-term care, etc.) in 13 Italian hospitals and 4 nursing homes in Italy with approximately 18,232 microbial surface samples collected by technique count plate.

All samples by microbiological samplings are performed following MEM methodology, but only 13,003 are samples obtained under the H7 protocol (7 hours after cleaning), which have been subjected to a matemathical process with the application of the 7/7 days protocol (cleaning performed all days).

The experimental study indicates that the use of probiotic microorganisms significantly reduces the pathogenic microbial load on contaminated surfaces, and that the effect is more prolonged and stable than that exerted by chemical disinfectants. An impact on the microbial surface remodeling in the nosocomial environment and reduction of infectious events has been demonstrated in correlation with species that mostly colonize the surface.

Consequently, surveillance of infections has shown a reduction in infectious events during the surface treatment with probiotic cleaning.

This study confirm the correlation between microbiological contamination of the environment and care-associated infections (HAIs) in public and the private facilities.

The present work investigated the effectiveness of a probiotic-based sanitation procedure for hard surfaces in both a contamination-controlled laboratory setup and in a real setting consisting of a hospital study-model. This study was based on the hypothesis that probiotic bacteria, defined as a preparation of viable microorganisms that bring a benefit to the host's health (19), could colonize surfaces and counteract the proliferation of other bacterial species (13), including those recognized as potential pathogens for humans.

Healthcare-Associated Infections (HAIs), which are the most frequent complications in healthcare facilities (1), represent a primary case of unwanted human side-effects related to direct or indirect contact with potential pathogens.

The evidence for a proof-of-principle application of this strategy cames from our results obtained in contamination-controlled settings in which we exploited commercially available pathogenic strains such as *Staphylococcus species, Eneterobacteriacea*, *Pseudomonas species, Acinetobacter species, Candida species* and *Clostridium difficile*.

The procedure resulted in a significant reduction in the referenced pathogen populations used, pointing towards the potential feasibility of this method in a real setting.

Our observations with the probiotic-based procedure indicate that its effectiveness in reducing and maintaining a low pathogen load was significantly more pronounced than that of a chemical-based disinfectant on all tested surfaces over time. The pathogen-lowering results of the probiotic-based treatment could be explained as an effect due to bio-stabilization.

The results indicated that i) a probiotic-based sanitation procedure was significantly more effective (up to about 80%) in reducing potentially pathogenic microbial loads than a traditional chlorine-based chemical protocol, and ii) the reduced microbial load was stably maintained at low levels throughout the 24 hours after the application, despite the presence of continuous and multiple sources of microbial re-contaminations due to external natural contributors such as patients, visitors, hospital staff and moving materials.

7 Conclusion:

The experimental study provides evidence that the strategy of bio-stabilization of the probiotic-based products are a reliable alternative to traditional chemical disinfection of surfaces, in particular in the correlation between microbiological contamination of the environment and care –associated infectiond (ICAs).

Although many disease control centers and the overall healthcare sector are aware of problems with resistant pathogens, new sustainable solutions and adequate monitoring techniques have not yet been implemented. This research clearly indicates the importance of monitoring pathogens throughout the healthcare sector publi and private and it presents an innovative and sustainable solution to resistant pathogens. The use of probiotic cleansing has proven experimentally to be more effective than conventional disinfection in the control of long-term infections.

Acknowledgements

This research was financially supported by Copma scrl Italy, Chrisal NV Belgium and Parxplastics Holand. Thanks as well to the boards of directors and ethics committees of all trial hospitals for allowing us to carry out this experimental study.

References:

Bibliography

1. La Placa (2010) Principi di Microbiologia Medica 12° ed. Ed. Esculapio ISBN 9788874883653
2. Suetens, C., Hopkins, S., Kolman, J., and Diaz Högberg, L. (2013) Point Prevalence Survey of Healthcare Associated Infections and Antimicrobial Use in European Acute Care Hospitals. Stockholm, Sweden: European Centre for Disease Prevention and Control.
3. Lautenbach E (2001). "Chapter 14. Impact of Changes in Antibiotic Use Practices on Nosocomial Infections and Antimicrobial Resistance—*Clostridium difficile* and Vancomycin-resistant Enterococcus (VRE)" (http://www.ahrq.g ov/clinic/ptsafety/chap14.htm). In Markowitz AJ. *Making Health Care Safer: A Critical Analysis of Patient Safety Practices* (http://www.ahrq.gov/clinic/ptsafety/). Agency for Healthcare Research and Quality.
4. *U.S. Centers for Disease Control and Prevention* (2016) "Division of Oral Health - Infection Control Glossary".. Retrieved 19 April 2016.
5. Bock, L.J., Wand, M.E., and Sutton, J.M. (2016) Varying activity of chlorhexidine-based disinfectants against Klebsiella pneumoniae clinical isolates and adapted strains. J Hosp Infect 93: 42–48.
6. Davies J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science 264: 375-382.
7. Cleaning and disinfecting Archived July 9, 2011, at the Wayback Machine., (2009), Mid Sussex District Council, UK.
8. Curtis J.DonskeyMDab Does improving surface cleaning and disinfection reduce health care-associated infections? American Journal of Infection Control. Volume 41, Issue 5, Supplement, May 2013, Pages S12-S19 https://doi.org/10.1016/j.ajic.2012.12.010.
9. Boyce JM (2016) Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrob Resist Infect Control. 2016 Apr 11;5:10. doi: 10.1186/s13756-016-0111-x. eCollection 2016.
10. Ghiglione JF, Martin-Laurent F, Pesce S. (2015) Microbial ecotoxicology: an emerging discipline facing contemporary environmental threats. Environ Sci Pollut Res; DOI 10.1007/s11356-015-5763-1
11. Carlone N., Pompei R. (2013) Microbiologia farmaceutica 2° ed. Ed. EdiSES 2013 ISBN 9788879597364
12. Schrezenmeir J, De Vrese M. 2001. Probiotics, Prebiotics, And Synbiotics--Approaching Definition. Am. J. Clin. Nutr. 73(2 Suppl):361s-364s.
13. *David Schlossberg (2015)* Clinical Infectious Disease*. Cambridge University Press. pp. 688–. ISBN 978-1-107-03891-2.*
14. AIDSinfo: Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents *(PDF). 2013-06-17. Retrieved 2015-05-09.*
15. Herman Goossens, Matus FerechPharm Robert Vander Stichele Monique Elseviers (2005) Outpatient antibiotic use in Europe and association with resistance: a cross-national database study The Lancet

Volume 365, Issue 9459, 12–18 February 2005, Pages 579-587 https://doi.org/10.1016/S0140-6736(05)17907-0

16. Weber, David J.[a,b]; Anderson, Deverick[c]; Rutala, William A.(2013)The role of the surface environment in healthcare-associated infections Current Opinion in Infectious Diseases: August 2013 - Volume 26 - Issue 4 - p 338–344 doi:1097/QCO.0b013e3283630f04
17. European Centre for Disease Prevention and Control (2016) *Point prevalence survey* of *healthcare-associated infections and antimicrobial use* in European acute care https://ecdc.europa.eu/.../point-prevalence-survey-healthcare-ass 12 ott 2016
18. Curtis J.DonskeyMDab (2013) Does improving surface cleaning and disinfection reduce health care-associated infections? American Journal of Infection Control. Volume 41, Issue 5, Supplement, May 2013, Pages S12-S19 https://doi.org/10.1016/j.ajic.2012.12.010.
19. Weber, D.J., and Rutala, W.A. (1997) Role of environmental contamination in the transmission of vancomycin-resistant enterococci. Infect Control Hosp Epidemiol 18: 306–309. DOI: 10.2307/30141222
20. Weber DJ[1], Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E (2010) Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control. 2010 Jun;38(5 Suppl 1):S25-33. doi: 10.1016/j.ajic.2010.04.196.
21. SJ Dancer (2009) The role of environmental cleaning in the control of hospital-acquired infection Journal of hospital Infection, 2009 – Elsevier https://doi.org/10.1016/j.jhin.2009.03.030
22. World Health Organization - HAI Fact Sheet 2012 / Center for Disease Control - http://www.cdc.gov/hai/pdfs/hai/scott_costpaper.pdf)
23. European Center for Disease Control (2013). Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011–2012 www.ecdc.europa.eu Stockholm, July 2013 ISBN 978-92-9193-485-0 doi 10.2900/86011
24. Dancer, S.J. (2011) Hospital cleaning in the 21st century. Eur J Clin Microbiol Infect Dis 30: 1473–1481. Eur J Clin Microbiol Infect Dis. 2011 Dec; 30(12):1473-81. doi: 10.1007/s10096-011-1250-x. Epub 2011 Apr 17.
25. A.Frabetti, A.Vandini, P.G.Balboni, F.Triolo, S.Mazzacane (2009) Experimental evaluation of the efficacy of sanitation procedures in operating rooms - American Journal of infection control – Vol. 37 n. 8 – pagg. 658-664 – October 2009
26. S. Mazzacane, A. Frabetti, A. Vandini, D. Migliori, P. Balboni (2007) L'igiene nei reparti ospedalieri: correlazioni tra le procedure di sanificazione ed i fattori di contaminazione-Conferenza Nazionale ANMDO 2007 – 12,14 Settembre 2007, Rimini – pubblicato sulla rivista L'Ospedale. ISBN 0030-6231
27. A.Frabetti, A.Vandini, D.Migliori, A. Cusumano, E.Righini, P.Balboni, S. Mazzacane (2006) Efficacia ed efficienza dei protocolli di pulizia e disinfezione in sale operatorie Congresso ANMDO 2006 - 21-24 settembre 2006, Lecce. ISBN 0030-6231

28. Carling, P.C., and Bartley, J.M. (2010) Evaluating hygienic cleaning in health care settings: what you do not know can harm your patients. Am J Infect Control 38: S41–S50. Am J Infect Control. 2010 Jun;38(5 Suppl 1):S41-50. doi: 10.1016/j.ajic.2010.03.004.
29. Carling, P.C., Parry, M.F., and Von Beheren, S.M. (2008) Identifying opportunities to enhance environmental cleaning in 23 acute care hospitals. Infect Control Hosp Epidemiol 29: 1–7. Infect Control Hosp Epidemiol. 2008 Jan;29(1):1-7. doi: 10.1086/524329.
30. Goodman, E.R., Platt, R., Bass, R., Onderdonk, A.B., Yokoe, D.S., and Huang, S.S. (2008) Impact of an environmental cleaning intervention on the presence of methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci on surfaces in intensive care unit rooms. Infect Control Hosp Epidemiol 29: 593–599. Infect Control Hosp Epidemiol. 2008 Jul;29(7):593-9. doi: 10.1086/588566.
31. Falagas ME, Makris GC (2009) Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis. J Hosp Infect 71: 301-306. J Hosp Infect. 2009 Apr;71(4):301-6. doi: 10.1016/j.jhin.2008.12.008. Epub 2009 Feb 6.
32. Garrett Hardin The Competitive Exclusion Principle *Science* 29 Apr 1960:Vol. 131, Issue 3409, pp. 1292-1297 DOI: 10.1126/science.131.3409.1292
33. Alberta Vandini, Alessia Frabetti, Paola Antonioli, Daniela Platano, Alessio Branchini, Maria Teresa Camerada, Luca Lanzoni, Pier Giorgio Balboni, Sante Mazzacane - "Reduction of the Microbiological Load on Hospital Surfaces Through Probiotic-Based Cleaning Procedures: A New Strategy to Control Nosocomial Infections", Journal of Microbiology & experimentation, October 6, 2014.
34. Vandini, A., Temmerman, R., Frabetti, A., Caselli, E., Antonioli, P., Balboni, P. G., Platano, D., Branchini, A. & Mazzacane, S. (2014). Hard Surface Biocontrol In Hospitals Using Microbial-Based Cleaning Products. Plos One, 9, E108598.
35. Vincenza La Fauci, Gaetano Bruno Costa, Francesca Anastasi, Alessio Facciola, Orazio Claudio Grillo, Raffaelle Squeri, "An Innovative Approach to Hospital Sanitization Using Probiotics: 'in vitro' and Field Trials" - Microbial & Biochemical Technology – 2015, 7:3, http://dx.doi.org/10.4172/1948-5948.1000198
36. E. Caselli, M. D'Accolti, A. Vandini, L. Lanzoni, M.T. Camerada, M. Coccagna, A. Branchini, P. Antonioli, P.G. Balboni, D. Di Luca, S.Mazzacane (2016) Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLOS ONE - DOI:10.1371/journal.pone.0148857 - February 17, 2016
37. S. Mazzacane, P.G. Balboni, A. Vandini, A. Frabetti, P.Antonioli (2012), "L'evoluzione delle procedure di sanificazione negli ospedali: prospettive di riduzione e controllo della carica batterica potenzialmente patogena mediante tecniche di biostabilizzazione", L'Ospedale, 02/2012: pagg. 78-83. ISBN 0030-6231
38. S. Mazzacane, P.G. Balboni, A. Vandini, A. Frabetti, P. Antonioli, M.C. Manzalini, M. Rovigatti (2011), "Sperimentazione di tecniche di biostabilizzazione nelle procedure di sanificazione di degenze ospedaliere", L'Ospedale, 04/11: pagg. 52-58. ISBN 0030-6231

39. Allegranzi B, Bagheri Nejad S, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011; 377(9761):228–41. Epub 2010/12/15. doi: S0140-6736(10)61458-4 [pii] doi: 10.1016/S0140- 6736(10)61458-4 PMID: 21146207.
40. Messineo A, Marsella LT (2015) Biological hazards and healthcare- associated infections in Italian healthcare facilities: some considerations on inspections and accountability. Ann Ig 27: 799-807.
41. Mancini A, Verdini D, La Vigna G, Recanatini C, Lombardi FE, et al. (2016) Retrospective analysis of nosocomial infections in an Italian tertiary care hospital. New Microbiol 39: 197-205. 2016, ISN 1121-7138
42. Flores-Mireles, AL; Walker, JN; Caparon, M; Hultgren, SJ (May 2015). "Urinary tract infections: epidemiology, mechanisms of infection and treatment options". Nature Reviews. Microbiology. 13 (5): 269–84. doi:10.1038/nrmicro3432. PMC 4457377 . PMID 25853778.
43. GBD 2015 Mortality and Causes of Death, Collaborators. (8 October 2016). "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015". Lancet. 388 (10053):1459–1544. doi:10.1016/S0140-6736(16)310121. PMID 27733281.
44. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, Torella M (June 2011). "Urinary tract infections in women". European journal of obstetrics, gynecology, and reproductive biology. 156 (2): 131–6. doi:10.1016/j.ejogrb.2011.01.028. PMID 21349630.
45. Abraham, Soman N.; Miao, Yuxuan (October 2015). "The nature of immune responses to urinary tract infections". Nature reviews. Immunology. 15 (10): 655–663. doi:10.1038/nri3887. ISSN 1474-1733. PMC 4926313 . PMID 26388331.
46. Axel KramerEmail author, Ingeborg Schwebke and Günter Kampf How long do nosocomial pathogens persist on inanimate surfaces? A systematic review *BMC Infectious Diseases* 2006 **6**:13 https://doi.org/10.1186/1471-2334-6-130
47. Hota, B. (2004) Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 39: 1182–1189. PMID:15486843 DOI:10.1086/424667
48. Kramer, A., Schwebke, I., and Kampf, G. (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 6: 130. PMID:16914034 DOI:10.1186/1471-2334-6-130
49. Weber, D.J., Rutala, W.A., Miller, M.B., Huslage, K., and Sickbert-Bennett, E. (2010) Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile* , and Acinetobacter species. *Am J Infect Control* 38: S25–S33. doi: 10.1016/j.ajic.2010.04.196.
50. David J.Weber, William A.Rutala, Melissa B.Miller KirkHuslage RN, EmilySickbert-Bennett Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. American Journal of Infection Control Volume 38, Issue 5, Supplement, June 2010, Pages S25-S33 https://doi.org/10.1016/j.ajic.2010.04.196

51. William R. Jarvis Infection Control: More Intensive Daily Disinfection? December 13, 2012 Medscape https://www.medscape.com/index/list_5427_0
52. P. Magiorakos, A. Srinivasan,R. B. Carey,Y. Carmeli, M. E. Falagas,C. G. Giske, S. Harbarth, J. Stelling, M. J. Struelens,A. Vatopoulos,T. Weber et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance 27 July 2011 DOI: 10.1111/j.1469-0691.2011.03570. Clinical Microbiology and Infection.
53. C. Lee Ventola,The Antibiotic Resistance Crisis Part 1: Causes and ThreatsP T. 2015 Apr; 40(4): 277–283. Journal List P T v.40(4); 2015 Apr PMC4378521
54. Centers for Disease Control and Prevention, Office of Infectious Disease Antibiotic resistance threats in the United States, (2013). Apr, 2013. Available at: http://www.cdc.gov/drugresistance/threat-report-2013
55. LH Aiken, W Sermeus, K Van den Heede, DM Sloane (2012) Patient safety, satisfaction, and quality of hospital care: cross sectional surveys of nurses and patients in 12 countries in Europe and the United States. *BMJ* 2012; 344 doi: https://doi.org/10.1136/bmj.e1717 BMJ 2012;344:e1717
56. Angela Huttner, Stephan Harbarth, Jean Carlet, Sara Cosgrove, Herman Goossens, Alison Holmes, Vinc ent Jarlier, Andreas Voss (2013) Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum *Antimicrobial Resistance and Infection Control*2013**2**:31 https://doi.org/10.1186/2047-2994-2-31
57. Karen L Tang, Niamh P Ca rey, Diego B Nóbrega, Susan C Cork, Paul E Ronksley, Herman W Barkema, Alicia J Polachek, Heather Ganshorn, Nishan Sharma, James D Kellner, William A Ghali (2017) Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet Health 2017; 1: e316–27 Published Online November 6, 2017 doi.org/10.1016/ S2542-5196(17)30141-9
58. Antibiotic Resistance Threats in the United States, CDC 2013 WHO: Antibiotic resistance a threat to global health security and the case for action, Side-event at the Sixty-sixth WHA, May 2013
59. CDC's annual *National and State Healthcare-Associated Infections Progress Report* (HAI Progress Report) published 2016 (https://www.cdc.gov/hai/surveillance/index.html)
60. Cornejo-Juarez, P., Vilar-Compte, D., Perez-Jimenez, C., Namendys-Silva, S. A., Sandoval-Hernandez, S. & Volkow-Fernandez, P. (2015) The Impact Of Hospital-Acquired Infections With Multidrug-Resistant Bacteria In An Oncology Intensive Care Unit. *Int J Infect Dis,* 31, 31-4
61. Caini, S., Hajdu, A., Kurcz, A. & Borocz, K. 2013. Hospital-Acquired Infections Due To Multidrug-Resistant Organisms In Hungary, 2005-2010. *Euro Surveill,* 18
62. Burden of Antibiotic Resistance. Action on Antibiotic Resistance (ReAct), 2012. http://www.reactgroup.org/uploads/publications/react-publications/ReAct-facts-burden-of-antibiotic-resistance-May-2012.pdf

63. Tackling drug-resistant infections globally: final report and recommendations.The Review on Antimicrobial Resistance chaired by Jim O'Neill. May 2016. http://amr-review.org/Publications
64. William A. Rutala (1996) APIC guideline for selection and use of disinfectants. American Journal of Infection Control. Volume 24, Issue 4, August 1996, Pages 313-342 https://doi.org/10.1016/S0196-6553(96)90066-8
65. Warren Litsky (1968) Disinfection, Sterilization, and Preservation Reviewed. Am J Public Health Nations Health. 1968 Nov; 58(11): 2175–2176. PMCID: PMC1229055
66. Seymour Stanton (2001) Disinfection, Sterilization, and Preservation Blook fifth edition (5th ed.) Lippincott WILLIAMS & WILKINS 2001 ISBN 0-683-30740-1
67. Sunil B. Somani, Nitin W.Ingole (2012) Formulation of kinetic model to predict disinfection of water by using natural herbs. International Journal of Environmental Sciences Volume 2 No.3, 2012 doi:10.6088/ijes.00202030021
68. Progress in Wastewater Disinfection Technology (1979) Proceedings of the National Symposium, Cincinnati, Ohio, September 18-20, 1978, by Albert D. Venosa Volume 1 June 1979 EPA ; 600/9-79-018
69. Rodney M. Donlan (2002) Biofilms: microbial life on surfaces Emerging infectious diseases, 2002 ncbi.nlm.nih.gov. Emerg Infect Dis. 2002 Sep; 8(9): 881–890. doi: 10.3201/eid0809.020063
70. Characklis WG, McFeters GA, Marshall KC. Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; 1990. p. 341–94.
71. López, Daniel; Vlamakis, Hera; Kolter, Roberto (2010). "Biofilms". *Cold Spring Harbor Perspectives in Biology*. 2 (7): a000398. doi:10.1101/cshperspect.a000398. ISSN 1943-0264. PMC 2890205 . PMID 20519345.
72. Weber, DJ; Rutala, WA (May 2013). "Self-disinfecting surfaces: review of current methodologies and future prospects.". *American journal of infection control*. 41 (5 Suppl): S31–5. PMID 23622745 (https://www.ncbi.nlm.nih. gov/pubmed/23622745). doi:10.1016/j.ajic.2012.12.005 (https://doi.org/10.1016%2Fj.ajic.2012.12.005).
73. Cookson, B., Mackenzie, D., Kafatos, G., Jans, B., Latour, K., Moro, M. L., Ricchizzi, E., Van De Mortel, M., Suetens, C. & Fabry, J. 2013. Development And Assessment Of National Performance Indicators For Infection Prevention And Control And Antimicrobial Stewardship In European Long-Term Care Facilities. *J Hosp Infect,* 85, 45-53.
74. S. Zanasi A. Vandini, N. Roveri, C Ventura (2016) "Le infezioni nella chirurgia protesica: Impianto antibatterico in lega di titanio rivestito di Ha - Lf - Zn ione. Studio preliminare L'Ospedale, vol. 3/2016 october 13th, 2016 0030-6231
75. S. Zanasi, A. Vandini, L. Costa, M. Fiore, M. Marchetti, C. Ventura (2016) Le infezioni nella chirurgia protesica: Inserto antibatterico in e-poly dopato con Zn ione) L'Ospedale, vol. 3/2016 october 13th, 2016 0030-6231

76. Ballardini A, Montesi M, Panseri S, Vandini A, P.G. Balboni, Tampieri A, Sprio S. (2017) New hydroxyapatite nanophases with enhanced osteogenic and anti-bacterial activity Journal of Biomedical Materials Research: Part A - JBMR-A-17-0074 J Biomed Mater Res A. 2017 Sep 29 DOI: 10.1002/jbm.a.36249
77. UC Davis (2006) Health System: Newroom. *UC Davis Pioneers Use of Microfiber Mops in Hospitals: Mops reduce injuries, kill more germs and reduce costs.* Archived 2010-07-06 at the Wayback Machine. June 23, 2006.
78. Magda Diab-Elschahawi,Ojan Assadian, Alexander Blacky, Maria Stadler, Elisabeth Pernicka, Jutta Berger, Helene Resch, Walter Koller (2010) Evaluation of the decontamination efficacy of new and reprocessed microfiber cleaning cloth compared with other commonly used cleaning cloths in the hospital American Journal of Infection Control Volume 38, Issue 4, May 2010, Pages 289-292 https://doi.org/10.1016/j.ajic.2009.09.006.
79. Leung M, Chan AH (March 2006). "Control and management of hospital indoor air quality (http://www.medscimon it.com/fulltxt.php?ICID=447117). *Med. Sci. Monit*. 12 (3): SR17 PMID 16501436 (https://www.ncbi.nlm.nih.go v/pubmed/16501436).
80. Steven W Kembel, Evan Jones, Jeff Kline, Dale Northcutt, Jason Stenson, Ann M Womack, Brendan JM Bohannan, G Z Brown, Jessica L Green Architectural design influences the diversity and structure of the built environment microbiome. Microbial Population and Community Ecology The ISME Journal (2012) 6, 1469-1479. doi:10.1038/ismej.2011.211
81. Arundel AV, Sterling EM, Biggin JH, Sterling TD. (1986). *Indirect health effects of relative humidity in indoor environments*. *Environ Health Perspect* 65: 351–361.
82. Atkinson J, Chartier Y, Pessoa-Silva CL, Jensen P, Li Y, Seto W-H (eds). (2009). *Natural Ventilation for Infection Control in Health-Care Settings*. World Health Organization: Geneva, Switzerland.
83. Escombe AR, Oeser CC, Gilman RH, Navincopa M, Ticona E, Pan W *et al*. (2007). Natural ventilation for the prevention of airborne contagion. *PLoS Med* 4: e68. | Article | PubMed |
84. Eames I, Tang JW, Li Y, Wilson P. (2009). Airborne transmission of disease in hospitals. *J R Soc Interface* 6(Suppl 6): S697–S702. | Article | PubMed | ISI |
85. Hospital Design and function, McGraw Hill, New York 1964.
86. M. Dettenkofer, S. Seegers, G. Antes, E. Motschall (2004) Does the Architecture of Hospital Facilities Influence Nosocomial Infection Rates? A Systematic Review Infection Control & Hospital Epidemiology Volume 25, Issue 1 January 2004 , pp. 21-https://doi.org/10.1086/502286
87. Endres JR, Clewell A, Jade KA, Farber T *et al* (2009) Safety assessment of a proprietary preparation of a novel Probiotic, *Bacillus coagulans*, as a food ingredient *Food Chem Toxicol.*
88. European Food Safety Authority and European Centre for Disease Prevention and Control. 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA Journal, 10(3):2597. http://www.efsa.europa.eu/de/efsajournal/pub/2597.htm

89. Floch Mh, Walker Wa, Madsen K, Sanders Me, Macfarlane Gt, Flint Hj, Dieleman La, Ringel Y, Guandalini S, Kelly Cp, Brandt Lj. *Recommendations For Probiotic Use-2011 Update.* J Clin Gastroenterol. 2011 Nov;45 Suppl:S168-71.
90. Schrezenmeir J, De Vrese M. 2001. *Probiotics, Prebiotics, And Synbiotics--Approaching Definition.* Am. J. Clin. Nutr. 73(2 Suppl):361s-364s.
91. Hong Ha, Duc Le H, Cutting Sm. 2005. *The Use Of Bacterial Spore Formers As Probiotics.* Fems Microbiol. Rev. 29:8138-35.
92. Wu, X. Y., M.J., W., Hornitzky, M. & Chin, J. 2006. Development Of A Group-Specific Pcr Combined With Ardra For The Identification Of Bacillus Species Of Environmental Significance. *J Microbiol Methods,* 64, 107-19.
93. Duc Le H, Hong Ha, Barbosa Tm, Henriques Ao, Cutting Sm. 2004. *Characterization Of Bacillus Probiotics Available For Human Use.* Appl. Environ. Microbiol. 70:2161-2171.
94. Sorokulova Ib, Pinchuk Iv, Denayrolles M, Osipova Ig, Huang Jm, Cutting Sm, Urdaci Mc. 2008. *The Safety Of Two Bacillus Probiotic Strains For Human Use.* Dig. Dis. Sci. 53:954-63.
95. Zweers JC, Barák I, Becher D, Driessen AJ, Hecker M, Kontinen VP, Saller MJ, Vavrová L, van Dijl Towards the development of Bacillus subtilis as a cell factory for membrane proteins and protein complexes. JM - Microb. Cell Fact. (2008) doi: 10.1186/1475-2859-7-10 PMCID: PMC2323362
96. Wattiau, P., Renard, M.-E., Ledent, P., Debois, V., Blackman, G. & Agathos, S. N. 2001. A Pcr Test To Identify Bacillus Subtilis And Closely Related Species And Its Application To The Monitoring Of Wastewater Biotreatment. *Appl Microbiol Biotechnol,* 56, 816-819.
97. Lee H[1], Kim HY. Lantibiotics, class I bacteriocins from the genus Bacillus. J Microbiol Biotechnol. 2011 Mar;21(3):229-35. PMID:21464591.
98. Chi H[1], Holo H[2].Synergistic Antimicrobial Activity Between the Broad Spectrum Bacteriocin Garvicin KS and Nisin, Farnesol and Polymyxin B Against Gram-Positive and Gram-Negative Bacteria.
99. *Mastromarino, Paola; Vitali, Beatrice; Mosca, Luciana (2013). "Bacterial vaginosis: a review on clinical trials with probiotics" (PDF). New Microbiologica. 36: 229–238. PMID 23912864.*
100. Falagas Me, Rafailidis Pi, Makris Gc. *Bacterial Interference For The Prevention And Treatment Of Infections.* Int J Antimicrob Agents. 2008 Jun;31(6):518-22.
101. A.Frabetti, A.Vandini, P.G.Balboni, F.Triolo, S.Mazzacane Experimental evaluation of the efficacy of sanitation procedures in operating rooms. American Journal of infection control – Vol. 37 n. 8 – pagg. 658-664 – October 2009.
102. A.Frabetti, A.Vandini, P.G.Balboni, F.Triolo, S.Mazzacane Experimental evaluation of the efficacy of sanitation procedures in operating rooms. American Journal of infection control – Vol. 37 n. 8 – pagg. 658-664 – October 2009.
103. S. Mazzacane, A. Frabetti, A. Vandini, D. Migliori, P. Balboni L'igiene nei reparti ospedalieri: correlazioni tra le procedure di sanificazione ed i fattori di contaminazione. Conferenza Nazionale ANMDO – 12,14 Settembre 2007, Rimini – pubblicato sulla rivista L'Ospedale.

104. A.Frabetti, A.Vandini, D.Migliori, A. Cusumano, E.Righini, P.Balboni, S. Mazzacane Efficacia ed efficienza dei protocolli di pulizia e disinfezione in sale operatorie Congresso ANMDO 2006 – Associazione Nazionale Medici Direzioni Ospedaliere - 21-24 settembre 2006, Lecce .
105. Frabetti, A.Vandini, S. Pantoja Rodriguez, F.Margelli, M. Cavicchioli, M.Migliori, D. Arujo Azevedo, , P.Balboni, S. Mazzacane. Microbiological risk in operating rooms: new strategies for infections surveillance Congresso Interazionale Environmental Risk, Bologna, settembre 2005.
106. Silvestri L, Petros AJ, Sarginson RE, de la Ca MA, Murray AE, van Saene HKF. Handwashing in the intensive care unit: a big measure with modest effects. J Hosp Infect 2005;59:172-9.
107. Sherlock O, O'Connell N, Creamer E, Humphreys H. Is it really clean? An evaluation of the efficacy of four methods for determining hospital cleanliness. Journal of Hospital Infection. 2009; 72(2): 140–6.
108. Lewis T , Griffith C, Gallo M, W einbren M. A modified A TP benchmark for evaluating the cleaning of some hospital environmental surfaces. Journal of Hospital Infection.2008; 69(2): 156–63.
109. Elizabeth A. Grice, Julia A. Segre (2011)The skin microbiome Nat Rev Microbiol. 2011 Apr; 9(4): 244–253. doi: 10.1038/nrmicro2537 PMCID: PMC3535073 NIHMSID: NIHMS424100.
110. Bhalla AMD, Pultz NJBS, Gries DMMD, Ray AJMD, Eckstein ECRN, David C. Aron MD, et al. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. Infection Control and Hospital Epidemiology. 2004;25(2):164–7.
111. McBryde ES, Bradley LC, Whitby M, McElwain DL (October 2004). "An investigation of contact transmission of methicillin-resistant Staphylococcus aureus". *J. Hosp. Infect*. 58 (2): 104–8. PMID 15474180 doi:10.1016/j.jhin.2004.06.010.
112. Holmdahl, Torsten (2017) Hospital Design and Room Decontamination for a Post-Antibiotic Era and an Era of Emerging Infectious Diseases—from a Macro to a Micro Perspective Published: 2017-01-0 Research Portal.
113. Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? Clin Infect Dis 2004;39:1182-9.
114. Pitzurra M, Savino A, Pasquarella C. Il monitoraggio ambientale microbiologico (MAM). Nota II. Ann Ig 1997; 9: 439-454.
115. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. J Hosp Infect 2000; 46: 241-256.
116. Dancer SJ.. How do we assess hospital clearing? A proposal for microbiological standard for suface hygiene in hospital. J. Hosp. Infect 2004; 56: 10-5
117. Hambraeus A., Bengtsson S., Laurell G. Bacterial contamination in a modern operating suite. 3. Importance of floor contamination as a source of airborne bacteria. J.Hyg.Camb. 1978; 80: 169-81
118. SaberYezliPhD[b]JamA.G.SalkeldBSc[b]Gary L.FrenchMD, FRCPath[a].Author links open overlay panel American Journal of Infection Control Volume 41, Issue 5, Supplement, May 2013, Pages S6-S11. https://doi.org/10.1016/j.ajic.2012.12.004.
119. Al-Hamad A, Maxwell S. How clean is clean? Proposed methods for hospital cleaning assessment.: J Hosp Infect. 2008 Dec;70(4):328-34. Epub 2008 Oct 9.

120. Griffith CJ, Cooper RA, Gilmore J, Davies C, Lewis M. An evaluation of hospital cleaning regimes and standards. J Hosp Infect. 2000 May;45(1):19-28 .
121. Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007 Jane D. Siegel, MD; Emily Rhinehart, RN MPH CIC; Marguerite Jackson, PhD; Linda Chiarello, RN MS; the Healthcare Infection Control Practices Advisory Committee.
122. Holmdahl, Torsten (2017)Hospital Design and Room Decontamination for a Post-Antibiotic Era and an Era of Emerging Infectious Diseases—from a Macro to a Micro Perspective Published: 2017-01-0 Research Portal.
123. EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives. *EFSA Journal* 226: 1-12.
124. Logan, N.A. (2004) Safety of Aerobic Endospore-Forming Bacteria. In *Bacterial Spore Formers: Probiotics and Emerging Applications* (Ricca E. et al., eds.). Horizon Bioscience, Wymondham, Norfolk, UK.: 93-105.
125. Hong, H.A., Huang, J-M., Khaneja, R., Hiep, L.V., Urdaci, M.C. & Cutting, S.M. (2008) The Safety of Bacillus subtilis and Bacillus indicus as food probiotics. *Journal of Applied Microbiology* 105: 510-520.
126. Sorokulova, I.B., Pinchuk, I.V., Denayrolles, M, Osipova, I.G., Huang, J.M., Cutting, S.M. & Urdaci, M.C. (2008) The Safety of Two Bacillus Probiotic Strains for Human Use. *Digestive Diseases and Sciences* 53: 954-963.
127. Sorokulova, I.B., Pinchuk, I.V., Denayrolles, M, Osipova, I.G., Huang, J.M., Cutting, S.M. & Urdaci, M.C. (2008) The Safety of Two Bacillus Probiotic Strains for Human.
128. Le H. Duc,Huynh A. Hong, Teresa M. Barbosa, Adriano O. Henriques and Simon M. Cutting Characterization of *Bacillus* Probiotics Available for Human Use doi: 10.1128/AEM.70.4.2161-2171.2004 Appl. Environ. Microbiol. April 2004 vol.70 no.4 2161-2171.
129. B. E. Kirsop Anne Doyle Maintenance of Microorganisms and Cultured Cells, Second Edition: A Manual of Laboratory Methods 2nd .ISBN-13: 978-0124103511 ISBN-10: 0124103510.
130. Boyce, J. M., Havill, N. L., Havill, H. L., Mangione, E., Dumigan, D. G. & Moore, B. A. 2011. Comparison Of Fluorescent Marker Systems With 2 Quantitative Methods Of Assessing Terminal Cleaning Practices. *Infect Control Hosp Epidemiol,* 32, 1187-93.
131. Katherine Donegan, Carl Matyac, Ramon Seidler and Arlene Porteous Evaluation of Methods for Sampling, Recovery, and Enumeration of Bacteria Applied to the Phylloplane. Appl Environ Microbiol. 1991 Jan; 57(1): 51–56. PMCID: PMC182663.
132. ISO 6888-2:1999 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -- Part 2: Technique using rabbit plasma fibrinogen agar medium.

133. Baird Parker, A.C. (1962). An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bact. 25 (1): 12-19. Beckers N.J. et al (1984). Cannad J. microbiol. 30: 470-474.
134. Iain L. Lamont, Lois W. Martin (2003) Identification and characterization of novel pyoverdine synthesis genes in *Pseudomonas aeruginosa* April 2003, Microbiology 149: 833-842, doi: 10.1099/mic.0.26085-0
135. Bauer, A. W., Kirby, W. M., Sherris, J. C. & Turck, M. 1966. Antibiotic Susceptibility Testing By A Standardized Single Disk Method. *Am J Clin Pathol,* 45, 493-6.
136. Clsi 2010. Performance Standards For Antimicrobial Susceptebility Testing: 20th Informational Supplement M100-S20. *Wayne, Pa: Clinical Laboratory Standard Institute (Clsi).*
137. Henderson DK (2006) Managing methicillin-resistant staphylococci: a paradigm for preventing nosocomial transmission of resistant organisms. Am J Infect Control. 2006 Jun;34(5 Suppl 1):S46-54: discussion S64-73.
138. Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H. Environmental reservoirs of methicillin-resistant Staphylococcus aureus in isolation rooms: correlation with patient isolates and implications for hospital hygiene. J Hosp Infect. 2006 Feb;62(2):187-94. Epub 2005 Nov 14.

Webliography

I. https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation
II. http://www.who.int/csr/bioriskreduction/infection_control/en/
III. www. Wikipedia® trademark of the Wikimedia Foundation, Inc., a non-profit organization edited on 17 May 2017
IV. https://www.cdc.gov/hai/surveillance/index.html
V. https://ecdc.europa.eu/en/surveillance-atlas-infectious-diseases
VI. https://www.gov.uk/government/collections/extended-spectrum-beta-lactamases-esbls-guidance-data-analysis
VII. https://www.theguardian.com/society/2017/feb/27/world-health-organisation-12-antibiotic-resistant-bacteria-threat-human-health
WHO names 12 bacteria that pose the greatest threat to human health Antibiotic resistance could make c-sections, transplants and chemotherapy too dangerous to perform, warns World Health Organisation
VIII. https://antibiotic.ecdc.europa.eu/en
IX. (http://www.salute.gov.it/portale/temi/p2_6.jsp?lingua=italiano&id=3558&area=biocidi&menu=biocidi)
X. European Committee for Standardization https://www.cen.eu/Pages/default.aspx
XI. UNI - Ente *Nazionale Italiano di* Unificazione http://www.uni.com
XII. www.*anmdo.org*
XIII. http://www.pvc.org/en/p/pvcs-physical-properties
XIV. http://www.kmdarchitects.com
XV. https://www.slideshare.net/dhobacyare/hospital-design-48571482
XVI. https://www.publichealthontario.ca/fr/eRepository/Public_Health_Inspectors_Guide_2013.pdf