

PROJECT REPORT

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PIP ALLERGY FREE MICROBIOLOGICAL VALIDATION

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INTRODUCTION

Within its range of probiotic cleaning products (Probiotics In Progress - PIP), **Chrisal** has developed a new textile spray – **PIP Allergy Free** – in order to strongly reduce the harmful effects of pathogens and dust mite allergens. In cooperation with Ghent University, Avecom and the Radisson SAS hotel Brussels, a comparative study was performed to verify the potential of the PIP Allergy Free to create a safe and healthy microbiological environment on the beds of the hotel.

In the performed case study, 2 rooms of the **Radisson SAS** hotel (Brussels) were treated during 1 month with Chrisal's PIP Allergy Free and microbiologically monitored by **Ghent University** and **Avecom**. Comparison was made with 2 similar rooms, subjective to the regular cleaning procedures of the hotel and not receiving the PIP Allergy Free treatment.

This report provides information on the prevalence of several bacterial groups in a hotel bed environment and clearly demonstrates the high efficiency of Chrisal's PIP Allergy Free in the control of (pathogenic) bacteria.

The report contains the following chapters:

- **PART 1: Project description**
 - o Product information: concept, safety, product range
 - o Study protocol: location, product application, microbiological analyses
- **PART 2: Project results**
- **PART 3: Conclusions**

PART 1

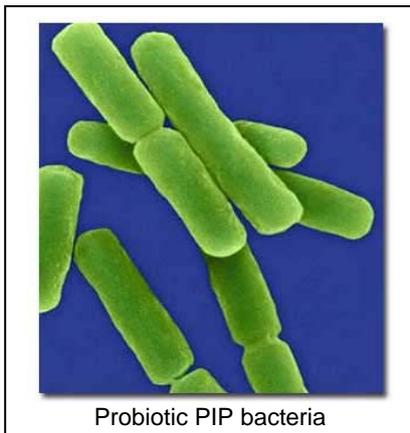
PROJECT DESCRIPTION

1. Product information

PIP Allergy Free belongs to the PIP product range of probiotic cleaning products, as developed by the company Chrisal. The mode of action and safety of the PIP products, as well as their advantages over disinfection are briefly explained in the following paragraph. Furthermore, the datasheet of the PIP Allergy Free is also presented.

a. Concept

A broad range of pathogenic (= disease causing) bacteria pose numerous health problems to humans. Some examples are *Clostridium difficile*, *E. coli*, *Enterococcus*, *Legionella*, *Listeria*, *Salmonella*, *Staphylococcus aureus* (MRSA) and *Streptococcus*. In addition to the dangers induced by these organisms in each of our (personal) environment, they are also responsible for large economic losses and costs (e.g. medical costs, health insurance). Using antibiotics and disinfectants, these problems could easily be controlled during the past decennia. However, the past years a rapidly increasing resistance against these 'miracle agents' has been noticed, to such an extent that a radical new approach is eminent.



By the creation of the **PIP – Probiotics In Progress – cleaning products**, Chrisal offers an innovative and sustainable solution to the emerging (resistance) problems with multiple pathogenic bacteria. The PIP philosophy is that of **microbial management**, in which no longer complete sterile environments are desired, but **a stable and healthy microbial community** is created. This can be achieved by means of probiotic bacteria. These are safe and useful bacteria, already known and exploited for many years in food and healthcare industry because of their health promoting properties to man and animal. All PIP products contain probiotic bacteria as a crucial ingredient, which possess the unique property of sporulation. This process makes it possible for these bacteria to survive harsh conditions and regain their activity as soon as environmental parameters improve. **PIP products are no disinfectants, but probiotic cleaning products.**

Especially in case of disinfectants, an important disadvantage is the unspecific action of these agents, killing both good and bad micro-organisms. This results in a surface free of organisms, allowing any remaining or newly arriving pathogen to bloom within a very limited timeframe. After all, each disinfection procedure leaves sufficient organic matter behind that may serve as carbohydrate and protein source to sustain a fast (pathogenic) recolonisation of the surface. Hence, disinfection results in a fast reduction of the number of micro-organisms of which, however, the effect is very short and unstable. Because of the current resistance problems, continuously increasing concentrations and

PIP Allergy Free – VALIDATION REPORT

frequencies of disinfectant have to be applied, which is detrimental to man and the environment due to their aggressive chemical nature.

Why are these problems not relevant to the probiotic PIP products?

During PIP cleaning, a layer of probiotic bacteria is placed on the treated surface, immediately occupying the 'field' by good bacteria. They will consume all remaining food sources, leaving nothing behind for potential pathogenic invaders looking for space and food. Because the probiotic PIP bacteria remain active for several days, the PIP cleaning procedure has a long lasting effect and creates stability in the microbiological results. **Given a minimal frequency of application, the products will replace most pathogenic bacteria by good bacteria, which will lead to a stable and healthy microbial (hospital) environment.** Because pathogenic organisms can not gain resistance against the PIP bacteria, the PIP cleaning products provide a sustainable solution to problems with various pathogenic bacteria.

The following table presents a conclusive comparison between disinfection and PIP cleaning:

<u>Disinfection/chemical cleaning</u>	<u>PIP cleaning</u>
- Risk at high number of pathogens	+ Constantly low number of pathogenic bacteria
- Short and local effect	+ Long lasting effect (stability)
- Resistance problems	+ No resistance possible (sustainability)
- Detrimental / unsafe products	+ Harmless / safe products
- Chemical / environment unfriendly	+ Biological / environment friendly
- Aggressive chemicals	+ Neutral composition

All PIP products are thoroughly validated by means of studies and/or clinical trials. The goal of this study was to evaluate the efficiency of the new **PIP Allergy Free** in order to create a safe and healthy microbial environment in hotel beds. An easy and short spraying of the **PIP Allergy Free** over the beds during each cleaning procedure was compared to beds only receiving the regular cleaning.

b. Safety aspects

All PIP products are completely safe to use for several reasons:

- The probiotic bacteria used in the PIP products are members of the genus *Bacillus* and belong to biosafety class 1, as listed by the American Type Culture Collection (ATCC). The following table presents all four biosafety classes:

Class	Description	Risc
1	Non-pathogenic micro-organisms	None
2	Micro-organisms and parasites that may cause disease, but with an unlikely spread and for which efficient prophylaxis or treatment exists.	Low
3	Micro-organisms and parasites that are able to spread and cause disease, but subjective to efficient prophylaxis or treatment	Average
4	Micro-organisms and parasietes with large scale spreading and serious illness, for which no prophylaxis or treatment exists.	High

- A number of probiotic *Bacillus* species have been granted the GRAS (Generally Recognized As Safe) label by the Food and Drug Administration (FDA) and can as such be used freely for human purposes.
- The PIP bacteria belong to the group of sporulating probiotics, of which over hundred commercial pharmaceutical and nutritional products are available for human oral consumption. A regular dose of these preparations is 10 billion bacteria per day, which is about 10.000x more concentrated than Chrisal's PIP products.
- Additional to the safety classification by ATCC, the producer of our bacterial strains performed a large number of toxicity test to guarantee the safety of our bacteria. No single toxic effect from our *Bacillus* strains was measured, as certified by the SGS testing company.
- Chrisal itself performed multiple safety test in collaboration with external and accredited laboratories. All PIP products are certified as safe to use.
- Members of the genus *Bacillus* are used intensively in different kinds of industries because of their high enzyme production capacity. Examples are washing powders, waste water treatment, food preservation,...

In conclusion, the probiotic PIP bacteria are perfectly safe to use. These organisms have been officially classified as save organisms and have been used for decades without any negative effect. Although during the course of this study the hotel guests did not come into contact with the cleaning products themselves, a direct contact with the PIP bacteria was possible by means of the treated surfaces. Given the fact that the amount of PIP bacteria is not higher compared to the average encountered microbiota on these surfaces, such contact only results in lesser pathogens that might be picked up by the guest.

c. Product data sheet

PIP Allergy Free

Improve Health quality

PIP Allergy Free is a textile spray to strongly reduce the harmful effects of germs and dust mite allergens. PIP Allergy Free creates a stable and healthy microbial environment through the introduction of probiotic bacteria. Due to its neutral composition, PIP Allergy Free is completely harmless and can be safely applied to all textile products. PIP Allergy Free is biologically degradable, non-inflammable and non-volatile.

PIP Allergy Free

Composition:	In conformance with E.C. regulations: Anionogenic surfactants <2% Additional content: Preservative and probiotic bacteria.
Operating instructions:	Always <u>shake</u> thoroughly before use. <u>Economically spray</u> the surfaces to be treated. Example: a 3 second spray to treat a bed. Always keep the spray can <u>vertical</u> during spraying.
pH:	Concentrate: 6,5
Product number/ Packaging:	PIP36070 J 400 mL
UN – Nr:	/
Protective measures:	Inhalation: / Skin: / Eyes: /
Risk remarks:	/
Safety remarks:	Spray can under pressure. Keep protected from direct sunlight and temperatures higher then 122°F. Don't perforate or heat the spray can. Don't spray in the direction of open fire or glowing objects. Keep away from children.

2. Study protocol

The goal of this study was to evaluate the efficiency of the new **PIP Allergy Free** in order to create a safe and healthy microbial environment in hotel beds. An easy and short spraying of the **PIP Allergy Free** over the beds during each cleaning procedure was compared to beds only receiving the regular cleaning.

In the Radisson hotel, Brussels, the hotel staff was interested in testing the **PIP Allergy Free** on the bed linen, in order to create certified pathogen-free rooms. A study was performed from half June until half July 2007, in order to evaluate the effect of daily spraying with the product **PIP Allergy Free** on the bed linen and to assess the decrease of pathogenic bacteria.

In total, four rooms were monitored during the four-week period: 2 rooms with regular cleaning (rooms 522 and 539) and 2 rooms with daily **PIP Allergy Free** treatment (rooms 515 and 516). The bacterial counts from all 4 rooms were compared in order to demonstrate the potential decrease of pathogenic bacteria due to **PIP Allergy Free** treatment.

The subsequent part of this report provides an overview of the study protocol and addresses the following items:

- Location: information on Radisson SAS, pilot hotel for this study
- Cleaning schedule: time schedule and hygienic guidelines about the cleaning during the course of this study.
- Microbial analyses: micro-organisms which were monitored and the sampling procedures applied.
- Report: the interpretation of the results.

a. Pilot hotel: Radisson SAS

Tests were performed at the 5-star SAS Radisson hotel in Brussels. The hotel already provides a number of Allergy-Free rooms for sensitive guests. In relation to this study, there exists an interest in expanding this service to pathogen-free rooms.

b. Treating schedule

For this study, four similar rooms on the 5th floor of the hotel were selected:

- 2 rooms served as control, with normal cleaning.
- 2 rooms were subjected to **PIP Allergy Free** treatment, additional to normal cleaning.

Note that all regular cleaning procedures were maintained during this study.

The **PIP Allergy Free** treatment only involved a 2 second spray on the mattress covers of the beds in the test rooms.

c. Microbial analyses

All microbiological analyses were carried out by the Laboratory of Microbial Ecology and Technology (LabMET - Ghent University), in collaboration with the consultancy company Avecom. The latter supervised the test protocols, the sampling, the analysis and also the final reporting.

All sampling points, frequencies and type of microbial analyses were identical for the 4 rooms throughout the study.

Sampling procedure:

Samples were taken by means of sterile contact plates of 30 cm², moisturized by means of 3 ml of sterile physiological solution per plate. After 3 minutes of contact with the surface, each plate was transferred to sterile Petri dishes and transported to the lab for microbial analyses. Each sampling was performed in triplicate in order to deliver statistically significant data.

Sampling points:

Sampling points were at all times identical for the four rooms. Each sampling day, 9 points were sampled on the mattress cover of the beds in every room.

Sampling time and frequency:

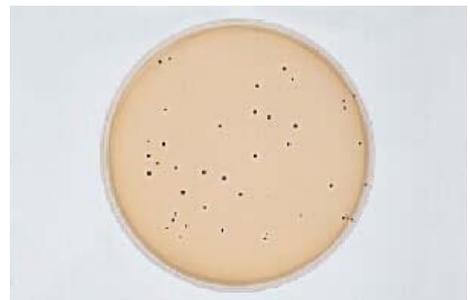
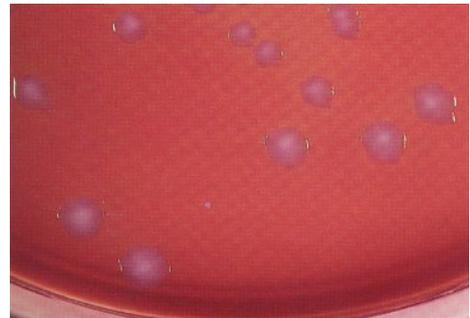
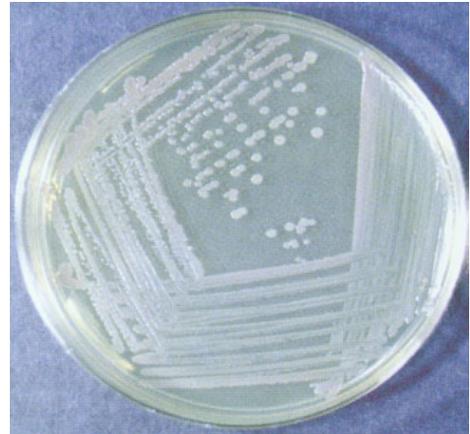
The actual treatment with Chrisal's **PIP Allergy Free** started on **Wednesday the 20th of June 2007 at 12.30 pm**. From that day onward, samples were taken twice a week. **Sampling time was 12.30 pm**.

Microbial analyses:

All samples were transported to the Laboratory of Microbial Ecology and Technology (LabMET) of the Ghent University. Upon arrival, contact plates were immediately placed on selective growth media for three minutes, after which these media were incubated at the proper temperature and atmosphere. After the proper incubation time for each of the organisms to determine, colonies on all plates were manually counted.

The following growth media were used:

- Trypticase Soy: Non selective medium for the determination of the **total count** of bacteria on the sampled surfaces. All colonies were counted. These values provide information on the amount of PIP bacteria that remain on the treated surfaces.
- McConkey: Selective medium for the quantification of **coliform bacteria**, with *Escherichia coli* as type organism. On this medium, all colonies were counted. This provides information on the fecal contamination of the sampled surfaces.
- Baird Parker: Selective medium for the determination of ***Staphylococcus aureus***. Positive counts are visible as brown, halo-surrounded colonies. These counts provide information on the potential MRSA load on the sampled surfaces.



d. Report

All communications and reports have been provided by LabMET, in collaboration with Avecom as indicated above. The obtained results were transferred to Chrisal, in order to evaluate the proceeding of the study concerning efficiency and safety to the guests and personnel.

After finishing the study, Avecom collected all of the generated data during the study in order to prepare the final report (i.e. the present document).

e. Conclusions

This study was initiated and financed by the company Chrisal. The goal of this study was to evaluate the efficiency of the new **PIP Allergy Free** in order to create a safe and healthy microbial environment in hotel beds. An easy and short spraying of the **PIP Allergy Free** over the beds during each cleaning procedure was compared to beds only receiving the regular cleaning. In order to obtain reliable results, all microbial analyses, data processing and reporting were outsourced to the joined team of LabMET and Avecom.

A presentation and discussion of the obtained results can be found in part 2 of this report.

PART 2

RESULTS

1. Introduction

This part of the report presents the results obtained during the course of the study.

An overview is given of the microbiological results of the four **fixed sampling points (i.e. rooms 515, 516, 522 and 539)**. These are presented as bar plots over time, with separate graphs for each of the evaluated bacterial groups: total count, coliform bacteria and *Staphylococcus aureus*. Each graph contains the number of colony forming units per square meter of surface (= **CFU/m²**). Values present the results from mattress sampling in the control rooms (black bars) and the PIP Allergy Free treated rooms (green bars).

All **results are the average values of triplicate sampling and analysis**. These threefold analyses provide a standard deviation, demonstrating the statistical significance of each measurement. These deviations are presented by means of error flags in the graphs.

2. Microbiological results

i) Total count

During the treatment with **PIP Allergy Free**, the total number of viable bacteria on the treated beds remained constantly above those of the untreated beds with a factor of about 25-fold. The evolution of the total count over the study period is shown in Fig 1. Mattresses in Rooms 515/516 were treated with PIP (green bars). Day 1 represents the initial situation before the actual start of the PIP-treatment. From day 2, a strong increase in total bacterial count was recorded. This was mainly due to the presence of the probiotic PIP bacteria, applied on the beds by means of the **PIP Allergy Free**. The PIP bacteria become the dominating bacteria on the mattresses.

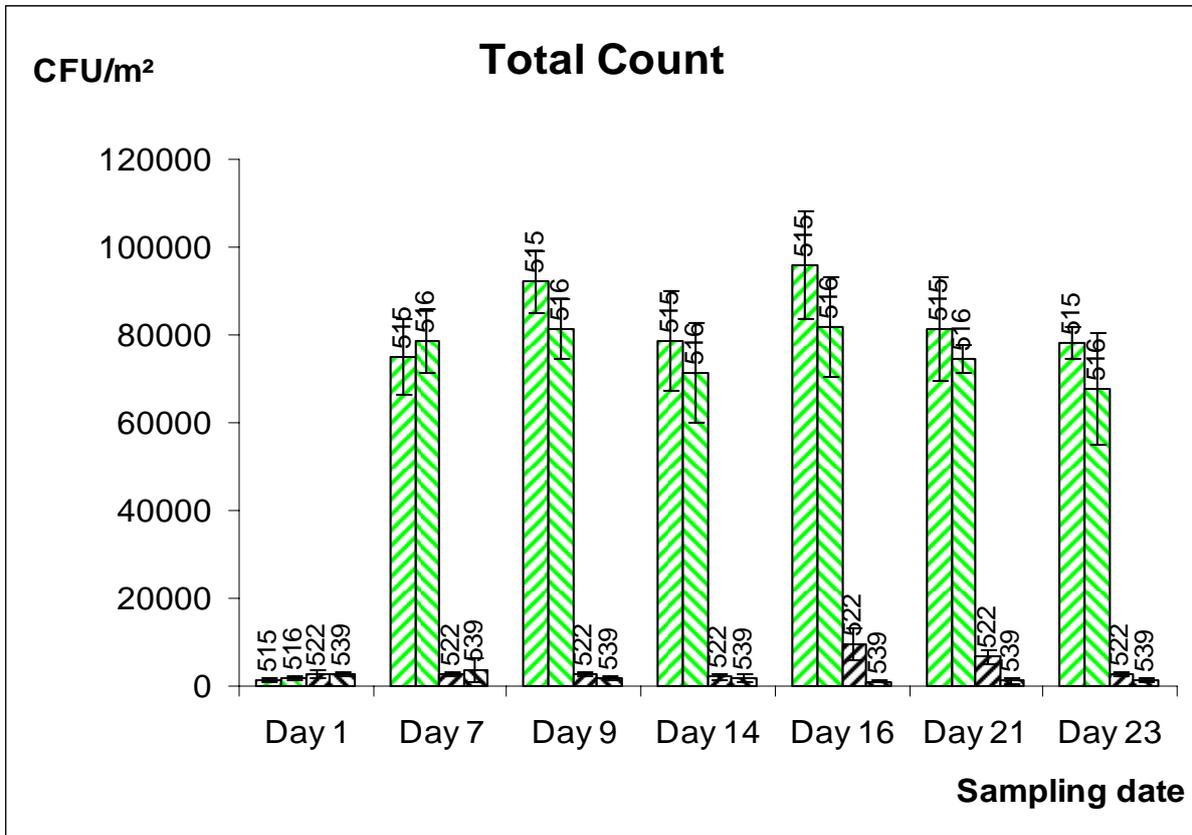


Fig 1. Total number of bacteria on the beds treated with the **PIP Allergy Free** (green bars, rooms 515 and 516), compared to the untreated beds (black bars, rooms 522 and 539).

ii) Coliform bacteria

The number of coliform bacteria decreased on the beds during treatment with **PIP Allergy Free**. Mattresses in Rooms 515/516 were treated with PIP. Day 1 represents the initial situation before the actual start of the PIP-treatment. During PIP treatment, the coliform bacteria decreased on average with 50%, compared to the untreated beds. The effect became stronger as the period of treatment prolonged.

The recorded decrease of coliform bacteria during **PIP Allergy Free** treatment indicates that a more hygienic environment on the beds is created.

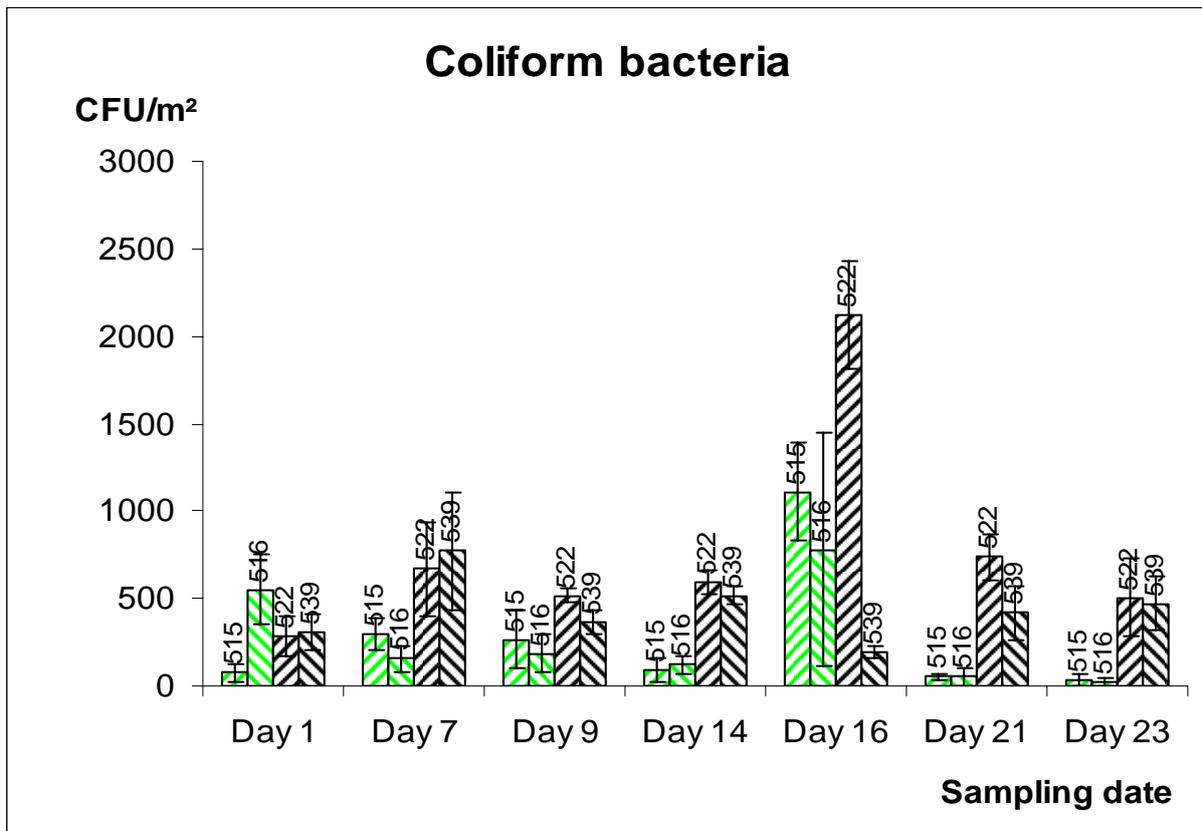


Fig 2. Number of coliform bacteria on the beds treated with the **PIP Allergy Free** (green bars, rooms 515 and 516), compared to the untreated beds (black bars, rooms 522 and 539).

iii) *Staphylococcus aureus*

In Fig 3, the evolution of the *Staphylococcus aureus* count is shown for the test period in the hotel. Mattresses in Rooms 515/516 were treated with PIP. Day 1 represents the initial situation before the actual start of the PIP-treatment. During PIP treatment, the number of *S. aureus* bacteria decreased on average with 84%, compared to the untreated beds.

The recorded decrease of *S. aureus* bacteria during **PIP Allergy Free** treatment indicates that a safer environment on the beds is created.

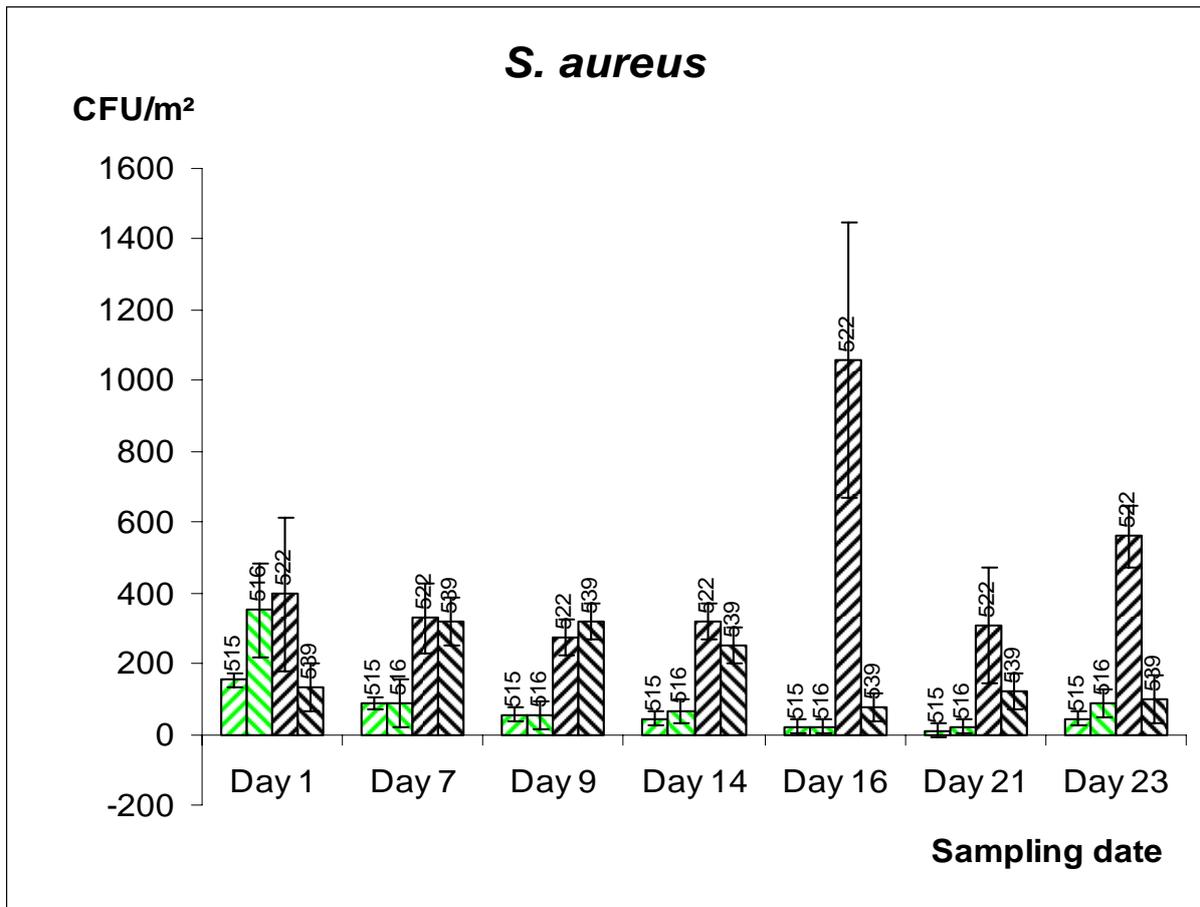


Fig 3. Number of viable *Staphylococcus aureus* cells on the beds treated with the **PIP Allergy Free** (green bars, rooms 515 and 516), compared to the untreated beds (black bars, rooms 522 and 539).

PART 3

CONCLUSIONS

Overall conclusion:

Daily spraying with Chrisal's **PIP Allergy Free** on the bed linen and mattresses of hotel beds, results in the following:

- The total number of bacteria on the mattresses increases. This is mainly attributable to the probiotic bacteria present in the **PIP Allergy Free**.
- Coliform bacteria (indicators for hygiene) decrease on average with 50% during the treatment with **PIP Allergy Free**.
- *Staphylococcus aureus* (opportunistic pathogen) decreases on average with 84% during **PIP Allergy Free** application.

The **PIP Allergy Free** is a straightforward way of applying the Probiotics-In-Progress concept. The probiotic PIP bacteria keep pathogenic bacteria, such as *Escherichia coli* (an indicator of fecal pollution) and *Staphylococcus aureus* (potential pathogen) under control. **PIP Allergy Free creates as such a safer and more hygienic environment in hotel rooms and beds. This was demonstrated in the SAS Radisson hotel during a 1 month case study.**

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