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Thesis Title

Environmental Health Indicators. Proposal of a measurement scale for verification of the effectiveness of cleaning/sanitizing processes/disinfection in nosocomial environment

Curriculum Architecture (SSD ING-IND/10)

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Premise

Preventive action plans developed in hospitals are mainly focused on infection control. This is of particular relevance due to the strong impact of nosocomial infections on economic and health costs for both healthcare systems and individuals, moreover they are indicators of the quality of service offered to patients. Infections often occur during hospital stay or after discharge of the patient admitted, thus leading to prolongation in the hospital stay and in the perduration of antibiotic therapy [1].

In high income countries, including Western Europe, about 5-15% of hospitalized patients develop at least one HAI (Healthcare Associated Infection) during the hospital stay [2,3,4]. This represents a massive additional financial burden for health systems, generates high costs for patients and their family, and causes unnecessary deaths. In according to prevalence study conducted by the ECDC (European Centre for Disease Control) in European acute care hospitals about 3.2 million patients acquire a healthcare infection associated to the hospital stay [5]. Furthermore, it is estimated that each year 4.1 million patients contract an HAI, and about 37,000 deaths occur as a direct consequence of these infections, while are a contributing factors to 110,000 [6]. In Italy, the HAIs incidence varies from 5 to 10% of overall and infections caused by antibiotic-resistant microorganisms are becoming increasingly more common, with a mortality rate of 20-30% [7]. Most frequently, HAIs include infestions that strike in the urinary tract, in surgical site, in the lungs, in blood (bacteriemia) and in the intestinal tract. Italian studies performed in over 50 healthcare structures confirmed these Infections as the most common and internal medicine wards are the most involved in the phenomenon [7,8].

World Health Organization Guide [9] defines the nosocomial infections as infections acquired during hospital stay which are not present or incubating at admission, but occurring after more than 48 hours from hospitalization.

According to some studies [1,10,11] the microorganisms responsible of healthcare-associated infection (HAI), such as *Staphylococcus* spp., in particular *Staphylococcus aureus*, *Enterobacteriaceae*, *Pseudomonas* spp., *Candida* spp. and *Acinetobacter* spp., are the ones that frequently survive on surfaces and that can be transmitted to patients as a consequence of direct contact or conveyed by health personnel and visitors and/or by contact between patients. According to the latest report by ECDC [5] “Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011-2012”, the microorganisms isolated from patients who developed a HAI (healthcare associated infections) are: *Escherichia coli* (15.9%), *Staphylococcus aureus* (12.3%), *Enterococcus* spp. (9.6%), *Pseudomonas aeruginosa* (8.9%), *Klebsiella* spp. (8.7%) and *Clostridium difficile* (5.4%). Also, on the list published by WHO (World Health Organization) in February 27th, 2017 it should be noted that the number of microorganisms who develop antibiotics resistance are growing. In order of priority has been reported *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*. These are Gram negative bacteria and can cause serious infections such as pneumonia and septicemia in immunocompromised patients, like transplant recipients or the elderly. The most dangerous bacterial strains have become even resistant to carbapenems, which are only class of antibiotics that can still effectively eliminate them. In the following list those patogens are mentioned: *Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter spp*, *Salmonella* spp. and *Neisseria gonorrhoeae.* Finally, *Streptococcus pneumoniae*, responsible for pneumonia and meningitis, *Haemophilus influenzae*, which causes ear infections, and *Shigella*, responsible for intestinal infections. According to the alarmist report "Review on Antimicrobial Resistance", drawn up by British Prime Minister David Cameron in 2014, it is expected that in 2050 the infections, for which appropriate drugs will be not available, may cause one death every 3 seconds, until it get to kill at least 10 million people [12].

Bacteria that cause hospital infections can be acquired in various ways or they are present in the normal human microbiota. These can cause endogenous infection following their broadcast from external sites, to tissue damage (wounds) or exponential growth due to inappropriate antibiotic therapies [9].

Different types of those microorganisms, in optimal microclimate conditions, can survive well on various objects, water and other fluids, linens, equipment and supplies used in the healthcare, as well as in fine particles and droplets generated during talking or coughing. Just consider that bacteria less than 10 μm in diameter remain in the air for several hours and they can be inhaled.

Microbial organisms and several types of particles are conveyed by air and can be inhaled or deposited directly into the patient or on adjacent surfaces [1,10,11]. Some studies have shown a positive correlation between the number of microorganisms and the amount of dust whose organic components, in favorable microclimatic conditions, are a source of nutrients for the development and proliferation of bacteria, fungi and mites [1,13].

Regarding the inorganic particles, the risk increases with size, exposure time and concentration. In fact, while the particles known as PM10 (about 10 μm in diameter) can be inhaled and placed in the upper respiratory tract, between nose and larynx, otherwise, fine particles (2,5-0,1 μm in diemeter) and ultrafine particles (0,1-0,01 μm in diameter) remain dispersed in the air and, being less subject to gravitational precipitation, can penetrate patch and/or breath, thus they can accumulate in the trachea, in lungs and the pulmonary alveoli, favoring the onset of various diseases, particularly those affecting the respiratory and cardiovascular system. The main sources from which the powders originate are humans and their indoor activity and/or production processes, which can release several micro-materials such as dead skin cells, hair, food residues and fibers. Further sources are wear or erosion of furnishings, finishes and construction materials.

The application of good hygiene practices, such as hand hygiene and the use of Individual Protection Devices (DPI) and proper application of healthcare practices can reduce the risk of transmitting or acquiring infections. Also, keeping the microclimatic parameters, such as temperature, relative humidity, wind speed and number of air changes within optimal values and the periodic maintenance of air conditioning systems, can inhibit growth of the microbial indicator resident there. To assess whether the contamination level in nosocomial environment is into acceptable limits, periodic monitoring actions are performed in order to identify the bacterial species present on surfaces and in the air. However as today, regarding hospital surfaces, standards or guidelines where threshold values are defined beyond which dangerous or unhealthiness conditions are established, does not exist. The limits existing as rifererence are not rapresentatives because they were obtained by tests carried out in high sterility environments, so they differ a lot from a healthcare environement where continuous recontamination phenomena are present. There is a need to evaluate the hygiene level through an objective assessment of the effectiveness of sanitization and the definition of the benchmarks. This explains the growing interest in the role assumed by the sanitation procedures in relation to the management of hospital infections (HAI), as proper sanitization procedures actions might help to control the proliferation of potentially pathogenic environmental microorganisms [14]. Finally, we must consider that chemical products, traditionally used in the sanitization practices, have a limited effectiveness over time, i.e. they immediately act after cleaning, they have a significant environmental impact and their widespread use may lead to selection of resistant microbial strains.

Research objectives

The main purpose of this research is to identify a scale of values of microbial contamination to be proposed as an objective measure of environmental hygiene in hospital stays. The intent is to define a sampling protocol and standardize the criteria for reading and interpretation of the results, considering not only the total number of microorganisms (Total Vital Count) as in literature, but to differentiate between the several strains of microorganisms responsible for the increased number of healthcare-associated infections. This will allow to monitor some changes in normal bacterial burden and implement contingency plans, identifying the potential source, such as an incorrect application of the healthcare associated practices, the failure to compliance with good hygiene practices, the ventilation systems malfunction, etc. The procedures and sanitizing products effectiveness will be assessed, comparing the CFU (Colony Forming Unit) values, sampled before and after use of probiotic products for daily cleaning compared to traditional chemical compounds, in order to detect the reduction and/or biostabilization of the microbial load in the short and long term. The collection of data obtained and implemented in a database that will constitute the basis for a study that can correlate the presence of some pathogenic species with infectious events that occur in the same departments and time periods.

CHAPTER I

1.1. Historical origins and evolution of health places.[15]

The etymology of the word ‘hospital’ indicates the dual function that this kind of structure had since ancient times, which is a needy shelter and sick care. In fact, the Latin word *hospitale* originates from the hospitality tradition and designates a place where guests or strangers are accommodated. While the Greek word *nosokomeion* means the place where the sick people are collected and treated. We cannot locate a precise historical period to place the inception of the hospital as a type of building dedicated specifically to dyagnosis and terapy of diseases, rather than a place consecrated to deity, to the reception of the poor and needy, not just sick. In fact, ancient medical practices were based on Greek-Roman concept of care of the body to prevent disease and as an aid to improve the conditions of the sick person, and often was a purely divine, and the prayer was the only way for healing, while the priests were limited to the use of empirical therapies. Near the temples, which have become place of hospitality, buildings were made that contained simple cells to accommodate the sick person along with some areas for physical exercise, the ablutions and the frictions for chronic patients or convalescent: a kind of primitive physical therapy. In Greek culture and later in the Roman period, the first "sanitary" structure is the *Asclepeium,* a temple dedicated to the God *Aesculapius.*These monumental temples were generally made up of a wide undifferentiated space where the sacred functions and the therapies alternated. In fact, both the sleep and the mystic contact with the deity were deemed essential to the healing process. In later period, restored by Romans, this health temple is divided into functional spaces and is surrounded by ancillary buildings such as an outdoor swimming pool and a two-story building with round basins for thermal treatments. The space distribution, the presence of internal and external staircases, and the hydraulic mechanism to replenish bathtubs water, indicates the existence of functional, safety and hygiene criteria. In the 5th century, Hippocrates (Coo 460 B.C. – Larissa 377 B.C.), founder of medical science, introduced the concept of "modern" medicine that not only takes on an increasingly "laic" connotation but becomes an empirical practice, a *téchne* based on the scientific method. His most famous written work is the “Oath” that encodes the medical ethics and still physicians and dentists take before beginning professional practice. This text is the first attempt to overcome the sacerdotal medicine in favor for patient's clinical study. In fact, he believed that only a global consideration of the entire context of the sick person's life provided insight and could defeat the disease, whose various manifestations might otherwise be destined to remain enigmatic. This examination must also be extended to the past (*anámnesis*, remember), in order to locate the pathology (*diágnosis*, knowledge) and reasonably to hypothesize the progress (*prógnosis*).

Often the patients were cared at home or at physician's home in places called *iatrieiae* (Greek) or *medicatrina,* these latter were nursing homes with some areas available for patients' observation in Rome. About the origins of the hospitals, beside the *Asklepieion* and the *iatreion*, the military infirmaries, named *valetudinarii*, deserved an important role: in those places, medicine became more practical and functional to resolve the problems posed by "military pathology". These infirmaries were designed and manufactured as fuctional structures to specific requirements during the war. The military infirmary represents the first example of designed-to-health activities strictly as evidenced by archaeological discovery of the Roman military infirmary at Novaesum near Dusseldorf (100 A.D.) (Fig 1). This is an example of the planning of a sanitary building, whose type results essentially from the activities performed. The constructive policy relies on rationalizing the path, on the deploying of common areas, such as vestibule, refectory, administration and by the concentric use of the recovery and treatment areas.

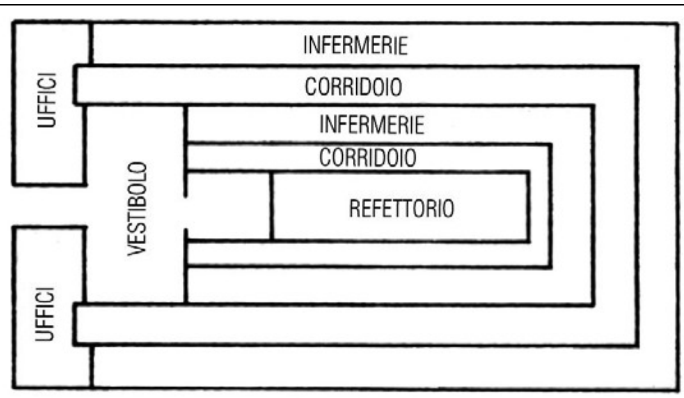


Fig 1. Map of the Roman military infirmary at Dusseldorf, 100 AD. (Capolonogo 2006)

The *valetudinarii,* generally rectangular shaped, consisted of rooms and hallways distributed around the building perimeter, in the middle were located the courtyard or enclosed spaces with different functions as the refectory. Medical thinking of ancient world reached its apex around the 2nd Century A.D. with Galen of Pergamum (Pergamum 129 - Rome 201). In continuation of the Hippocratic principles and giving relevance to the direct observation of the sick person, Galen developed a doctrinaire system that actually paralyzed the research progress and the advancement of the medical field with its rigidity and dogmatism, until the late 17th century.

A true healthcare system was created in Europe during the Middle Ages, about as a result of the consolidation of Christianity and around the aethical principles of charity. In fact, following the Nicaea Council, convened by the Roman Emperor Constantine I in 325 A.D., it was determined that in the bishoprics and monasteries of each city, a poorhouse and sick people shelter should be built. The " hospitable places ", named *Xenodochia* and differentiated in according to the type of heathcare provided spread. In 534 A.D., an update of the Justitian *Codex* is drawn up by order of the Byzantine (East Roman Empire) emperor Justinian, in wich a series of institutions ere listed, each of which with its own purpose: the orphanage, the geriatric homes and the hospital. Then, from the next fourth century onwards, following the establishment of the mendicant, religious and military orders, the shelters had to be set up near the monasteries, Episcopal sees and, generally, along the main communication routes and pilgrims' itineraries, named *Hospitale Pauperum et Pellegrinorum*, where the poor and sick people were put together. Monastic hospitals were inside in care facilities of the monasteries, where areas of the building were designated and organized for the purpose. In case of new construction, the architecture of care centres was based on the Basilical type. The medieval hospital building typology is therefore subject to the characteristic of the site it where it was built, and in monasteries the healer role was carried on by the priest who administered the therapies not only on the body but mainly on the soul. Into the infirmaries of the Abbey, at the same time of prayers, pharmacological and surgical therapies were performed, the first having been made as herbal preparations such as lotions and potions, the latter being consisted in salassi, engravings and burns with iron red-hot. These conventual or episcopal hospitals functioned as unsorted containers where beds were prepared, and healthcare took place at the chapel or at the altars on the sides of the main nave. Such places were located away from the centers of daily life. From 1000 onwards, happened the birth of several major medical schools, such as the one of Salerno and subsequently the one of

Bologna, the last considered the prototype of the first universities that between the 13th and the 14th centuries were actually built. Because of the scarcity of places to study and exchange knowledges in medical field, these schools became a magnet for scholars from all over Europe. But the therapies actually consisted mainly in body hygiene. Among the notable organisms in early Christian you can mention the great infirmary annex to the Benedictine convent of Saint Gall (820 A.D.). It is a complex structured in a way that the religious body (the Abbey Church with cloister) constituted the core around which were articulated a number of outbuildings such as rooms, refectories, poorhouses, restrooms, doctor’s lodgings, schools, according to a concept of modernity that anticipates the pavilions hospital. Observing the plant shown in Fig. 2 it is clear that the complex was not built like a single block but the individual buildings were annexed later, adding them one after another according to the possibility and necessity. The school was made up of twelve places for lectures, grouped around a Central Hall divided into two parts. The convent was known as intellectual center, as shown by the extensive collection of books preserved in the library.

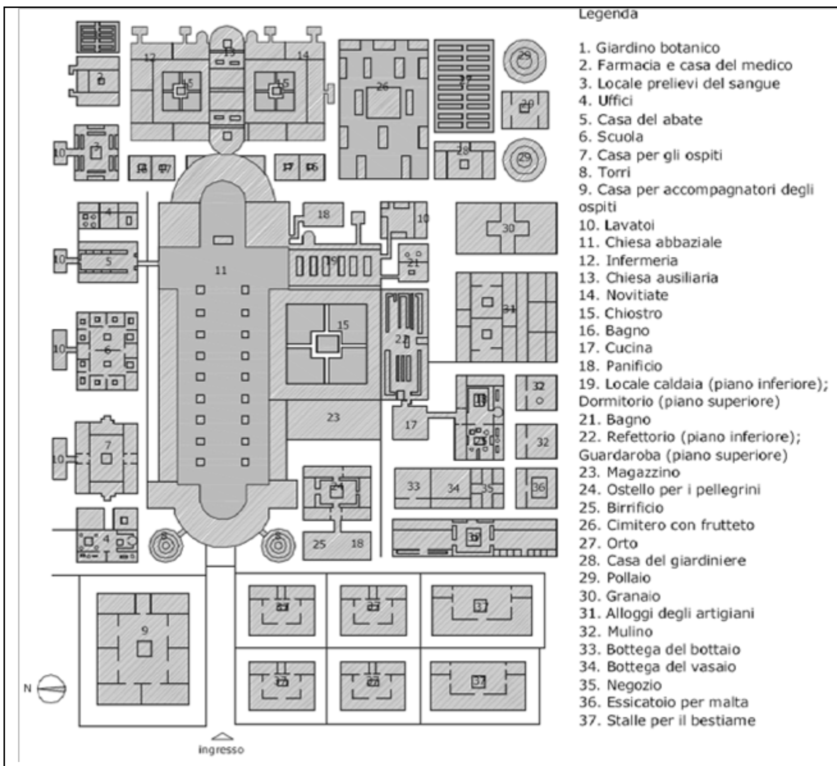
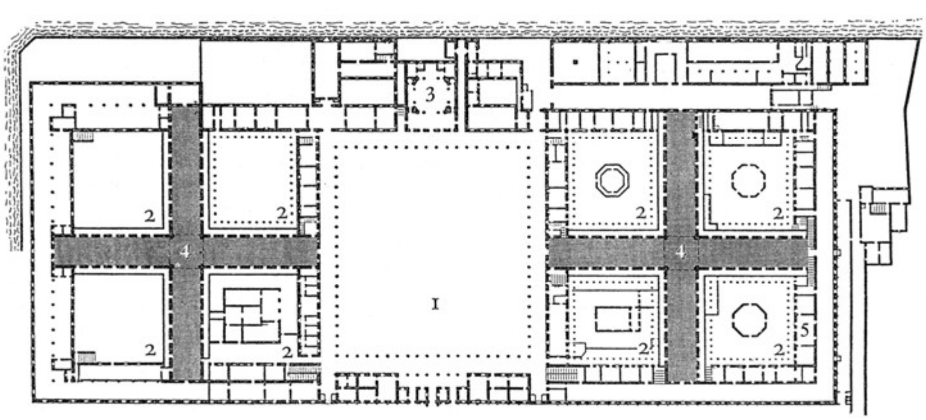


Fig. 2. Plan of the Abbey of St. Gall (Switzerland), 13th century. (Capolongo 2006)

The health complex is much more developed than the needs of the convent, so it is reasonable to think that this was a real hospital able to accommodate the sick people in an organized manner. It's only around the mid-14th century, with the spread of the plague and the consolidation of the secularism for medical profession, that the sick people were actually separated from the poor. On this occasion the State intervened to establish health departiments with the task, through officials and inspectors, to keep tabs on the possible sources of infection. So the healthcare is transferred from the Church to the State, which from now on will be in charge to organize, to monitor and to administrate the hospital.

Between the 14th and the 15th centuries, a transformation of health places has taken place, that became different from those of care. In addition to the distinction between the poor and the sick person, among these the differences between acute, chronic and infected patients were pointed out. These different kinds of ill people were associated with different hospitals or spaces of the hospital building where appropriate care and terapy were done. In such way, the chronic shelters were born and also the hospitals for the acute patients and the hospitals for the infected. During the Renaissance happened many political, religious and social changes, the vision about the man and about the nature varied and, consequently, the concept of hospital is modified, becoming a place with its own organizational, spatial and functional characteristics that make it to stand out from other building types. In the mid-15th century the current hospital type is characterized by the rooms of the Greek or Latin cross form with the altar at the head of the main aisle or in the intersection between the arms. An examples is the Saint Lucas Hospital of Brescia (1447), the Hospital San Matteo of Pavia (1448), and the civil hospital of Mantova. At the same time as the cruise type, were still widely used is the building types with a nave and rectangular courtyard. The first, derived from Romanesque-Gothic architecture, was used mainly for small facilities, such as Brunelleschi's Hospital of the Innocents (1419). The type with courtyard such as the Saint Louis hospital of Paris in the early 1600s, according to some texts, was inspired clearly by monasteries and churches cloisters, but according to other authors it originates instead from the cross-shaped scheme inscribed in a square. In Milan, following the economy revival and the reorganization of urban and social reality Antonio di Pietro Averlino named Filarete (sculptor, architect and architectural theorist Italian Florence, 1400 about - Rome 1469) starts an urban and architectural transformation of the city, in order to achieve the building of the ideal city of Sforzinda, commisioned by the duke Francesco Sforza. This project was named "Hospital Reform” and was wanted by the Archbishop Rampini, in the schedule of reorganization of the care functions of the city also included the construction of the *Cà Granda* hospital. The *Cà Granda* facility (Fig. 3) is conceived and designed for the care and terapy of acute patients, while chronic those were admitted to other hospitals, which are in turn intended to special categories: fools, abandoned children, etc. Filarete's project serves the needs of efficiency of the hospital organism and the new concept of separation and classification of patients led to the creation of a new architectural typology: the infirmaries with crossing, that although represent an innovation in the arrangement of the spaces yet they still refer to tradition. The Filarete's plan foresaw two crossings, one for men and another for women, divided by a rectangular courtyard with a church in the Center. Filarete recovered the features of the Renaissance building: the orthogonality, the symmetry, the order, the search for morphological and spatial balance between the parties, and he studied a square form module repeated ten times: four modules for two crossing and two for the central courtyard. It is a large building, arranged symmetrically to a central axis that traverses a big arcaded courtyard and ends with a church. On th other side of the courtyard there are two square modules featuring a crossing central building dedicated to hospital stays, four smaller and squared courtyards, with spaces dedicated to *officinae* and houses arranged around the perimeter. The "module" placed to the south was made immediately, the one to the North was added some centuries later. In the middle of the two buildings there is an altar.



1. CENTRAL COURTYARD
2. SIDE COURTYARDS
3. CHAPEL
4. CROSS FOR SICK PEOPLE

Fig. 3. *Cà Granda* Hospital, Milan 1456. (Capolongo, 2006)

At the point of intersection of the arms of the infirmaries, the altar was placed so that the residents could follow the mass from their own bed, showing a continuity with medieval religious tradition.

For the first time the architecture is functional therapy, by the use of advanced building techniques, such as water and sewage disposal, the sanitary facilities (sanitation), ventilation and placement of the furniture. One of the features that came up with Filarete was to equip the infirmaries with toilets. Each bed was placed between two access doors to the latrines so this order was insitituted: one sick person, one bed, one latrine. In addition to the gateway to the latrine, between a bed and the other one, a small cabinet is built in the wall, with a sloping bottom and a small gutter, so that the bog could be clean by water streaming. To ensure cleanliness of the latrine's channel, in addition to dark water, the water from the Naviglio is scrolled through a system of '*sifonature'* and also a system to convey rainwater from downpipes also was made. Filarete solved the ventilaztion problem in the infirmaries, without creating annoying air currents, that was considered dangerous for the sick person, by placing windows to a higher altitude, so the cold air entering through windows streamed above the service corridor (being it much less tall than the infirmary, tended to go down, while the "foul", warmer air coming from the infirmary, tended to rise, thus creating a flow that allowed air circulation. The years between the sixteenth and eighteenth centuries were a period of stagnation, from a socio-political perspective, which iwas reflected in the medical culture, and finally, on the possible hospital innovations. This was a period of political and religious tensions. The general economic and social conditions worsened, the religion struggled and problems such the unemployment, the spread of the vagrancy and the banditry, induced the Europeans governments to adopt restrictive measures. In an effort to clean up the streets of the cities from vagabonds, paupers, orphans and brigands, different structures were adapted to accommodate each of these categories of individuals, considered unproductive and thorny. In the countries of the Reform the ex-convents that were left empty were used as shelters, while Catholic countries waited until the French Revolution and the Napoleonic suppressions to have the same features. As regard to medical science, in this period of attempts to innovate, the studies about human anatomy awere detailed, lively debates took place, people and information exchange in the European circulated and, schools for foreign students were born. Until the 17th century, with the emergence of scientific thought, the diseases were divided by type (classification) and medical experiences were catalogued according to the experimental method, with a more analytical approach, and were organised by sectors. In this period a number of instruments for measuring and observation was invented: thermometers, hygrometers and microscopes etc., which will facilitate the exploration of the human body and the understanding of how it works. Aside from a few exceptions, such as the project of a radial plan hospital designed by Antoine Desgodets towards the end of the seventeenth century, the achievements of the 17th century remained anchored to the classical forms of the cross plan facilities, more or less variously assembled and arranged around a courtyard. In the 18th century the union of hospital and universities took place and the number of teachers attending the hospital wards increased. The sanitation in care places was very poor and such problem became itself cause of the worsening of physical condition of sick people. Also, the quality of the hospitals was assessed on the capacity to accommodate the greatest number of people which led to an unprecedented overcrowding in nosocomial facilities. The clinical method, which is also based on subdivision and identification of diseases, spreaded rapidly throughout Europe. The diagnostics became equivalent in importance to therapy, and in Padua, Italy, Professor and doctor Morgagni iperformed dissecting practice art exerts upon the bodies of deceased patients, in which tissues, and only in those, he believed to have may detect the consequences of illnesses suffered in life, unlike the dissection was carried out essentially on the executed people's bodies, in other words on basically healthy samples, whose body didn't not reveal any trace of morbidity. The French Revolution of 1789 and the fire in 1772 of the Hotel Dieu in Paris, were the events that gave impetus to the renewal of the Organization of health facilities and for the invention of the "pavilion" type in France. The new model provided the breakdown of the single building in multiple buildings arranged in a complex. In this way it was possible to isolate different kind of diseases and solve the problem of contagion. The design criteria were the followings: the decentralization of hospitals, placed preferably on the outskirts or isolated; to reduction in the capacity (1200/1500 beds); the choice of the pavilions model with courtyard and with a distance of at least twice the height of buildings; the buildings orientation in order to allow for better ventilation and natural lighting; maximum height of buildings stabilized in three floors, including two to be used as infirmaries; the height of the windows was extended up to the ceiling to remove stale air; the plan ceilings; independent restroom (latrines, washrooms, kitchenettes, staff places) in each infirmary; the separation of departments for men and women by quaranteeing every ill his/her bed; maximum capacity of the infirmaries of 34-36 beds arranged in two rows. These criteria, established a few years earlier than 1789, were exported and spreaded throughout Europe with the Revolution and Napoleonic campaigns, and they remained fundamental in the construction of hospitals until the mid-20th century. The main characteristics of this architectural type were: the relationship between built-up area and the green area, the connection system (external paths, surface covered connections, basement or underground connections, etc.) and the single hospital ward (the capacity, relocation of the restroom, the orientation etc.). Although such renovations in the hospital field were attributed to the French in literature, they were already used in England as a consequence of Religious Reform and the suppression of the monasteries that housed the poor and infirm. Since the middle of the century, also because of the divulgation work accomplished by Florence Nightingale (Florence, 1820- London 1910, founder of nursing care) as well as other sector experts, the "pavilions" hospitals multiplied. In order to make the nurse surveillance aneasier and more efficient task, it was suggested to reserve a dedicated area, located in the entryway of each aisle, that was designed for a capacity of 30 beds, placed on the two long sides of the facilities. The cuisine was placed next to the room and it was reserved for the nurses, while to the opposite side, the restrooms and single rooms reserved for less serious patients were placed. The gravest patients, in need of greater attention, were placed in proximity of the infirmary and in decreasing order of severity. Along the walls wide windows were opened, to ensure a high standard of lighting and ventilation. These are the so-called "Nightingale Wards" that constitute the dominant model for the creation and development of the system of the wards in "pavilions" hospitals. The new philosophy behind hospital buildings was based on maximum possible hygiene achieved through ventilation, lighting, separation of the facilities, the delocalization from population centers, and it also led to a phenomenon of progressive abstraction of the environment that assumed a psychological character of sterility and non-involvement with the patient. At the beginning of the nineteenth century in England, another very important change took place: the healthcare specialization depending on the type of disease. Towards the end of the century the hospital was built in separate pavilions to promote lighting and ventilation; these buildings were preferred because of the lower incidence of infections and because of the shortest duration of hospitalizations. Often these were one-story buildings and therefore they were very extensive, with infirmaries consisting in about 30 beds flanked by some single isolation room. The pavilions needed to occupy a greater space with additional construction costs, but a greater space meaned also that the staff was obliged to take longer routes to reach the restrooms. To preserve the utility of these facilities it was necessary to limit the planimetric expansion by increasing the number of floors and aisles. Typically, the pavilions built around the turn of the century had two floors with two aisles in each floor placed in the head of the building in order to capture the lighting at least on three sides. The pavilions type was improved further with new medical discoveries. The healtcare and restroom were separated by hospital stay, placing them in separate buildings: each pavilion is designed to a single specialty, or to care or to tasks. This constructive tendency is reversed since the end of the last century and beyond, bringing the healthcare areas and service areas in contact with each specialty, in the same pavilion, grown benefit for the staff and the costs of the facilities. To preserve the utility of these facilities it was necessary to limit the planimetric expansion by increasing the number of floors and aisles. The placement of the restroom in the central area of the pavilion leads to the hypothesis of two schemes. The "Linear" scheme, which includes the central atrium and the stairs, the utility rooms, and the places for the staff and the healthcare, while the rooms intended for convalescents' stay and infirmaries are placed symmetrically with respect to the transverse axis; and the "H" scheme, wich includes the central area, the healthcare facilities and restroom, while the wings host an infirmary each. The disadvantages of this building type that host up to 30 beds, were the contagion, the duration of the medical examination, as well as disorder caused by overcrowding, therefore, the four-storey pavilions were proposed and the there was a reduction of the beds number to 16-18 in each infirmary. Over time, the principle of integration between the restroom and terapy areas became widespread, since their separation entailed a sharp discomfort for the sick person and the staff, due to the long distances to be covered and therefore it was suggested the placement of the latter in the pavilions, often in the middle. Some buildings had a plan in which the central area was isolated from the rest of the building, according to a "T" scheme: it is an evolution that was made possible by a greater knowing about the sterility problems. The end of the 19th century was a period of great discoveries in the diagnostics and therapeutic field, many progress hsa been made in disciplines such as organic chemistry, cell theory, microbiology, research on cell alteration by Virchovche, which manageed to locate the disease in the alteration of the cellular structure of a tissue of an organ, the asepsis, bacteriology and vaccination discoveries by Pasteur, and so the health concept makes its way as a right for all citizens and makes clear the relationship existing betweeen health and social conditions of the patient. In 1846, the first painless surgical operation in medical surgery records is performed, with the employment of anesthesia. Towards the mid-19th century with the invention of the stethoscope there is a radical transformation in the relationship between doctor and patient. In this climate the experimental medicine makes its way, resulting in the birth of modern research laboratories and analysis and affirmation of chemical-based remedies. Finally, in 1895 Wilhelm Conrad Röntgen's discovery of x-rays, marks the beginning of Radiology era, which today is branch of the imaging diagnostic. When the pavilion hospital model goes to crisis they opted for the "monoblock" hospital or skyscraper hospital as a solution. This is conceivable as the superposition of an indefinite number of floors where all the wards, the therapy and diagnosis rooms and general services are incorporated in one floor or more connected through vertical accesses. This kind of building is located in high building and housing density, since it is difficult to find areas of appropriate size and the construction and purchase of land are very expensive. The monoblock hospitals features the following technical innovations: a steel skeleton structure with large windows; a new foundations system; a mechanical or electrical elevator systems. These structures can be built from scratch, or renew or replace the old buildings. These are buildings dominated by the usefulness and sobriety principle, without any concession towards the decoration or luxury. The diagnostic and therapeutic methods and laboratory analysis gain importance, while the wards are divided by the kind of illness. In the '30s and '50s the hospital architecture is articulated in several styles in North America and European countries, while emerging from a common rationalist approach, based on the idea of " *machine à guerir* ", and that is expressed in net volumes of buildings, in the breadth of the glass windows, in the flat roof, but also monumental sizes that emphasizes its institutional role. American hospitals are grown for about 12-30 floors, with rooms of 1 to 3 beds, while in Europe the height reached by the monoblock hospitals is generally lower, the facilities can to reach up to 15 floors. In Italy, following the Royal Decree July 20, 1939 "instructions for the hospital buildings", it was determined that the maximum height has to be equal to seven floors. The Italian facilities differ from those U.S.A. and European in the planimetry for frequent adoption of triple body, differently from quintuple body (consisting of three bands of separate environments by a dual path for services places in the central sector). The "poliblocco" kind, with "T" scheme (Fig. 4) is mainly developed in Italy in compliance with legislation of 1939 which recommend a height not exceeding seven floors. This model includes a small number of buildings, which are grouped and connected, high on average five to seven floors, where the restroom, the treatments and the hospital stays are placed, with reciprocal relationships similar to those of the monoblock.

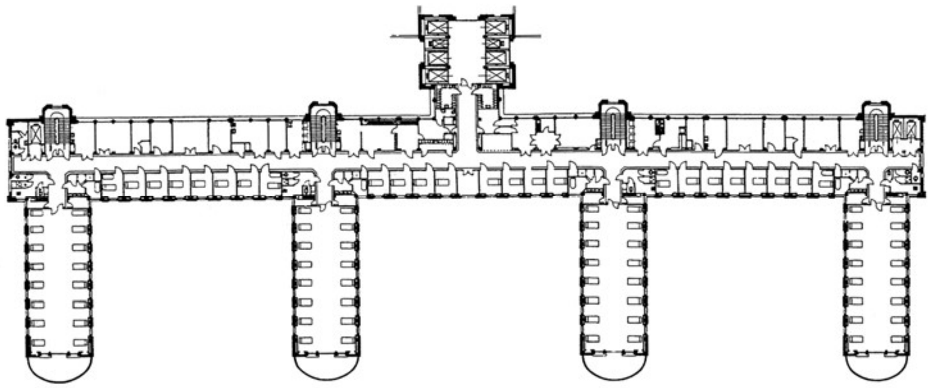


Fig.4. T scheme plant. (Capolongo, 2006)

The Italian experience is characterised by the adoption of the monoblock type unit for small and medium-sized hospitals and the poliblocco type to larger hospitals, in order to avoid an overcrowding in the wards and to services a single architectural body. The evolution of poliblocco is represented by the "*radial*" Hospital where the hallways are deleted or the unnecessary volumes, facilitating contact between departments and inpatient services, among doctors of different specialties. The volumes and surfaces analysis, with the same beds, reveals significant savings resulting from the adoption of the radial kind, in comparison to other kind existing in the same period. In this period the plate-tower hospital appears designed as an articulate body into which stays are placed in a vertical tower, while the care spaces and services (diagnosys) for the public are in a horizontal plate, so they could be easily reached from inside and outside without disturbing to patients. Simple and compact, the structure is quite cheap. Since the late 1950s and for three decades after, the United Kingdom starts a series of administrative and technical experiences that make it the most advanced country in the world for research in the field of hospitals. After the war, the models to be aspired to hospitals reconstruction are the United States, Switzerland and the Scandinavian countries: they bring special attention to the connections inside the structure, giving rise to tower of typological choices, with compact planimetric joints and gathered around a core of elevators. In 1953, after the foundation of the "National Health Service", the British Government publish the "Hospital Building Operation Handbook", a manual containing the programming instructions and the procedures to be followed for the construction of healthcare buildings. Among the new criteria for hospital spaces organization, it prevails a design that consider not only current needs but also those that could be introduced in the future. It is a large scale planning of possible transformations and expansions of the hospital complex over time, producing two different design approaches. The first is the so-called “indeterminacy principle” theorized by John Weeks, wich propose a design based on organizational and functional criteria constantly evolving, such as to allow the changes also required in terms of technological requirements, engineering requirements and building codes (Fig. 5).

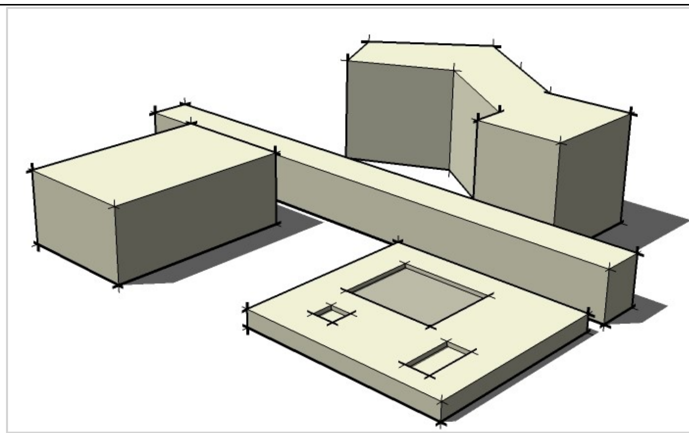


Fig.5. Scheme in according to the indeterminacy principle. (Capolongo, 2006)

The hospital complex was divided into single buildings with plant of geometric shape diversified and united generally by a horizontal connection, creating an apparent disorder.

During the same period in England is developed a theory according to which the body is conceived as a "single box" able to allow interchangeability both in a functional and an engineering way (Fig. 6).

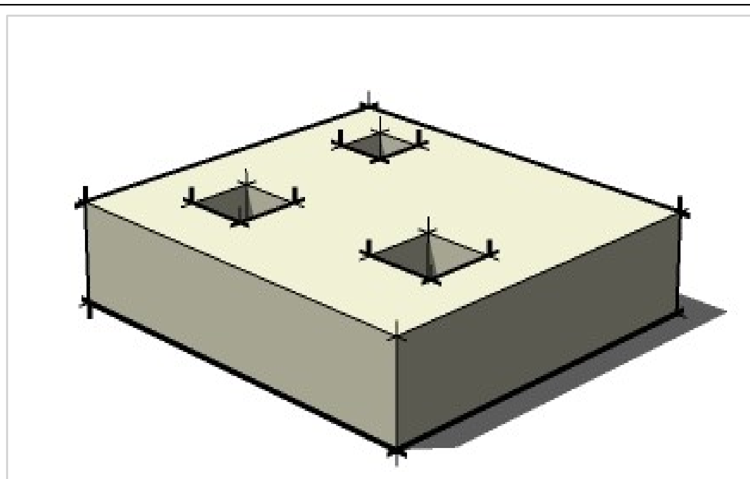


Fig.6 Scheme of the unique container. (Capolongo, 2006)

In the late 1960’s the universal building concept is used to define a large and complex container in terms of engineering and technology. It's about building systems, characterized by their degree of rigidity or morphological adaptability where some structural elements are primary and will be kept constant, other elements can undergo the transformations at a low cost and small inconveniences. The potential limits of this "megastructure" are related to the management that, in view of the technological and installation complexity, is quite expensive. During these years in United Kingdom and North America the compact and undifferentiated structures were born, workable in sequential phases, where the spaces of the hospital stays and the diagnosis and care spaces are connected by horizontal paths. As the medical services increased in the hospital stays, the central space expands, and the rectangular plan is replaced by square module (the racetrack square, the block plancube). The compact plan, which involves the artificial lighting and the air conditioning systems, was opposed by the medical staff, as well as criticized as architectural achievement. At the base of this type still there was the reduction principle of the routes, leading ti less travelling time by using connections in different directions and the mechanization of the materials transport. The preference of horizontality in hospital projects is due mainly to the integration with the outside environmental and it is possible also through the realization of large internal courtyards. A very important aspect in the development of compact horizontal hospital and then in its evolution, has been the control of the construction and management costs, evaluating the realization phases and the transformations which can be disavantageous for the hospital. The "Best Buy" program, based on the assumption of economic health settlements at high building standards, it proposed interventions of limited size (maximum capacity 450/500 p.l.) and with a simplified technology leaving most spaces for the lighting and natural ventilation. The Ministry of Health in an effort to improve the quality of the hospital equipment and reduce the construction costs, organized a complete plan which included the operations cycle related to the construction of a hospital: the identification of the user needs, the design and the construction, the management verification. This leads to the development of the "Harness" system and then after a few decades, to the "Nucleus" system. The "Harness” model was conceived not only as an example of hospital design, but also as a method of administration, structured in free blocks at the extremity and arranged along a linear travel (Fig. 7).

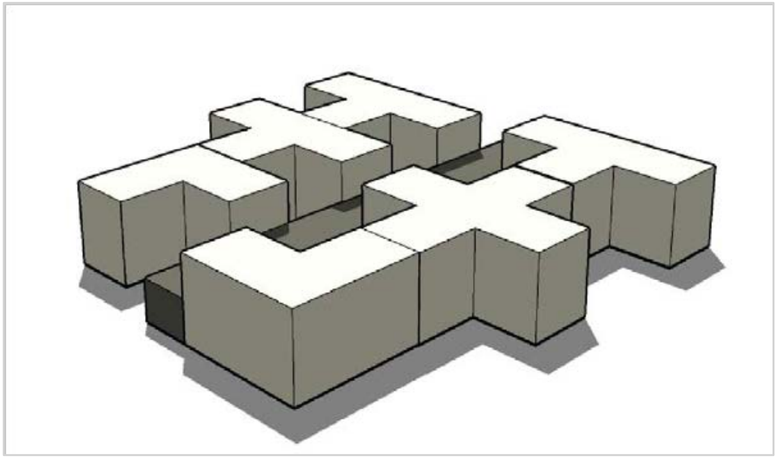


Fig.7. Scheme of the Harness project. (Capolongo, 2006)

The unit, even if different sizes, were arranged in a square grid and combined in various ways and up to four floors in heigth, along the perimeter of all units are enclosed courtyards from wich it receives light and natural ventilation. The critical review of the Harness system gave rise to a new modular design named "Nucleus".

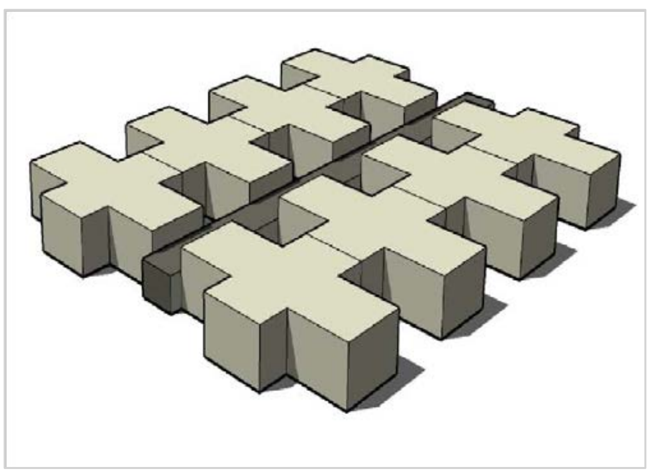


Fig.8. scheme of the Nucleus project. (Capolongo, 2006)

The "Nucleus" scheme (Fig. 8) has been designed to offer the opportunity to build a hospital in sequential phases, allowing the addition of individual units in various combinations. Such a system could also be used to expand existing hospitals. As the"Harness" system it is based on the aggregation of pre-planned units around a linear journey axis, but it is different, since each unit is a star-shaped block of identical dimensions ti the others. If placed next to each other, these blocks produce a checkerboard of constant geometry. The system is concepted to build up to three floors, but preferably two, with light and natural ventilation in most of the environments. The "Nucleus " model recieved several critics due to lack of flexibility nor the ability to adapt the spaces to several activities in hospitals, specifically laboratories and in operating rooms.

At the end of the 19th century, in the construction sector priority is given to the functional and technological aspects. In the United States it is witnessed the failure of the "modern hospital machine" that causes stress to patients, staff and visitors. It highlights the confusion created by the system of paths in large hospitals, the noise of the public spaces, the depersonalization of patient rooms. In UK and France, where the models of industrialized policy had been promoted, now they were dropped, giving space to architectural quality. The system of architectural competitions about hospital building was born and also in Italy was used to Project Financing for important interventions in some regions. The image of the hospital changes. The aesthetic quality and comfort are of important in health policy and market conditions, with different impact depending on the context: overcharges attributable to staff costs are unmanageable for megastructures; the competition by private sector leads the providers to consider the advantages of an attractive facility; the introduction into the hospital of the “Quality System” and customer orientation; an increasing incidence of the outpatient and daily care; the outsourcing of some support and service functions and, the introduction of commercial services for the patients and visitors. The structure has changed, the industrial spaces assume less importance, the wards look according to hotel standards, while those destined to outpatient terapy have a look that is more comfortable. In the USA there are the “malls” Healthcare facilities, but in France it is claimed a continuity in contemporary design with the “modern thinking hospital for the synthesis that draws between functional organization and reflections on urbanity and architecture" (C. Fermand). In UK there are uprising questions about the household nature of the hospital (Peter Blundell Jones) and in Italy, in 2000, the Ministry of Health offers a Decalogue for hospitals in which the humanization and urbanity are mentioned first. The very rapid evolution of the nosocomial technologies has ensured that the hospitals built in the ‘60s and ‘70s have undergone a considerable obsolescence, as well as those in the ‘80s are been considerably renovated, while the facilities in ‘90s have not kept up with the new architectural type of day hospital and day-surgery. The current trend is to reorganize the existing spaces, strengthen and enlarge the plant design and installation. The humanization of the hospitals leads to the renovation of the rooms, with more spaces, the use of the most advanced technologies and less cold and more personalized furnitures. The medical organization requires a greater fragmentation of the space, each isolated from the others, but with one management. These places also offer the greatest flexibility to adapt over time to the progresses in medicine and medical technology. Finally, the interventions will concern the reception and the patient's room that will increasingly seem a hotel room.

In Italy there are many obsolete hospitals and they are typologically and technologically inadequate to meet the needs in terms of functionality, comfort and safety. In addition, there are several health facilities built completely in years, but still not in use. The Ministerial Decree from 12 December 2000 promised adequate clinical and medical services in the facilities which could guarantee performance levels of comfort unmatched in hotel services and the redevelopment of heritage buildings. The hospital design provides for the insertion of new structures in an urban context that not has not to be affected by traffic congestion, thus the consequent reduction of acoustic and environmental pollution; the placement of the building bodies has to bedone with low environmental impact and they have to be inserted in green areas and with large parking lots. Many pavilion hospitals, in the requalify and modernization projects, undergo an environmental integration with the existing landscape and they easily adapt to the functionality concepts and the hotel standards. In Italy, the legislation that prevents the construction of the buildings height up to seven floors made sure they are erected according to a more structured plan and the poliblocco type prevails. The problem we are facing is that one of the facility position in relation to the already existing urban fabric. It is about hospitals built once in outlying areas, but now they have been incorporated by the city, although at the time they fulfilled the environmental comfort characteristics considered today so important. Now, attention is payed to the arrangement of the green areas and the areas for parking lots to encourage adequate accessibility; but also the patients discomfort forced them to live with the noise and smog produced by continuous and unregulated vehicle traffic. In this respect the formal and typological hospital buildings included the reduction of the beds number and the consequent contraction of the necessary space, the transfer of the non-essential services outside of the hospital and an increased architectural attention to the the deans with the least environmental impact.

The hospital takes on a new urban significance (Renzo Piano, new model of Hospital, Ministry of Health, Rome, 21 March 2001.) not so much for its architecture but to social dynamics within it. So the more the hospital will be able to expand outwards, less traumatic psychological leap will be that the patient will have to address at the time of admission. Also in this case the use of green spaces also opens outside plays an important role in planning to create a more flexible and transparent fruiction. The hospital stays should be made to be available to visitors and helper in terms of times so as not to create excessive and unjustified isolation of the patient, without neglecting all aspects related to the safety and healthiness of the spaces, including the hospital stays. In modern hospitals, particular attention is paid to personal privacy, comfort and pleasantness of the spaces in wich the patient covers the fundamental and central part in his physical and psychological complexity (Ministerial Decree of 12 December 2000). The hospital become an urban place because of streets, galleries and shopping center. These spaces make it exceed the typical isolation that the patients had in healthcare facilities. The humanization becomes a therapeutic function.

The Ministerial Decree of 12 December 2000 does not give the structural design lines, but indicates the actions which should be performed first, regarding the elevations and floor plans, in order to promote the understanding of the implementation of the proposed guidelines. The hospitals have average to small dimensions with most beds dedicated to the intensive care unit and shorter hospital stays. The structure is designed with telematic links fast letting between the various departments and at the same time it is the fulcrum of the health network of the land in contact with family doctors and the different diagnostic centers.

The electronic systems dedicated to the patient will have a direct long distance relationship with any other health care facility and among the sick person at home and the hospital. The health care system as a whole is undergoing a transformation whereby contemporary hospital must be a flexible structure ready to adapt quickly to new systems of organization and efficiency.

The concept of health has changed over time as well as the needs patients and the care modalities have changed. The hospital is structured to be a scientific and technological center of diagnosis and therapy, prevention and investigation, experimentation and research. This transformation is evident in the local subdivision, the hospitalization spaces decrease and increase those regarding research and therapies, but especially in the combination of different types of plate – monoblock. In the plate buildings the spaces can expand to the future functional needs, such as diagnostic and research spaces, while in the monoblock lie mostly hospital stays, managed double bodies, triple or quintuple. In the contemporary hospital are placed also the day hospital and day-surgery wards, which require according to the reorganization involving containement in Legislative Decree 502/92, a different approach to the methods of care and hospitalization of patients.

The day hospital and day surgery are created as alternatives to hospitalization for those interventions where the hospital is not indispensable. These two systems are part-time admissions[1] and the sevices is related to diagnostic, rehabilitative and therapeutic interventions in nature. The classification of the day hospital may also occur depending on the specialty treated as Pediatrics, Oncology, Nephrology, etc. The two services can be framed institutionally within the existing hospital facilities in contiguity with their ordinary units. These formulas daytime care may entail benefits both economically, as in a matter of fact, they can reduce costs by 50% as long as the hotel expenses are eliminated, both on the human and social aspect of the sick person, which can carry out their activities except on days dedicated to care. Also the medical tests answers are immediate and take place throughout the day. This involves a greater commitment on the part of health professionals and patient selection and according to the overall physical condition and age.

1.2. Indoor contamination

The World Health Organization (WHO,1948) defines the environment as a physical and human factors integrated system that has a significant impat on the health of the community. The concept of health should be understood not only as the absence of actual disease but as a state of physical, mental and social wellbeing [15]. It is clear the relationship between man - environment - wellbeing and how the environmental quality might affect the wellbeing of its guests. Hence the attention to design and organization, in fact the condition of well-being is primarily reflected in the structural quality of the building and is derived from careful planning and design of the facilities, with the building and maintenance supported by technological and engineering choices suitable for the proper management of the health service [15]. For this

[1] The day surgery requires a hospital stay not exceeding 12 hours as established by the first legislative reference in L.595/85 and D.M. 19/03/88. The day-surgery finds reference in the national health plan 1992/94 and is activated for surgical activity in a shelter that does not exceed the period of 24 hours.

reason, some design solutions are defined not only to that take into account the functionality and efficiency of the health service but also comfort offered to patients, visitors and hospital staff (doctors, nurses, administrative, technical). To meet the welfare, different aspects must be taken into account, such as the thermal, acoustic, visual, psychological and social comfort, and the quality of the confined air. The perception of well-being may vary according to age, state of health, constitution and culture. The acoustic well-being is characterized by absence of sources of sound-pollution exceeding a certain quantity, while the visual one is determined by the nature and intensity of natural and artificial light, and by the colors used, within the hospital stays, including furnishings and facilities. In fact, a good lighting plays a pivotal role in preventing eye strain, but also to ensure physical and mental wellbeing. In the specific case of hospital stays, the windows preferably should be extended until ground level to encourage the views outside, and allowing natural light to penetrate in order to facilitate the activities carried out within of the rooms. The natural light can inhibit the presence of photosensitive bacteria, mold and mites, the latter responsible for many forms of allergies. The concept of comfort extends to even the materials the furnishing is made of, in respect of patient's health and safety, they ahs to be easily washable and sterilisable, fire resistant, bacteriostatic and fungicides (with treatment), and organized to avoid obstacle to the comfort, the safety and the activities carried out by users [15]. The new building codes, the regulations and the green building guidelines have emphasized the concept of sustainability. In fact, recent studies [16] confirmed that the environmental quality problems in confined spaces have a direct effect on the comfort and health, as well as on the productivity of occupants [17]. ASHRAE standards[2] 55 of 2010 (ASHRAE 2010, American Society of Heating, Refrigerating and Air-Conditioning Engineers) and the ISO 7730 standard of 1994 define the thermal comfort as " the mood that expresses satisfaction with the thermal environment where he is located" [17]. In Italy, the reference values of temperature, humidity and air velocity in hospital are designated accordingly to the UNI 10339/1995 standard, currently under review, as shown in the table below (table 1).

[2] ASHRAE, founded in 1894, is a global society with the scope of advancing human well-being through sustainable technology for the built environment. The Society and its members focus on building systems, energy efficiency, indoor air quality, refrigeration and sustainability within the industry.

Microclimatic Characteristics in areas of the hospital stays (UNI 10339:2005)	
winter indoor temperature	21° C-23° C
summer temperature inside	25°-27° C
relative humidity:	40%-60%
number of air changes/hour	2 v/h for bedrooms (also not forced to existing structures)
air velocity	0.05-0.15 m/s
Heating	0.05 to 0.10 m/s
Cooling	0.05 to 0.15 m/s
pressure	positive or neutral for hospital room

Table 1. Microclimatic parameters in hospital stays (UNI 10339:2005).

The moisture increases the perception of hot and cold, because the air, being a good conductor of heat, can cause discomfort. In addition, a value of relative humidity below 20% increases the presence of dust in the air, thus bacteria in air-floating suspension [15] and raise the possibility of formation of electrical charges, while RH values more than 60%, temperatures and insufficient air changes determine optimal conditions for germs proliferation, resulting in condensation and molds on cold walls, causing inconvenience and risks to human health. Another important parameter is the ventilation. This is determined by the difference in pressure and temperature of the air between two or more adjacent areas, because the air tends to move from high pressure/temperature areas to low pressure/temperature ones, with a speed of movement directly proportionated to the difference between the two areas. The ventilation in confined environments can be natural or forced by the use of ventilation and air conditioning systems. The air velocity should not exceed 0.50 m/s to not create annoying air currents and stagnation that can create discomfort such as the sick building syndrome (SBS). To avoid this the air changes are set at least to 15 vol/h in order to allow the recirculation (the outside air mixed with air taken from the same premises after an appropriate reprocessing) or by inside admission of outside air. An acceptable air quality has to meet the regulations imposed by the competent authority, but also it has to be in compliance with the requirement of occupants' comfort, and this it is not always achieved.

ASHRAE 62/04 standard *"indoor air quality is deemed acceptable when in it there are not harmful pollutants in detrimental concentrations, as determined by the competent authorities, and when a large percentage of people (80 % or more) does not express dissatisfaction towards it*". (ASHRAE, 2004)

Indoor pollution is considered as the presence, in confined spaces, of chemical, physical and biological pollutants that aern't naturally present in outdoor air of high quality ecological systems [18]. Indoor contaminants may consist of particles of various kind and provenance: pollen, spores, mites, dust, mold, bacteria, *viruses,* gases and other particles. In particular, with regard to indoor environments, the training processes are represented by wear on floors, walls, ceilings and furnishings generally, by activities carried out in the premises, by health and sanitation practices, by the influx of people and their clothing.

Depending on the nature and size of the particles, they are distinguished as [19, 20]:

• aerosol, formed by solid or liquid particles suspended in the air and with a diameter of less than 1 micron (µm);

• mists, formed by droplets with diameter less than 2 microns;

• fumes, formed by solid particles with diameter less than 1 micron and released usually by chemical and metallurgical processes;

• smokes, formed by solid particles with diameter less than 2 microns and transported by gas mixtures;

• dusts, consisting of solid particles with diameter between 0.25 and 0.5 microns;

• sands, formed by the solid particles with a diameter greater than 500 microns.

The patients represent the groups most at risk because already debilitated. The atmospheric particulate, in fact, may be responsible for the onset of various diseases of the respiratory tract. The particles can be divided according to size in:

• ultrafine: particles with an aerodynamic diameter between 0.01 and 0.1 µm, as sulphurous anhydride (SO_2), ammonia (NH_3), nitrogen oxides (NO_X) and combustion products;

• fine: particles with an aerodynamic diameter between 0.1 and 2.5 µm, as sulphates, nitrates, ammonium ions, elemental and organic carbon, but also particles of biological origin like fungal spores, yeasts, bacteria etc.

• coarse: particles with an aerodynamic diameter between 2.5 and 100 µm; essentially produced by mechanical processes such as erosion, mechanical or wind resuspension and grinding.

In function to their diameter, these particles can penetrate and deposit in different areas of the human respiratory tract, from the oropharyngeal cavity until the pulmonary alveoli, promoting the development of possible pathologies. Generally, in a workplace, the pathologies related to environment and caused by the pollutants presence within the facilities, are called building-related illnesses (BRI). If the symptoms occur only upon the acess, by users, into that particular environment and then disappear upon exiting, then we talk about sick building syndrome (SBS). In the hospital setting, the focus on indoor air pollution is higher also because of more frequent possibility of contracting various diseases, including professional ones. Before depositing themselves, the particles can remain suspended in air for long periods of time and can be transported over long distances from the place of origin, depending on their weight (size and shape), temperature, air currents flow (convections) and moisture, but they can also be conveyed by the people present in the environment.

Dust deposition processes on surfaces can occur as a result of:

• Brownian deposition of the particles. Before settling on the surface, the small particles (0.01 µm in diameter), as a result of collisions with air molecules in thermal agitation, will begin to move with different velocity, and randomly, in all directions (Brownian motion). Such movements depend on air temperature and time; in fact, they increase with both.

• Phoretic deposition that depends on different gradients of the temperature and vapor concentration, near the surfaces. It stands out in:

-thermophoretic deposition (0.01 < d < 1 μm), the phenomenon is due to different temperature gradients between the surface and the adjacent air mass. The particle in a gaseous medium (air), in proximity to a surface, is subject to collisions by air molecules, thus less intense near a colder zone (lower temperature), but more intense in a warmer zone (higher temperature). This means that the particle in question moves to the area where receives less collisions, namely the coldest one.

-diffusiophoresis (0.01 < d <1 μm) and Stefan flow. The deposition for a specific humidity (SH) gradients caused by condensation in the micropores is due to the resultant between diffusiophoresis and Stefan flow. The diffusiophoresis is due to the diffusion of a particular gas into another. In the atmosphere, this process is tied to localized concentrations of water vapor due to evaporation or condensation. The continuous condensation or evaporation can lead to an accumulation or deficiency in dry air and to avoid this, it is necessary to transport in a hydrodynamic way the moist air surrounding, named flow of Stefan. The balance between the flow of Stefan and diffusiophoretic flow during condensation permittes the deposition of particles on the surface, the opposite happens during evaporation.

• Inertial deposition. This depend on the particles transported by air circulation that smak down (d > 1 μm, maximum efficacy: 4 - 5 μm) or by the thermal inhomogeneities induced in the vicinity of a surface. The particle, by its inaction, let the flow of air to carries itself and goes on to impact with an obstacle. The deposition can take place by: friction near an area which generates turbulence, pressure differences due to air currents, thermal inhomogeneities that generate convection.

• Gravitational deposition. the particles deposite because of their weight (d < 1-2 μm) according to Stokes' law[3] . The process in the presence of convective motions is invalid, because the particles remain in suspension longer and undergo repositionings.

• Electrostatic deposition (indep. of d), due to the formation of a force field.

• Photophoretic deposition force (indep. of d). The particles, struck by a beam of intense light, start a movement in the direction of light propagation

[3]Stokes' Law: the sedimentation rate in the air is proportional to the square of the particle diameter.

The environmental humidity influences the diameter, and the particle deposition, in fact high values of relative humidity determine heavier particles that fall quickly to the ground and consequent time propagation redution up in the air. Besides being an excellent conductor, the moisture allows electric charges to disperse preventing accumulation and the formation of electrostatic fields that attract dust.

When a particle hits on an area, it not necessarily adheres on this, but it could also simply bounce or be raised by turbulence and convection, that is caused by differences in temperature between air mass, floor, walls and ceiling. In fact, the warm air near the ground, tends to rise towards higher strata, according to trajectories determined by temperature conditions of the walls and ceiling. In the case of gravitational deposition, the particles remain on the surface until a mechanical action just uplifts them from the ground, eg. the passage of people, the use of cloths for dusting, etc. If there is rubbing or contact, an electrostatic field is formed and the adherence is due to the electrostatic attraction among bodies with opposite charges, according to Coulomb's law. The adhesion is a physico-chemical phenomenon of attraction among different molecules that occurs after contact. The risk of contracting infections from contaminated surfaces in hospital stays is still an object of debate among the scientific community [14], surely these surfaces act as reservoirs for microorganisms [21] even for a long time. The microorganisms, in addition to being carried as aerosol, may be conveyed by the staff or the other patients, through a cross-contamination of the high-touch surfaces or by direct contact. In fact, according to some studies [14] the contaminated surfaces can facilitate the transmission of healthcare-associated pathogens such as *Clostridium difficile, Acinetobacter* spp., *Staphylococcus aureus,* which represent a risk factor of contracting infections. For these reasons, the compliance of hand hygiene and the sanitizing procedures play an important role into prevention and monitoring plans.

CHAPTER II

2.1. State of the art

The degree of environmental hygiene is often evalueted on a visual and olfactory perception, in fact there are no generally accepted standards to measure the effect of a detergent or the effectiveness of sanitization procedures. In literature the tests conducted to assess the effectiveness of routine cleanings are rather scarce, although several studies have demonstrated the importance of environmental hygiene in developing infections. In fact, a study [22] published in 2010 reports that most surfaces close to patients are clean inadequately than specified in existing hospital procedures and it has confirmed that patients admitted to rooms previously occupied by patients with nosocomial pathogens have a greater risk (73%) of acquiring the same pathology related to such microorganism than patients not occupying these kind of rooms [22].

Other studies have shown that less than 50% of the surfaces present in the hospital room are cleaned and sanitized properly when using chemical disinfectants [14, 23]. Hence the need to more training of hospital staff, to the use of control checklist and methods to evaluate the performance of cleaning using fluorescent markers. In the United Kingdom it was conducted a study [14] to assess the cleanliness within hospital and to evaluate the equipment used for sanitising. Failure to adequately decontaminate reusable materials permits survival of bacteria, including the spores, which may then contaminate the next surface to be cleaned. The trial included the use of chemical disinfectants, UV devices and fluorescent markers, to provide the feedback on the completeness of the cleanliness, while for microbiological control, executed immediately after the traditional cleaning and after disinfection with UV, using swabs. In this way it has been shown that the correct execution of the cleaning helps to reduce the pathogenic microbial load, though in the case of traditional products, only immediately after disinfection; highlighting the importance of hand hygiene by staff and the high-touch surfaces as a possible reservoir of transmission of potential pathogens [14]. Finally, the use of chemical products may induce the increase in bacterial resistance, since those disinfectants are designed to eliminate a large proportion of microbial species, but they do not kill all of them. Some organisms and bacterial spores actually do survive and they likely will be the more resistant member of the community [24]. The environment can become a reservoir for microorganisms that can survive on surfaces for days, increasing the risk to other patients of acquire infections when heathcare practices are not carried out properly. So, in the development of the surveillance systems and monitoring to contain the microbial load there is a need to standardize the methods and criteria of sampling, reading and interpreting of results obtained by defining a values scale of the acceptability of microbial contamination. In literature, there are few references on the evaluation of the microbial load in hospital, because the research conducted so far were developed for needs related to highly sterile environments (clean rooms, operating rooms). In 2010 the CDC (European Center for Disease Prevention and Control) of Atlanta proposes to carry out monitorings to assess the effectiveness of cleaning and so improve the quality of the environment. In Italy, with regard to hospital surfaces monitoring there are not the rules or the specific guidelines or otherwise are only partial texts. In fact, for the definition of the methods, the guidelines ISPESL 2009 and the UNI EN ISO 14698-1:2004 standard refer to controlled environments such as operating rooms or clean rooms, while the INAIL guidelines relate primarily to workplaces, while the nosocomial stays can be compared to low-risk environments, such as hospital aisles, services and offices. For the establishment of the limits of acceptability the issue is further complicated because existing legislation only provides values that are purely indicative and that refer to the Total Counts, not discriminating between the potentially pathogens and responsibles of nosocomial infections microorganisms.

Generally, the active sampling is considered the most useful way to evaluate the risk arising from inhalable aerosol, while passive sampling is more reliable to evaluate the risks arising from deposition of the particles [25]. Active sampling is performed using impact samplers, such as the SAS (Surface Air System), conveying known volumes of air on agar plates containing a culture medium that is chosen by the operator depending on the kind of organism to identify; that way a targeted microbial sampling an be carried out depending by the characteristics of the environment to be monitored. The plates sampled are incubated at 37°C for 48 hours and at 25° C for additional 24 hours, after which the colonies developed are counted and the results are expressed as colony forming units (CFU) per m^3 of air (CFU/m^3). The effectiveness of the method may be affected by factors like distribution uneven of the microorganisms in the air, their size and the different deposition velocity of viable particles, temperature and humidity, as well as the volumes of air sampled. Another instrument, usually used in food, dairy or pharmaceutical industrie, is the sampler of Reuter (Reuter Centrifugal air Sampler or RCS), wich is able to suck in different volumes of air on a strip consisting of an agar substrate. The strip tends to become saturated quickly and can give rise to overlapping and inhibition of the colonies, making it less easy to identify pure cultures.

Currently the assessment of surfaces contamination, is performed using different techniques: Petri plates, sterile swabs or sponge, slides, Rodac plates (Replicate Organism Detection And Counting), and molecular techniques such as the DGGE (Denaturing Gradient Gel Electrophoresis) and microarray sequencing, although the more precise ones are too expensive to be used regularly.

Contact plates allow you to determine the value of CFU referred to the contact area of the plate in relation to the area of sampling [26].

In Italy, as indicated in the guidelines *INAIL-CONTARP*, sampling is performed by two different operators on the same sampling sites and in areas with similar characteristics. Contact plates allow you to determine the value of CFU referred to the contact area of the plate in relation to the area of collection [26]. It is also recommended to run, for each sampling point and medium kind, a triple sampling plan, so it is possible to calculate the mathematical mean and have a more accurate estimate of the microbial load. In fact, the mathematical mean identifies the possible outliers, caused by transient conditions that are unpredictable, like air currents, transit of persons, etc., but also the fluctuations due to human error.

The sampling method involves a light pressure on the plates surface placed on the area to be analyzed.

Sampling can also be performed with a standardized weight applicator equipped with timers (Rodac-Weight, International PBI), in order to guarantee a more uniform pressure on the plate surface and therefore a greater reproducibility and comparability of the data. Such way it is possible to evaluate the walls and surfaces of medical equipment, especially the ones that are close to the patient (switches, handles, footboards, bed sides, telephones, sinks, handrails etc.). In particular situations, as when the surfaces to be monitored are wet, uneven or not easily accessible, it resorts to use swabs or nitrocellulose membranes, rather than plates. Although is actually prefrable to limit the use of swabs, because the results are unlikely to be standardised and comparable with those obtained with contact plates.

The determination of the microbial load may also be done by using the technique of bioluminescence. This is based on a biochemical phenomenon due to the reaction between the enzyme luciferase and ATP molecule produced by alive animal and plant cells to accumulate energy. This method allows to count the total number of viable microorganisms in a very short period of time, but is unable to discriminate the kind and species. As for the passive surfaces sampling we generally use plates (Petri) containing culture medium, in which the microorganisms are collected by sedimentation and, after appropriate incubation, they are counted. The limit of this method is that it does not allow to draw a correlation between number of microorganisms and a known volume of air. However, the plates have the advantage of being sterile, cheap, easily available and the results are reproducible and reliable. In fact, this method allows to simultaneous sampling in different locations in the same area and it permits to collect and compare data obtained by several operators in different places. To this day the acceptability limits of contamination in low-risk environments, are defined by the Microbial Air Index (IMA) [26, 27, 28], which involves samples at varying degrees of contamination, timetable defined and for a number of years, in order to determine the maximum and minimum levels of contamination. For the collection of microorganisms 90 mm sedimentation Petri plates were used, that have been left open and exposed to the air according to the key 1/1/1 (for 1 hour, 1 metre above the floor, about 1 meter away from walls or obstacles). When finished, the plate is closed with a sterile lid and incubated at 37° C in thermostat for 48 hours and at 25°C for another 24 hours. Then, we proceed with the counting of the number of colonies grown and the results are expressed in CFU (Colony Forming Units)/cm^2 or CFU/dm^2, corresponding to the IMA (Microbial Air Index) [26].

This index, depending on the kind of environment considered (Table 2) contains different values, but it considers only the total number of microorganisms (TVC, Total Vital Count) actually present, not distinguishing between microorganisms that are actually pathogens to human health from those with low health-related risk. In addition, the acceptability limits as regard the surfaces refer to measurements carried out in operating rooms immediately after sanitizing treatment [27]. Another attempt has been made to define the limits through the use of the Rodac plates or swabs for determining microbial surface index (IMS) (Table 4) [1] as reported in INAIL 2017 guidelines [29] for the surfaces evaluation in surgeries and on the basis of previous references, it has considered three contamination classes: acceptable, uncertain and altered (Table 3). As regards the total bacterial count a value > 5 cfu/cm^2 was considered as an altered situation, while for the fungi this limite is set to 3 cfu/cm^2. The samplings were taken in 3 different working periods: before the start of the work, during and at the end of the shift, after the cleaning. The values shown for bacteria, correspond to those set out as standard by Dancer *et Alii* [14,30] for the hospitals (Total Aerobic colony counts of <2.5 to 5 CFU per cm^2 on hand touch sites and <1 CFU/cm^2 hospital pathogens, e.g. MRSA, VRE, C. *difficile*, etc.), while the values of the *Staphylococcus* spp. have been obtained analysing the microbial quality of air and surfaces during 30 drives performed by ambulances (Luksamijarulkul e Pipitsangjan 2015) [26]. The results obtained were higher than the adopted standard (<5 cfu / cm^2) but showed a significant correlation between bacterial and fungal contamination in the air and on the surfaces that were analyzed [28].

Environment	**(CFU/plate)**
High risk environments Ultra clean room, protective insulation, operating rooms for ear prosthesis, some working operations of the electronics and pharmaceutical industry.	5
High risk environments Clean rooms, operating rooms for general surgery, reanimation, dialysis, some processing of the electronics and pharmaceutical industry, microbiology laboratories	25
Medium-risk environments Clinic, laboratories, food industries, kitchens, restaurants, factories	50
Low-risk environments Aisles of hospitals, services, offices	75

Table 2. Index Microbiological Air (IMA) (INAIL, 2017)

EC GMP (2003, 2008)			**Pitzurra (1997)**	**ISPESL (2009)**
Class	***UFC/plate 55 mm***	***UFC/cm^2***	***UFC/cm^2***	***UFC/plate 55 mm (ufc/cm^2)***
A	<1	<1	–	–
B	5	0,21	0,04[a]	5 (0,21) [e]
C	25	1,05	1,00[b]	15 (0,62) [e]
D	50	2,10	2,00[c]	50 (2,10) [f]
			4,00[d]	

Pitzurra et al, 1997: [a]ultraclean room; [b]clean room; [c]clinicsi; [d]aisles

ISPESL, 2009: [e] operating rooms and other critical environments; [f]hospital stays post-operative, intensive care, neonatology

Tabella 3. Surface contamination threshold values according to: *European Commission Guide to Good Manufacturing Practice (2003, 2008), Pitzurra et al. (1997), ISPESL (2009)*

Expanding and reviewing previous data, the participants in the study described above [28] have established a reference standard by calculating the median values obtained by samplings performed at the beginning and end of the work shift, in the work surfaces of the dental offices (≤ 0.64 CFU/cm2) and switches (≤ 0.64 CFU/cm2) [31]. The authors also defined, for both areas, an alert value, calculating it like 75th percentile of median values, respectively of 1.48 CFU/cm^2 and 1.31 CFU/cm^2.

IMS CFU/cm^2	**CLASSIFICATION**		
	Acceptable	**Dubious**	**Altered**
Total microbial count and *Staphylococcus spp.* (CFU/cm^2)	0	1 ÷ 5	> 5
Enterobacteriaceae and *Pseudomonas aeruginosa* (CFU/cm^2)	0 ÷ 2	-	> 2
Fungal loads (yeast and mould) total (CFU/cm^2)	0	1 ÷ 3	> 3

Table 4. Reference values for the classification of surfaces (IMS) in relation to the microbial contamination of outpatient environments offered by Carson et al. (INAIL 2017)

2.2. Materials and methods

This research is part of a very complex study which has included the continuous monitoring of patients admitted to 7 different hospitals, to monitor the incidence of health care-related infections arising during the trial period, to record the locations and kind of microorganism responsible for HAIs, in order to find possible correlations with data obtained by microbiological sampling. Furthermore, in parallel to Rodac plates sampling, microbiological samples were collected with sterile swabs to be analyzed using the quantitative method of real time PCR (qPCR) with microarray to characterize the resistances. The resistance of the *Staphylococcus aureus* strains has been also analyzed by traditional microbiology with the Kirby-Bauer method of antibiograms. Aforementioned topics are not covered in this work, which focuses exclusively on environmental monitoring with Rodac plates, carried out to collect statistically significant values in order to define a possible microbiological quality index.

The chosen parameters as indicators of the level of environmental hygiene are the species of microorganisms, usually present on the surfaces of hospitals and responsible of HAIs. The measure is the statistical distribution of the number of colonies of microorganisms per m^2 of surface. Another aspect that is considered is assessing sanitation procedures performed using traditional methods and with the PCHS system based on probiotics, non-pathogenic microorganisms belonging to the genus *Bacillus.* Product safety has been demonstrated by a study that showed the genetic stability of the *Bacillus* used and the absence of infection risk for exposure on patients in contact with surfaces sanitized with probiotics [32,33,34]. It has been shown that probiotic based detergents can reduce the surfaces contamination up to 90% more than the chemicals [32,35] and the pathogens resistances [33] induced by excessive conventional sanitation. Not to mention that the chemical cleaners are effective immediately after cleaning, while the sanitizing action of probiotics endures over time, in fact the monitoring performed to 7 hours daily cleaning practices.

The search has involved seven Italian hospitals for a continuous period of 18 months in total. The study included [36] an initial phase in which hospitals have kept traditional sanitization procedures with chlorine-based chemicals for a period of 6 months; in the next phase, the sanitation was performed by applying cleaning agents containing probiotics, including some time off 2-3 months between traditional and PCHS system for stabilization of the new method. Hospitals were divided into two groups, in 3 of these the monitoring is started by January 2016, while other 3 are entered the search with a delay of 6 and 9 months. Finally, the seventh hospital has retained for the entire period (12 months) the traditional protocol and is served as an external control. Each hospital has participated with the wards chosen from those listed below: Medicine (internal – General), Geriatrics/Neurology/Cardiology, Gastroenterology, Nephrology, and theirr number varies from 2 to 4, depending on the size and availability of structure. Health directions have agreed not to introduce changes in health practices and prevention programs adopted by individual hospitals, not to affect normal activities already in place, in order to obtain reliable data and understand how the sanitisation procedures can affect the occurrence and kind of healthcare associated infections.

Sampling was performed in each facility on a monthly basis, in six or three randomly selected rooms, respectively for hospitals with less or more than 100 patients. In each room three different areas have been sampled, those chosen for their criticality: floor, footboard and sink. Each point has been sampled in duplicate or triplicate (2 or 3 culture medium for the same microorganism, for all selected microorganisms on the same point) for a total number of 42 or 21 plates for each pathogen researched, as shown in the Tables below (Table 5, Table 6).

Facilities with more than 100 beds:

Sampling point	Room 1	Room 2	Room 3	Room 4	Room 5	Room 6
Floor	3	2	2	3	2	2
Bed footboard	3	2	2	3	2	2
Bathroom sink	3	2	2	3	2	2
Total for room	9	6	6	9	6	6
Total samples (single pathogen)	42					

Table 5. Scheme of the monthly sampling carried out by Rodac plates in healthcare facilities with more than 100 beds.

Facilities with less than 100 beds:

Sampling point	Room 1	Room 2	Room 3
Floor	3	2	2
Bed footboard	3	2	2
Bathroom sink	3	2	2
Total for room	9	6	6
Total samples (single pathogen)	21		

Table 6. Scheme of the monthly sampling carried out by Rodac plates in healthcare facilities with less than 100 beds.

The sampling was carried out with contact Rodac plates (Replicate Organism Direct Agar Contact) of 55 mm in diameter containing selective media suitable for the growth of microorganisms, which are described in the following table (Table 7).

Microorganisms	Culture medium
Total Bacteria	Tryptic Soy Agar (TSA)
Staphylococcus spp and *Staphylococcus aureus*	Baird Parker Agar
Enterobacteriaceae	Mac Conkey Agar
Acinetobacter spp.	Herellea Agar
Clostridium difficile	*Clostridium difficile* Agar
Pseudomonas spp.	Cetrimide Agar
Candida spp.	Sabouraud Agar + Chloramphenicol
Aspergillus spp.	Sabouraud Agar + Chloramphenicol

Table 7. List of microorganisms chosen as indicators and their culture media.

After placing the plate on the surface, a pressure is exerted upon it, equally and constantly for 10 seconds. The plates, 14 (in double: 2 * 7 agar plates) or 21 (in triple: 3 * 7 agar plates) for sampling point, were positioned close to each other. The sampling was performed after 7 hours from morning cleaning. The samples were kept refrigerated until arrival at the laboratory, which happened as soon as possible or in any case within 24 hours of sampling [26]. Then the plates were placed in an incubator at the temperature of 37° C, the TSA medium for 24 hours while all other soils for 48 hours.

Finally, the *Sabouraud* Agar was left for additional 24 hours at room temperature (25° C). Once grown, the colonies have been photographed under biohazard hood with NIKON istrument (Model C-LEDS), subsequently counted and the values obtained were entered in a database implemented gradually. The photos were stored as document and available for any check.

The species identification was performed using 90 mm Petri plates:

- Mannitol Salt Agar (MSA): selective medium for *Staphylococcus aureus*
- Mueller Hinton non selective
- Mac Conkey Agar: selective medium for *Enterobacteriaceae*

-Cetrimide agar: selective medium for *Pseudomonas* spp.

- Biochemical tests for identification of *Enterobacteriaceae* (Enterotube) and other Gram negatives (Oxi-ferm tube)
- Biochemical tests for identification of *Staphylococcus aureus* (CCS system 18R).
- Oxidase test

The database in access, was transferred to a specially created online system, where have been given the following data: names of samplers, town, name, Department, date and time of sampling, sampling point, material constituent, kind of culture medium used, name of the microorganisms, number of colonies and photos. The system allows you to export data to a Microsoft excel file for further processing. The values obtained from the colony counting are expressed in CFU/m^2 (colony-forming units per m^2 of surface), which is obtained by the formula:

CFU/m^2 = **N**/23.75 * 10000

N is the number of colonies on plate

23.75 is Rodac plate diameter

CHAPTER III

3.1 Results and discussion

Recent studies have shown that hospital pathogens including Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomicine-resistant *Enterococci* (VRE), *Pseudomonas* spp., *Acinetobacter* spp. and virus (eg. Norovirus) persist on the inanimate surfaces, for a period ranging from some days to several weeks; e.g. the spores of *Clostridium difficile* can survive on environmental surfaces for months, with their infectious ability still active [33]. Therefore, the risk of acquiring infections increases for patients located in shared rooms or locals previously occupied by colonized or infected people.

Some of these microorganisms like *Staphylococcus aureus* are normally present on the skin and nasopharyngeal mucous membranes of the adults and in the immunocompromised patients and they can induce infectios developement and can be conveyed by contact directed with hands and objects, or by the form of droplets diffused by sneezing and coughing or migrate at different sites-borne pathogens if there is in the presence of wounds. *Enterobacteriaceae* and *Clostridium difficile* are naturally located in gastrointestinal tract, as well as *Candida albicans* residing in vaginal, gastrointestinal and oropharyngeal mucous membranes.

The massive use of antibiotics and an oversanification with chemicals products can increase drug resistance rate. Particulary attention arises to sanitizing methods, mainly because the wards are not enclosed local, thus recontamination continues. Hence the need to maintain the microbial load below hygienically acceptable limits, even as time passes by, in comparsion to everyday sanitization. For these reasons monitoring was performed at time of the maximum microbial colonization, i.e. just before the afternoon sanitisation which usually, in the facilities chosen, is carried out 7 hours after morning cleaning.

As described in the previous chapter the microorganisms used as indicators were:

Staphylococcus spp. and *Staphylococcus aureus* grown on Baird Parker Agar

Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp. etc.) grown on MacConkey Agar

Acinetobacter spp. grown on Herellea Agar

Pseudomonas spp. grown on Cetrimide Agar

Clostridium difficile grown on *Clostridium difficile* Agar

Candida spp. grown on Sabouraud Agar + Cloramphenicol

Aspergillus spp. grown on Sabouraud Agar + Cloramphenicol

While the *Bacillus* spp. colonies have been counted on TSA.

The microbiological quality index (MQI) was obtained considering the CFU of all the pathogens detected on the different selective media. Statistical analysis was performed considering the data without distinguishing by department and/or sampling point, either by evaluating the progress of all pathogens for each hospital, and all hospitals by single sampling point or by single microorganism. The trend was observed for 12 months, highlighting the differences between the period in which daily cleanings were carried out using a traditional method and the one in which the probiotic system was applied. The proposed limits were obtained computing the median value of the pathogens sum. This was calculated by adding, for each sampling point (in the same room of the same hospital), together pathogens colonies, chosen as indicators of microbiological quality (list above) and grown on the selective culture media. The data obtained during the application of the two different types of sanitization protocol were compared and to define the limit of environmental contamination the values of the method that was most effective with which the best results were obtained: the probiotic system. As observable also in the following statistics evaluations, the median value corresponding to acceptability limite was established to be < 10,000 CFU/m^2. In fact, following the experimental results obtained in other Italian hospitals over the years and according to literature references [14], the value of 10,000 CFU/m^2 was proposed as threshold limit of acceptability (MQI, Microbial quality index) for the total load, intended as the result of the median value of the sum of pathogens that have grown on the different elective culture media for their isolation.

The results are charted with a box plot, providing the following functions:

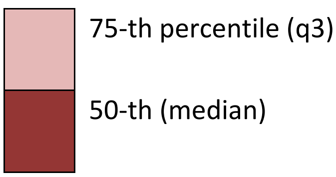
a) minimum and maximum values (min and max), which respectively stands for the lowest and highest value of the data series;

b) the first and third quartile (q1 and q3): the values within which the 25% and 75% of the data values are grouped;

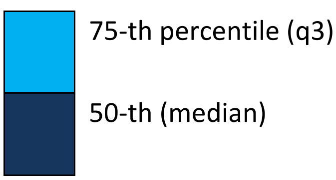
c) the median: representing the 50% of the data.

The width of the box indicates the data dispersion, relating to the third quartile and the median. Maximum and minimum limits are represented by the upper and lower lines outside the box. For better understanding, the legend for interpreting the chart box 1 is shown below.

Traditional procedures:



PCHS:



Hospitals monitored were named with sequential numbers: Hospital 1, Hospital 2, Hospital 3, Hospital 4, Hospital 5, Hospital 6 and Hospital 7. Hospital 1, 2 and 3 were the first to be monitored (January 2016) and are also facilities that contain more than 100 patients, where 42 sampling points for each microorganism were carried out. After 6 months, Hospital 4 and 5 (21 sampling points for each microorganism) were added to the research plan, while hospital 6 was enrolled in September 2016. In all facilities the second phase, which has involved the use of probiotic products, started in January 2017. Starting from June 2016 hospital 7 was monitored, where cleaning is carried out exclusively with chemicals and it was used as an external control [36]. The Hospitals from 4 to 7 are facilities with less than 100 in patients –

beds, therefore 21 sampling points for each microorganism were carried out. Chart 1 shows the values of the pathogen sum of all hospitals monitored, control (Hospital 7) including. The values of the microbial contamination, obtained during the first phase (6 months) where traditional products (T) have been used, are a lot higher than those detected during the second phase (6 months) in which the probiotics (P) were applied, as shown in Table 1 and it is graphically represented in Chart 1. In fact, the median value is definitely above the indicated limit value of 10,000 CFU.

	Hospital 1 T	Hospital 1 P	Hospital 2 T	Hospital 2 P	Hospital 3 T	Hospital 3 P	Hospital 4 T	Hospital 4 P	Hospital 5 T	Hospital 5 P	Hospital 6 T	Hospital 6 P	Hospital 7 T	Hospital 7 P
max (maximum value)	253,895	167,579	252,211	168,421	277,053	149,053	224,421	89,263	255,579	160,000	219,368	108,211	126,316	140,211
q3 (75% of the values)	44,105	11,895	60,737	17,684	71,263	18,947	78,632	11,789	86,211	31,474	57,474	7,684	42,105	58,526
median (50% of the values)	17,053	2,947	21,895	4,632	26,105	8,211	28,632	4,632	60,632	12,632	22,737	842	18,947	34,316
q1 (25% of the values)	2,211	0	3,684	842	8,000	1,263	2,947	526	6,421	2,526	6,737	0	2,211	5,579
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1. Statistical values of all indicators microorganisms in each hospital, in the traditional (T) and PCHS (P) system. Hospital 7 has been chosen as a control and it has maintained the traditional protocol during the whole period of monitoring.

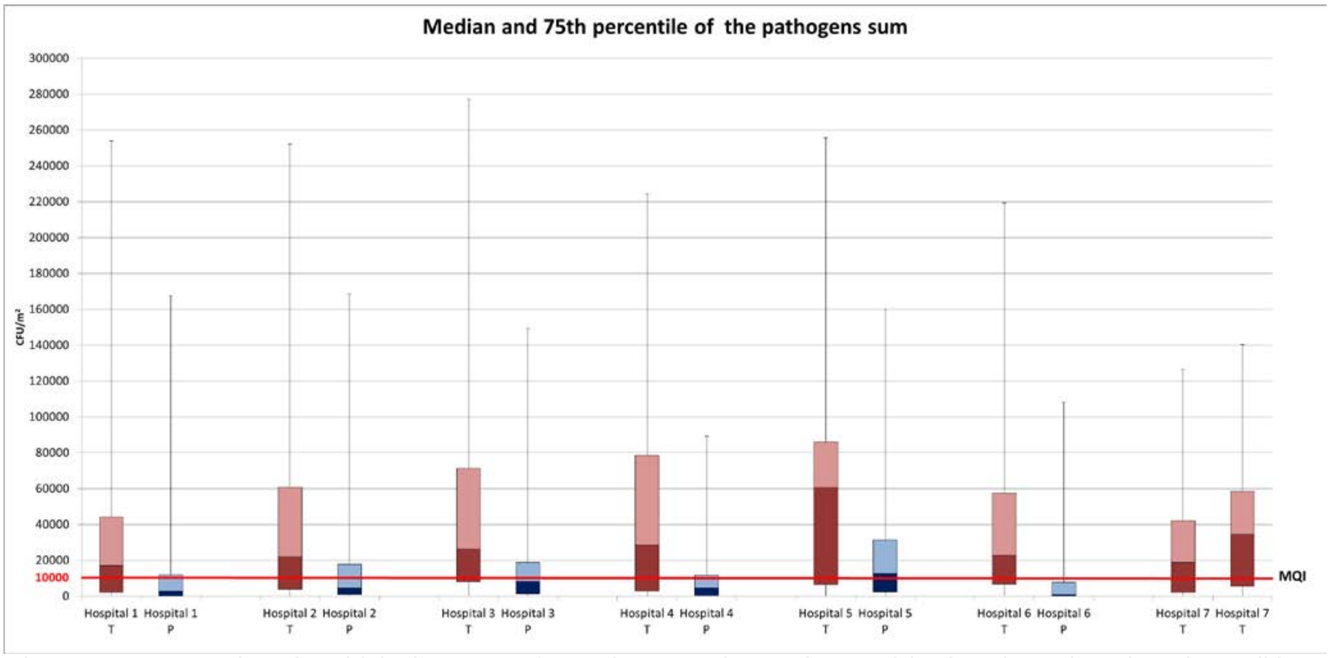


Chart 1. Box plot chart in which the CFU of all microorganisms, detected in the single hospitals in traditional period T (pink and red box, which represent third quartile and median respectively) and while applying the PCHS: P (light blue and blue box, which represent third quartile and median respectively) are compared. Red line represents the limit of 10,000 CFU (MQI limit).

In the second period in which the cleanings were performed by PCHS, the median of the sum of the detected pathogens is below the threshold value, with the exception of hospital 5, which started with a situation with high levels of contamination. It is proving that the method PCHS, after 7 hours from morning cleaning is effective in reducing the microbial growth.

Chart 2 was created considering the median values of the sum of pathogens of every single hospital. In this case, we observed the trend of total microbial load during the whole monitoring period sequentially, starting from phase 1 with the traditional (T) protocol and continuing with phase 2 when PCHS was applied. Samplings were performed monthly.

	Month 1- T	Month 2- T	Month 3- T	Month 4- T	Month 5- T	Month 6- T	Month 1- P	Month 2- P	Month 3- P	Month 4- P	Month 5- P	Month 6- P
Hospital 1	16,632	13,263	8,211	25,263	21,263	21,263	4,000	1,263	2,737	3,789	5,684	1,263
Hospital 2	20,632	38,526	10,316	14,947	28,421	25,263	5,053	3,789	5,053	3,579	6,316	4,842
Hospital 3	13,053	27,368	32,842	28,632	23,789	43,368	15,368	6,947	5,053	9,263	12,842	3,158
Hospital 4	64,000	53,053	13,474	23,579	20,632	24,000	5,895	6,316	4,632	421	4,632	3,368
Hospital 5	59,368	39,579	60,211	61,053	84,632	53,053	8,421	16,421	12,211	3,368	15,158	11,368
Hospital 6				22,737	25,053	18,105	5,684	1,895	5,684	211	421	421
Traditional protocol												
Hospital 7	25,263	42,105	18,526	11,789	14,737	2,947	21,053	13,895	34,105	23,158	42,105	48,421

Table 2. Statistical values for all microorganisms detected during the whole period. In the table are highlighted in pink the traditional months, while in blue the PCHS months. The hospital 7 maintains the traditional cleaning procedures throughout the whole period.

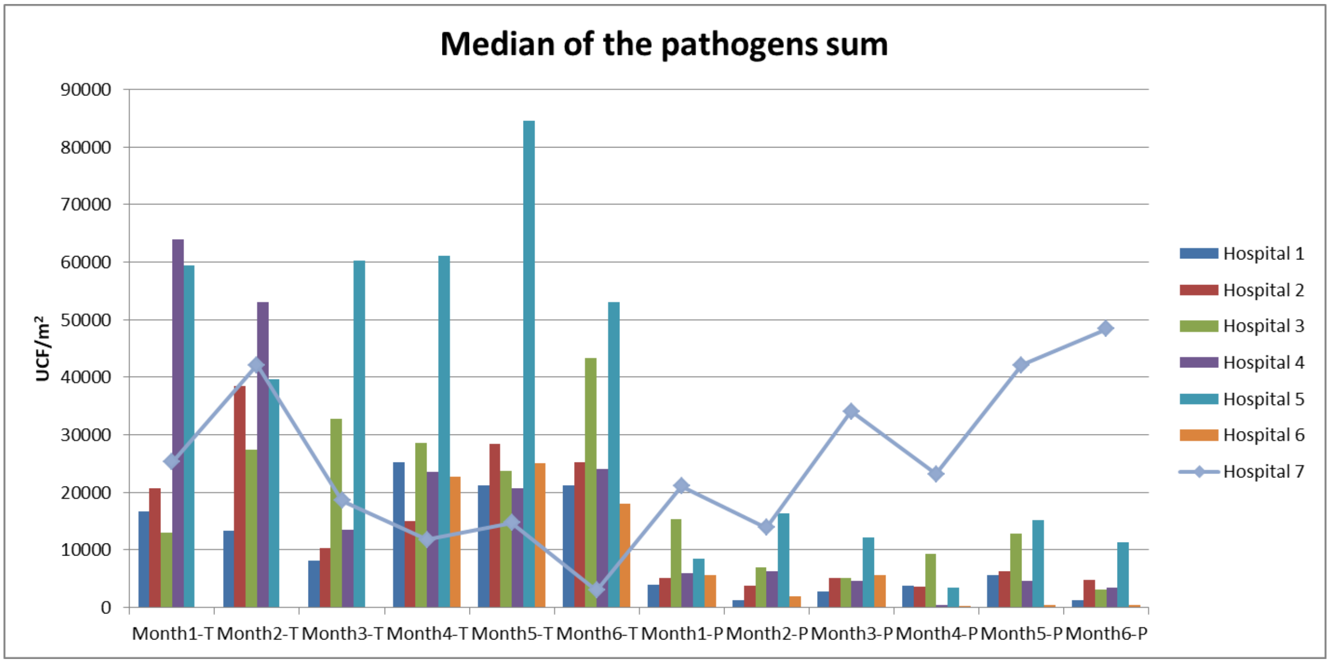


Chart 2. Trend of the sum of the single pathogens in the entire monitoring period. The first phase is distinguished from the second one, in which probiotic products were used, by the letters T (Traditional) and P (Probiotic system). Hospital 7 is represented by a line with markers chart in order to show the different trend. The pathogens of the six hospital decreases in the second phase, while in the control hospital (hospital 7) there is an exponential growth of the microbial load.

Graph 2 shows the trend of the microbial load in the various hospitals (bar chart), compared with the CFU detected in control hospital (line chart), which is represented here with a marked line to differentiate it. The results obtained during phase 2 indicate an effective reduction of the pathogenic load, as you can observe in table 3, where for each hospital the percentage reduction was calculated according to the following formula:

Reducing % = [(xf-xi)/xi] * 100 xf = final value; xi = initial value

	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Hospital 1	-75.9%	-90.5%	-66.7%	-85.0%	-73.3%	-94.1%
Hospital 2	-75.5%	-90.2%	-51.0%	-76.1%	-77.8%	-80.8%
Hospital 3	17.7%	-74.6%	-84.6%	-67.6%	-46.0%	-92.7%
Hospital 4	-90.8%	-88.1%	-65.6%	-98.2%	-77.6%	-86.0%
Hospital 5	-85.8%	-58.5%	-79.7%	-94.5%	-82.1%	-78.6%
Hospital 6				-99.1%	-98.3%	-97.7%
Hospital 7	-16.7%	-67.0%	84.1%	96.4%	185.7%	1542.9%

Table 3. Percentage reduction of the median values obtened from the sum of all pathogens of single hospital. The hospital 7 maintains the traditional cleaning procedures throughout the whole period.

The effectiveness of the method of sanitation is evident especially in those structures, as the hospital 5, where the initial contamination was quite high, with a microbial load of 61,053 CFU/m^2, 84,632 CFU/m^2 and 53,053 CFU/m^2, respectively in the month 4T, month 5T and month 6T (Table 2). While in the corresponding period for the phase 2, the microbial load underwent an appreciable decrease in a range of 78.6% to 94.5 % (Table 3), with median values of 3,368 CFU/m^2, 15,158 CFU/m^2 and 11,368 CFU/m^2 respectively in the month 4P, month 5P and month 6P (Table 2).

As demonstrated in numerous studies [14], traditional products are notoriously ineffective for decontamination purposes, as their action is able to immediately reduce the number of pathogens but their effectiveness is limited to a brief period of time immediately after cleaning, without forgetting that an excessive hygiene can induce the development of drug resistance phoenomena. The Probiotic – based sanitizing system, as well as being environmental friendly and safe for human health [33,34], seems to be a solution to this problem.

In fact, probiotic products, as demonstrated by the following charts, by colonizing the environment in which they are introduced, reduce and stabilize the potentially pathogenic bacteria. Laboratory tests were conducted on a quarterly basis in order to determinate in vitro the development of antiobiotic-resistancees using the Kirby-Bauer method which provides for the use of antibiograms to evaluate the resistance of *Staphylococcus aureus* to some commonly used antibiotics (OXOID, UK): Penicillin G (10iu), Ampicillin (10mcg), Vancomycin (5mcg), Oxacillin (5mcg), Cefotaxime (30mcg), Imipenem (10mcg). It has been shown that not only the presence of such microorganisms has diminished, but also their resistance or however the number of drug-sensitive pathogens has increased.

In the charts 3 and 4, the trends of median values of *Bacillus* spp. are compared and the trends of the pathogens sum during the 12 months of monitoring, arranged in a time sequence. The chart 3 shows the trend of the microbial load (red line) in the control hospital (Hospital 7) which remains high throughout the period, while the *Bacillus* values are close to zero (blue line).

Hospital 7	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Bacillus spp.	0	0	0	0	0	0	0	0	0	0	0	842
Pathogens sum	25,263	42,105	18,526	11,789	14,737	2,947	21,053	13,895	34,105	23,158	42,105	48,421

Table 4. Median values of *Bacillus* spp. and sum of pathogens in control hospital 7.

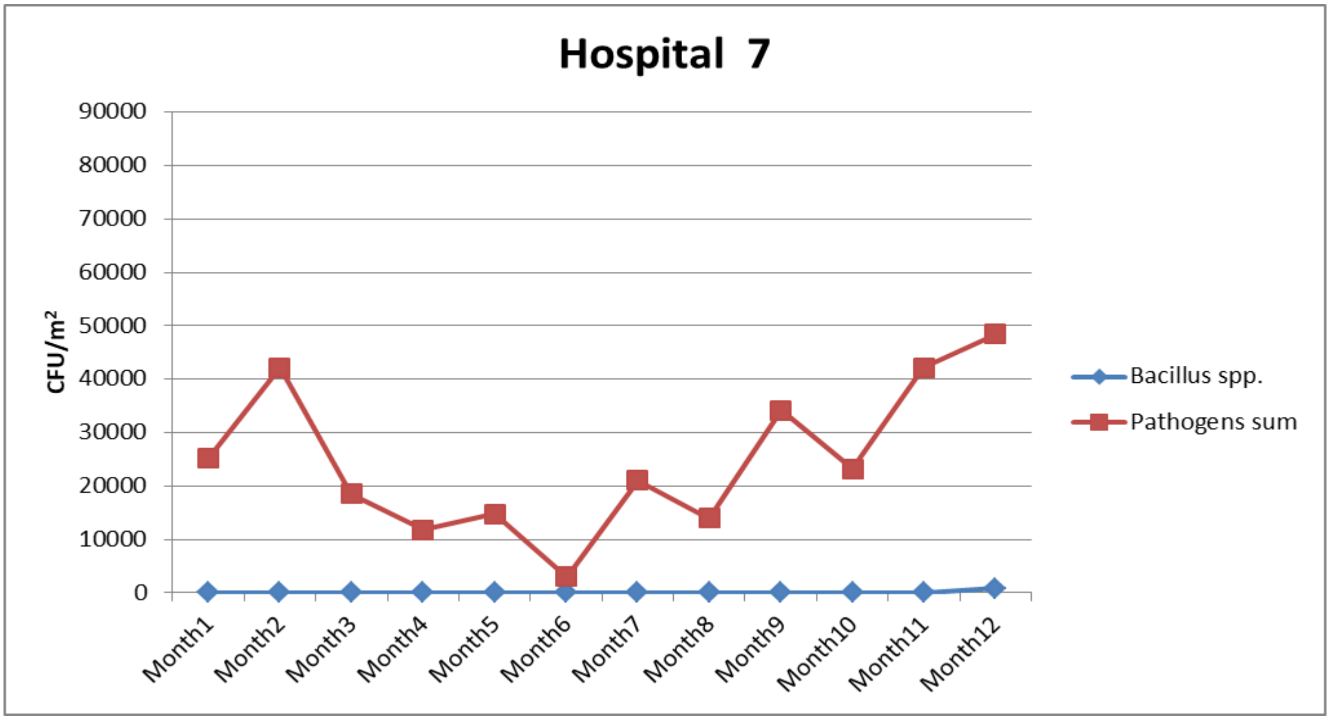


Chart 3. Comparison between median values of *Bacillus* spp. and the sum of pathogens for the whole monitoring period.

The chart 4 shows, as the colonies number of the pathogens decreases, the detection in all hospitals decreases considerably in the 6 months of PCHS (Chart 4, Month 7 – Month 12, blue line) respected to first six months (Chart 4, Month 1 – Month 6, red line), where the cleaning practices have been carried out using chemicals. While the *Bacillus* colonies number gradually increases, replacing the pathogens according to the principle of biological competition (competitive exclusion principle) which creates a biostabilization effect.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
***Bacillus* spp.**	211	421	0	0	421	421	32,211	38,737	53,053	64,000	54,737	57,474
Pathogens sum	18,737	27,789	13,263	24,632	27,158	24,421	6,947	5,053	5,053	3,158	4,632	2,947

Table 5. Median values of *Bacillus* spp. and pathogens sum of all hospitals, excluding the control.

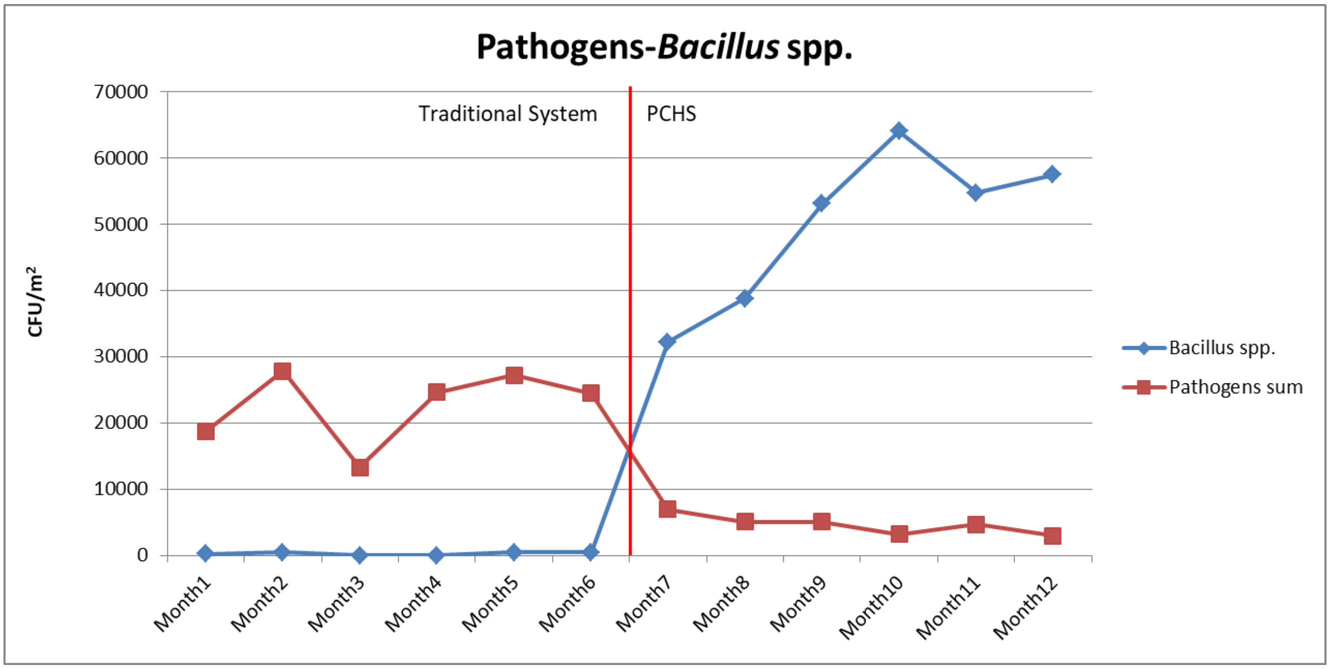


Chart 4. Representation of the comparison between median values of *Bacillus* spp. (Table 5) and the sum of pathogens in all hospitals for the whole monitoring period.

In order to evaluate the level of contamination, three surface sampling points were chosen into the hospital room: sink, floor and footboard. As it can be seen in graph 5, during the first six months of monitoring, when chemical products were used, the levels of contamination of the sink and the floor were high, while in the second phase in which probiotics were applied (from the 7th to 12th month), these values have decreased considerably. This decrease, even if less evident, has also affected the footboard sampling point, as shown in the values calcuted in table 6 and graphically represented in chart 5.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Sink	82,737	72,842	36,211	46,947	80,842	83,158	8,000	5,474	5,053	11,789	12,211	9,474
Floor	26,316	41,263	31,158	40,211	32,632	38,737	12,842	9,684	8,632	5,053	12,211	6,947
Footboard	2,526	2,737	2,316	1,474	2,526	3,158	842	421	842	211	211	421

Table 6. Median values of all hospitals, excluding control, for sampling point throughout the monitoring period. Month1-Month 6: months of cleaning with the traditional method; Month7-Month12: months in which the system based on probiotics was applied.

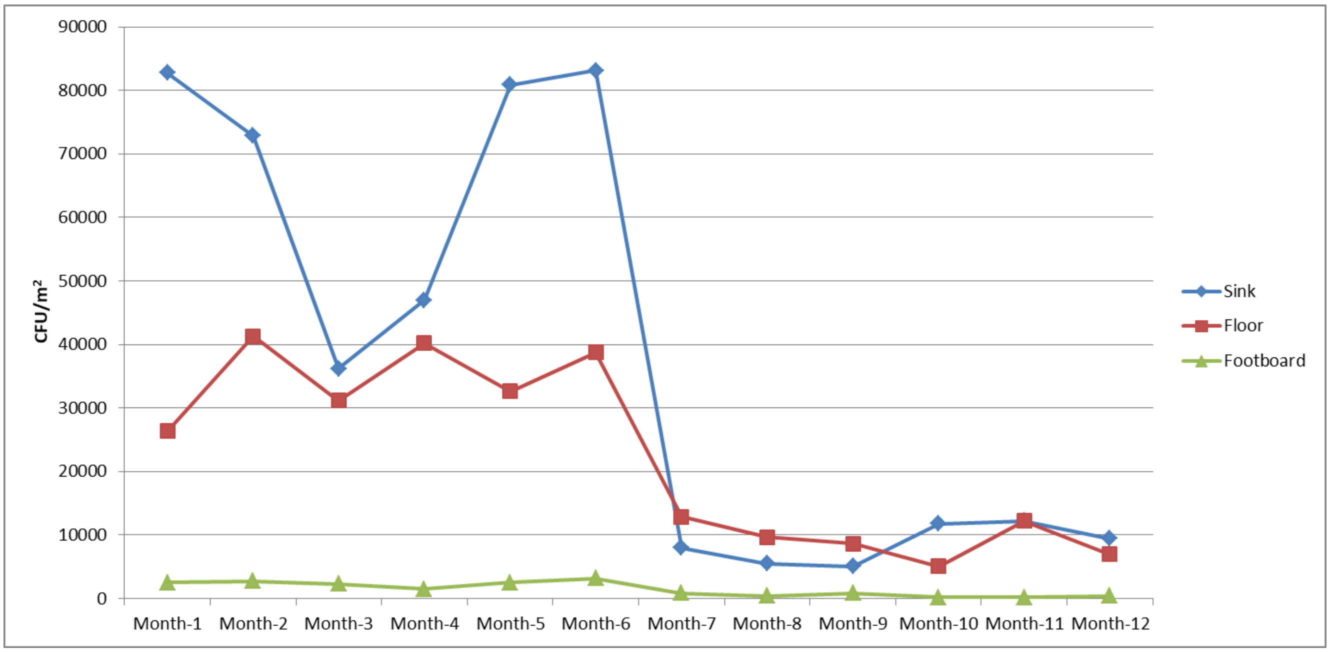


Chart 5. Graphic representation of median values of all hospitals (Table 6), excluding the control, for sampling point throughout the monitoring period. Month1-Month 6: months of cleaning with the traditional method; Month7-Month12: months in which the system based on probiotics was applied.

This decrease of the microbial load is even more evident if we compare the values obtained in the traditional (T) and during the application of probiotics (P) in the same months, as shown in chart 6.

In the traditional period, the sink is a particularly critical spot for microbial contamination (Chart 6, from Month1-T to Month6-T) not only because of microclimatic conditions that are established, but also because of its use and of the human activities carried out on it. While in the PCHS period the contamination level decreases exponentially, from initial values included in a range of 83,158 CFU/m^2 - 36,211 CFU/m^2 to values of 12,211 CFU/m^2 - 5,053 CFU/m^2,

	Month 1-T	Month 2-T	Month 3-T	Month 4-T	Month 5-T	Month 6-T	Month 7-P	Month 8-P	Month 9-P	Month 10-P	Month 11-P	Month 12-P
Sink	82,737	72,842	36,211	46,947	80,842	83,158	8,000	5,474	5,053	11,789	12,211	9,474
Floor	26,316	41,263	31,158	40,211	32,632	38,737	12,842	9,684	8,632	5,053	12,211	6,947
Footboard	2,526	2,737	2,316	1,474	2,526	3,158	842	421	842	211	211	421

Table 7. Median values for sampling point and comparison between traditional months (from Month1-T to Month6-T) and months of PCHS (from Month7-P to Month12-P). This table shows the medians of the pathogens sum of all the hospitals, excluding the control (hospital 7).

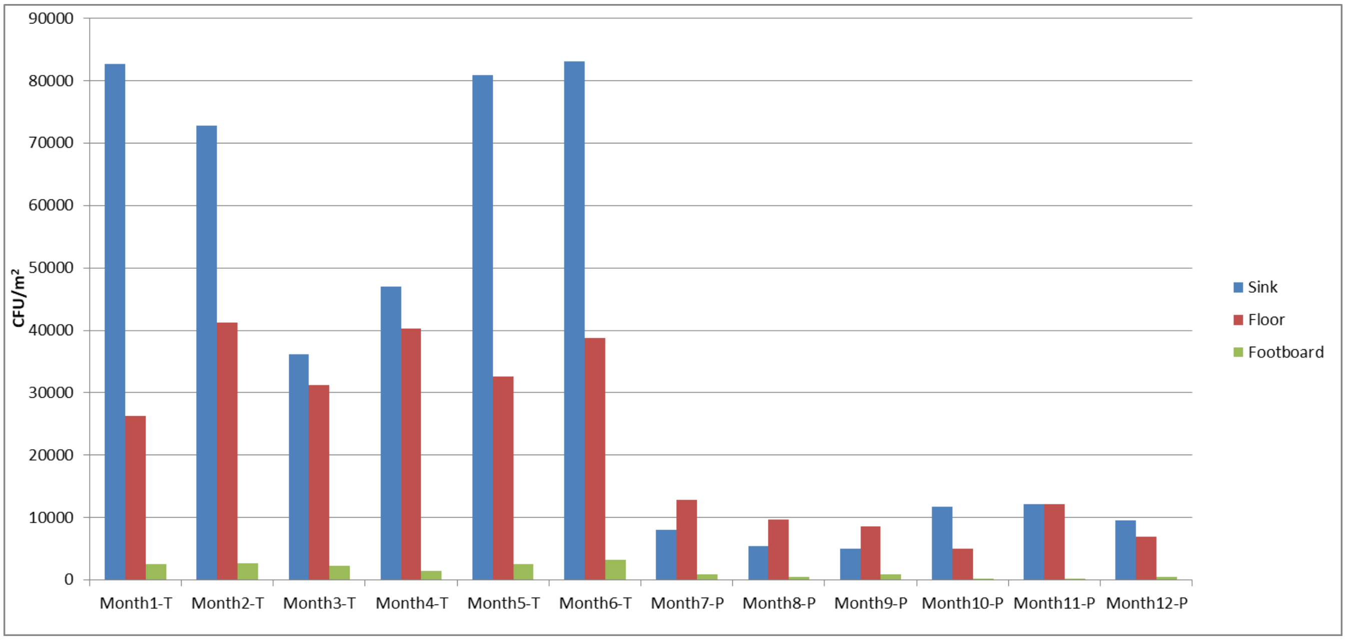


Chart 6. Graphic representation of median values for point of sampling and comparison between traditional months (from Month1-T to Month6T) and months of PCHS (from Month7-P to Month12-P), escluding the control hospital (hospital 7).

To demonstrate the consistency of data, the statistical values for a single sampling point are shown below, comparing the median values of all the monitored hospitals versus the control hospital.

Regarding the sink (Table 8, Chart 7) there is an evident reduction of microbial load in phase 2 and in the data values, increasing the difference with the control.

Sink	Month1	Month2	Month3	Month4	Month5	Month6	Month7	Month8	Month9	Month10	Month11	Month12
Hospitals	82,737	72,842	36,211	46,947	80,842	83,158	8,000	5,474	5,053	11,789	12,211	9,474
Control	25,263	42,105	13,474	11,368	22,316	1,684	18,526	9,263	32,421	22,737	46,316	32,842

Table 8. Median values for sampling point (sink), comparing the CFU detected in all hospitals and in control in the 6 months in which the sanitation chemicals have been used (from Month1 to Month 6) and the further 6 months (from Month 7 to Month 12) of probiotics application.

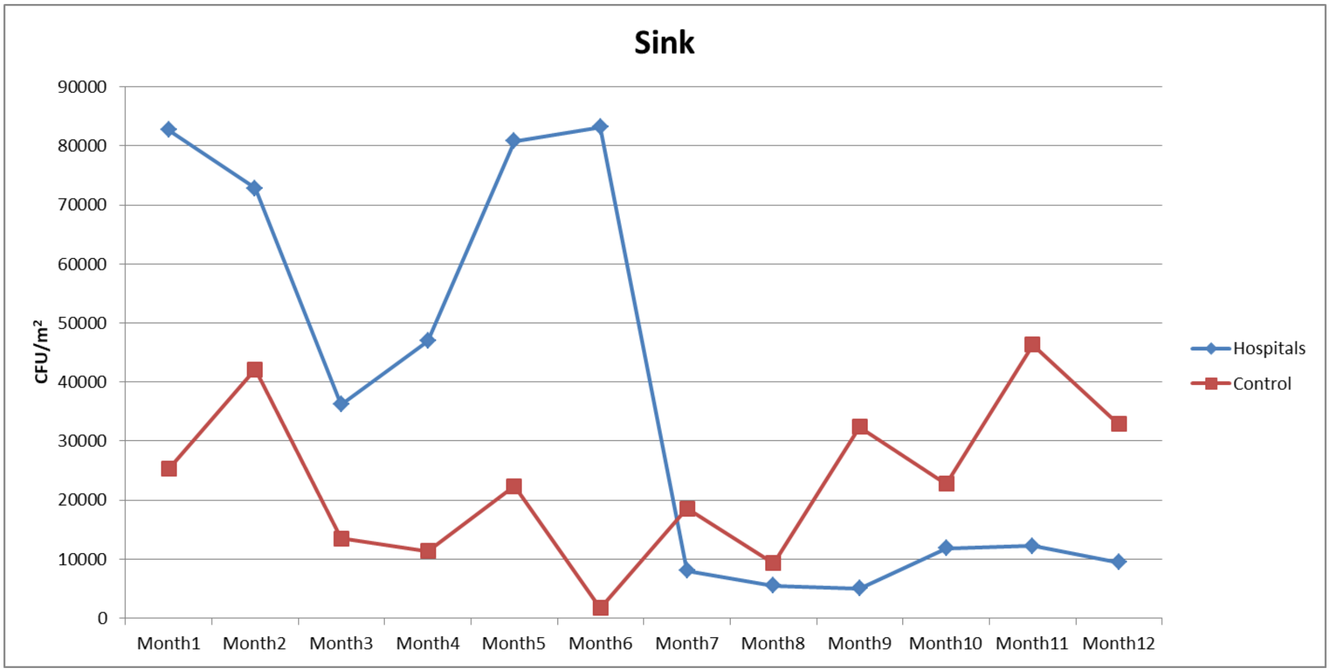


Chart 7. Graphic representation of median values of the sink throughout the period monitored and comparison between the CFU of all hospitals and control hospital. The chart show the microbial load detected in the initial 6 months of the traditional sanitizing (from Month1 to Month 6) and in the 6 months later application of the probiotics (from Month 7 to Month 12).

Regarding the sampling point “floor”, in phase 1 the trend of microbial load is similar between control hospital and all other hospitals (Table 9, Chart 8), while in the second phase due to the drastic decrease in median values of all hospitals, from maximum value of 41,263 CFU/m^2 to minumum one of 5,053 CFU/m^2 (Table 9), it corresponds to an exponential increase in the control.

Floor	Month1	Month2	Month3	Month4	Month5	Month6	Month7	Month8	Month9	Month10	Month11	Month12
Hospitals	26,316	41,263	31,158	40,211	32,632	38,737	12,842	9,684	8,632	5,053	12,211	6,947
Control	42,105	53,053	34,526	48,000	19,789	32,842	57,263	45,053	43,789	72,842	85,895	76,211

Table 9. Median values for sampling point (floor) comparing the CFU detected in all hospitals and in control in the 6 months in which the sanitation chemicals have been used (from Month1 to Month 6) and the further 6 months (from Month 7 to Month 12) of probiotics application.

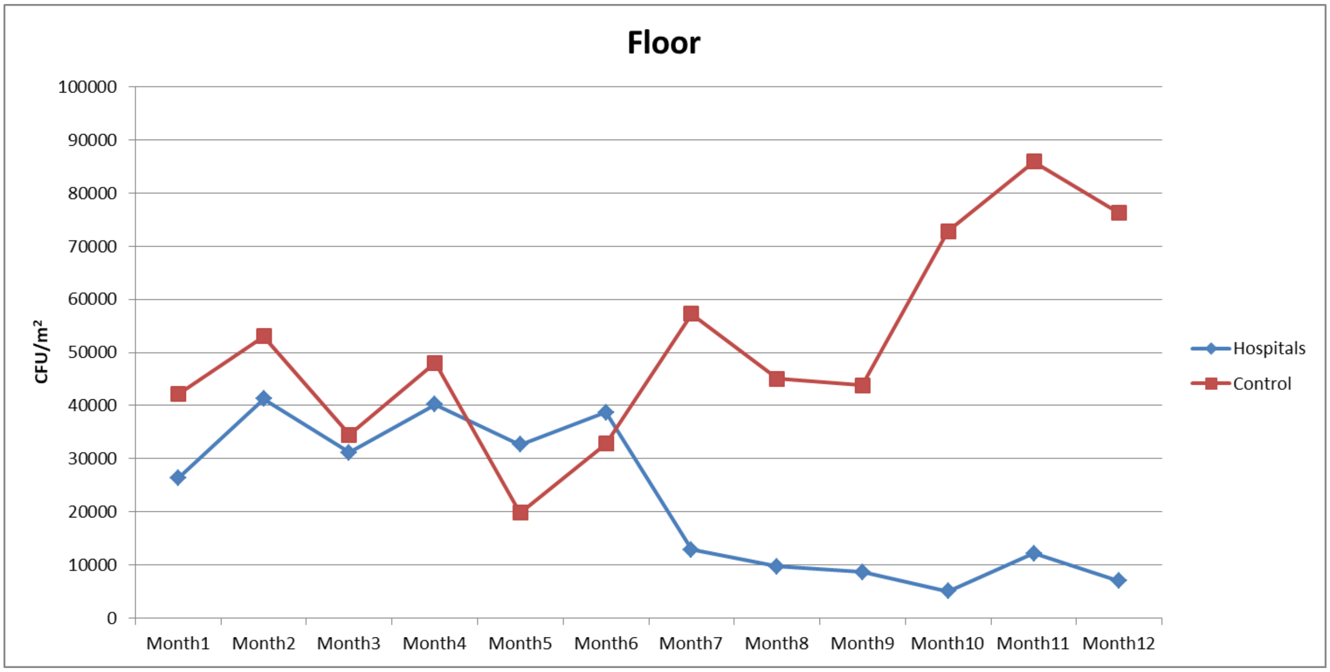


Chart 8. Graphic representation of median values of the floor throughout the period monitored and comparison between the CFU of all hospitals and control hospital. The chart show the microbial load detected in the initial 6 months of the traditional sanitizing (from Month1 to Month 6) and in the 6 months after application of the probiotics (from Month 7 to Month 12).

Finally, the sample point “footboard” in control hospital has 3 obvious peaks in the 2nd, 9th and 11th month (8,421 and 8,000 CFU/m^2), while the other hospitals values are below 3.158 CFU/m^2 as detected in the traditional. Also in this case, in phase 2 (from month 7 to month 12) the contamination decreases in an evident manner, with a maximum value of 842 CFU/m^2 and a minimum value of 211 CFU/m^2 (Table 10).

Footboard	Month1	Month2	Month3	Month4	Month5	Month6	Month7	Month8	Month9	Month10	Month11	Month12
Hospitals	2,526	2,737	2,316	1,474	2,526	3,158	842	421	842	211	211	421
Control	2,947	8,421	6,316	1,684	2,105	421	1,263	1,263	8,000	421	5,895	6,737

Table 10. Median values per sampling point (footboard) comparing the CFU detected in all hospitals and in control in the 6 months in which the sanitation chemicals have been used (from Month1 to Month 6) and the further 6 months (from Month 7 to Month 12) of probiotics application.

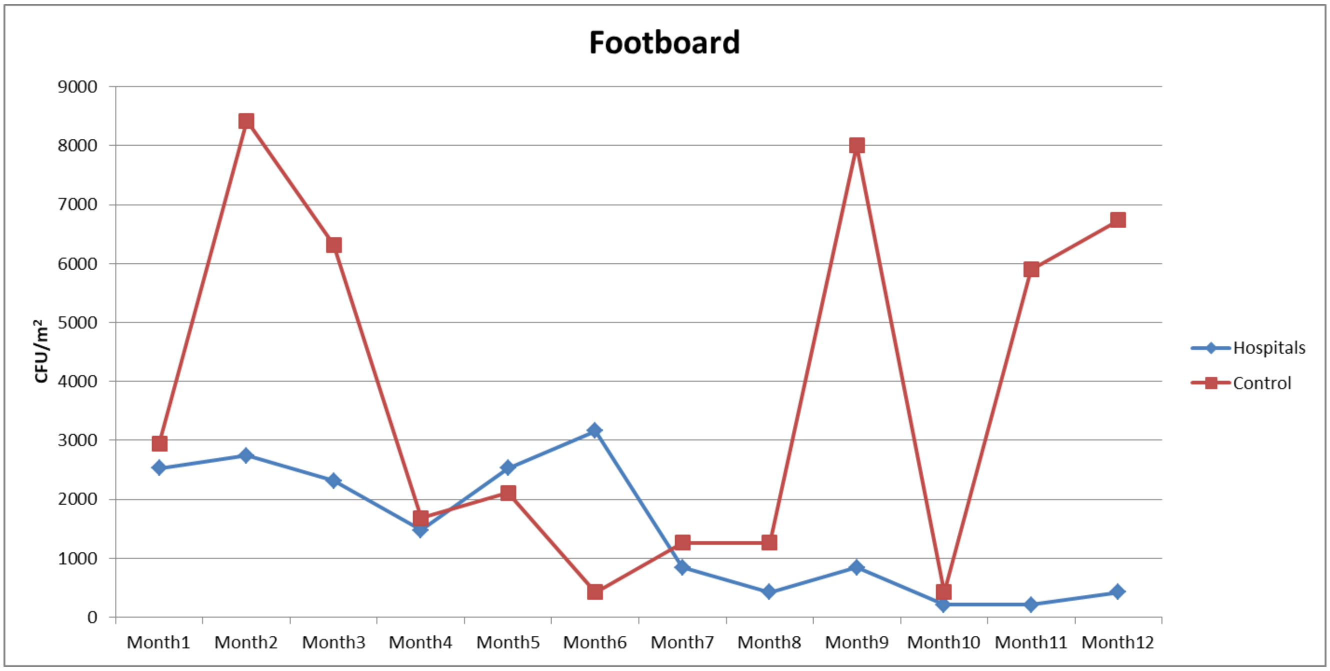


Chart 9. Graphic representation of median values of the footboard throughout the period monitored and comparison between the CFU of all hospitals and control hospital. The chart show the microbial load detected in the initial 6 months of the traditional sanitizing (from Month1 to Month 6) and in the 6 months later application of the probiotics (from Month 7 to Month 12) median values per point of sampling and comparison between all hospitals and control.

In order to define a threshold for microbial contamination, the data were processed considering the single pathogens detected in all 6 hospitals in which both traditional sanitization systems and probiotic products (PCHS) were used. The values used in the elaborations correspond to the microorganisms grown and counted on the specific media for their isolation. The tables and graphs shown below were constructed considering all the colonies detected in phase 1 in which traditional hygienic products were used (from Month1-T to Month6-T) and in the following phase 2 during the application of probiotics (from the month 1-P per month 6-P). The charts below were built by box plots, as described earlier. The boxes represent the median values (blue box) and the q3 (light blue box), the 75% of the values. As can be seen from the following elaborations, the value of the pathogens sum relies, above all, on the presence of *Staphylococcus* spp., in fact the median values for all the other microorganisms that were chosen as indicators are equal to zero for the entire monitoring period. The high median values of *Staphylococcus* spp., detected in phase 1 (from month 1-T to month 6-T) and included in a range between 26,947 CFU/m^2 and 10,737 CFU/m^2, decreases to values between 6,105 CFU/m^2 and 1,684 CFU/m^2 (Table 11, Chart 10). There is an obvious decrease in pathogenic load (Table 11, Chart 10) in phase 2 where the median values are below 10,000 CFU/m^2.

***Staphylococcus* spp.**	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 P
max (maximum value)	101,053	98,526	84,211	84,211	84,211	93,474	84,211	84,211	84,211	84,211	84,211	98,947
q3 (75% of the values)	58,316	63,789	51,579	51,053	73,368	62,842	17,158	9,684	14,211	12,947	17,684	12,632
median (50% of the values)	15,158	26,947	10,737	20,421	24,211	21,474	6,105	4,211	4,632	2,526	3,368	1,684
q1 (25% of the values)	3,053	4,211	2,421	2,526	4,842	5,158	842	421	421	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 11. Median values of *Staphylococcus* spp. during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

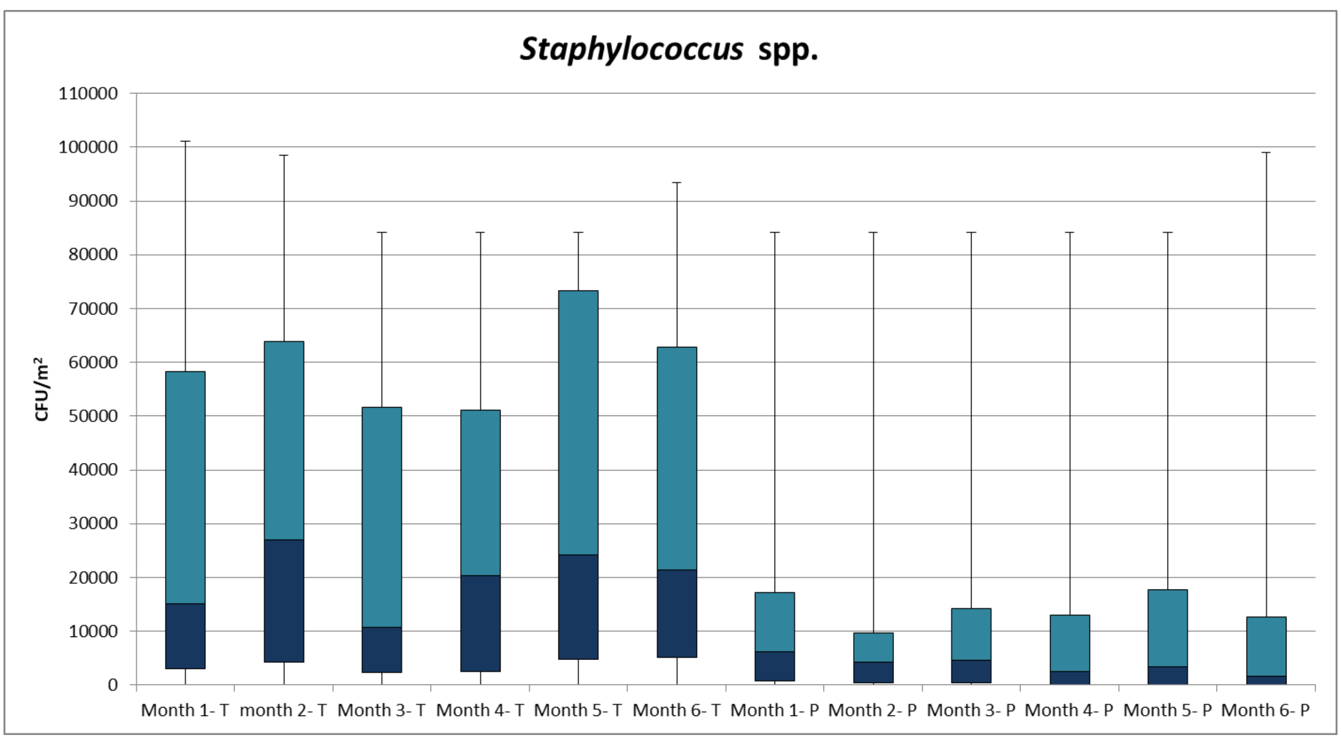


Chart 10. Representation of statistical values for *Staphylococcus* spp.

If you look at the data of *St. aureus* (Table 12, Chart 11), the values of q3 detected in phase 1 are below 1,263 CFU/m^2, while in phase 2 the decrease is equal to 100%. Also the median values are equal to zero.

Staphylococcus aureus	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 P
max (maximum value)	10,947	10,105	27,368	10,947	4,632	82,947	4,632	2,526	842	2,526	1,263	842
q3 (75% of the values)	1,263	1,263	842	842	842	842	421	0	0	0	0	0
median (50% of the values)	421	421	211	421	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 12. Median values of *Staphylococcus aureus* during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

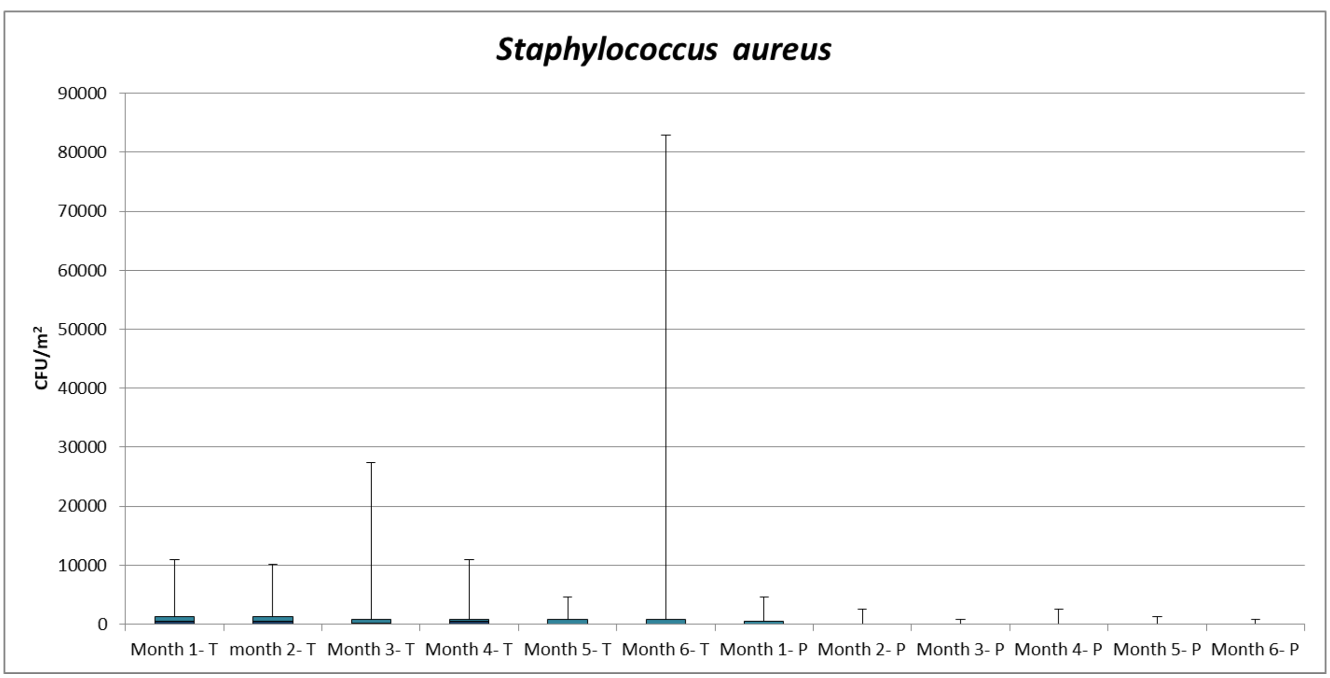


Chart 11. Representation of statistical values for *Staphylococcus aureus.*

The following there are the values of the microorganisms and their graphical representation: Enterobacteriaceae (Table 13, Chart 12), *Acinetobacter* spp. (Table 14, Chart 13), *Pseudomonas* spp (Table 15, Chart 14), *Clostridium difficile* (Table 16, Chart 15), *Candida* spp. (Table 17, Chart 16) and *Aspergillus* spp. (Tab 18, Chart 17). As mentioned, the median values of these pathogens are zero, but we can observe the decrease in the heat of q3 with regard to *Clostridium difficile*. in chart 15 (Table 16) and *Candida* spp. (Table 17, Chart 16).

Enterobacteriaceae	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	84,211	61,895	84,211	84,211	84,211	84,211	21,474	13,895	4,632	24,000	84,211	8,421
q3 (75% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 13. Median values of *Enterobacteriaceae* during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

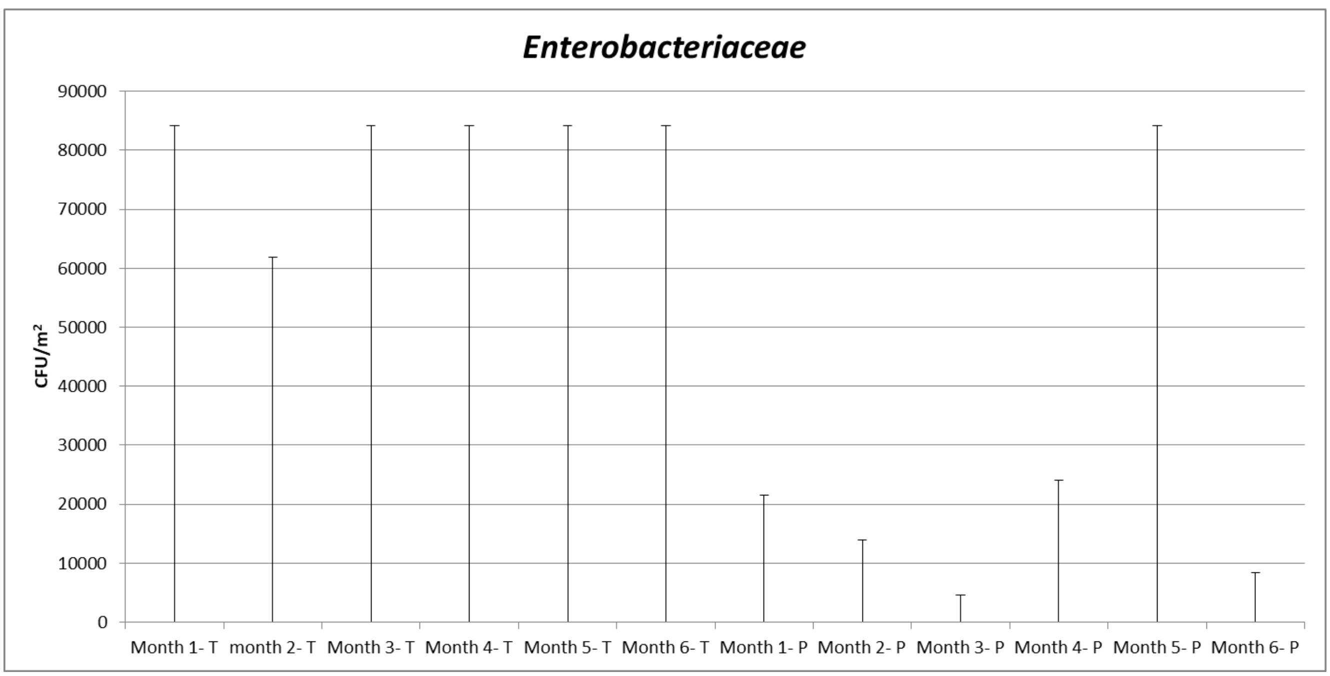


Chart 12. Representation of statistical values for *Enterobacteriaceae*

Acinetobacter spp.	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	84,211	84,211	34,947	84,211	84,211	84,211	83,368	48,421	17,263	3,368	34,947	6,316
q3 (75% of the values)	0	105	0	0	0	0	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 14. Median values of *Acinetobacter* spp. during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

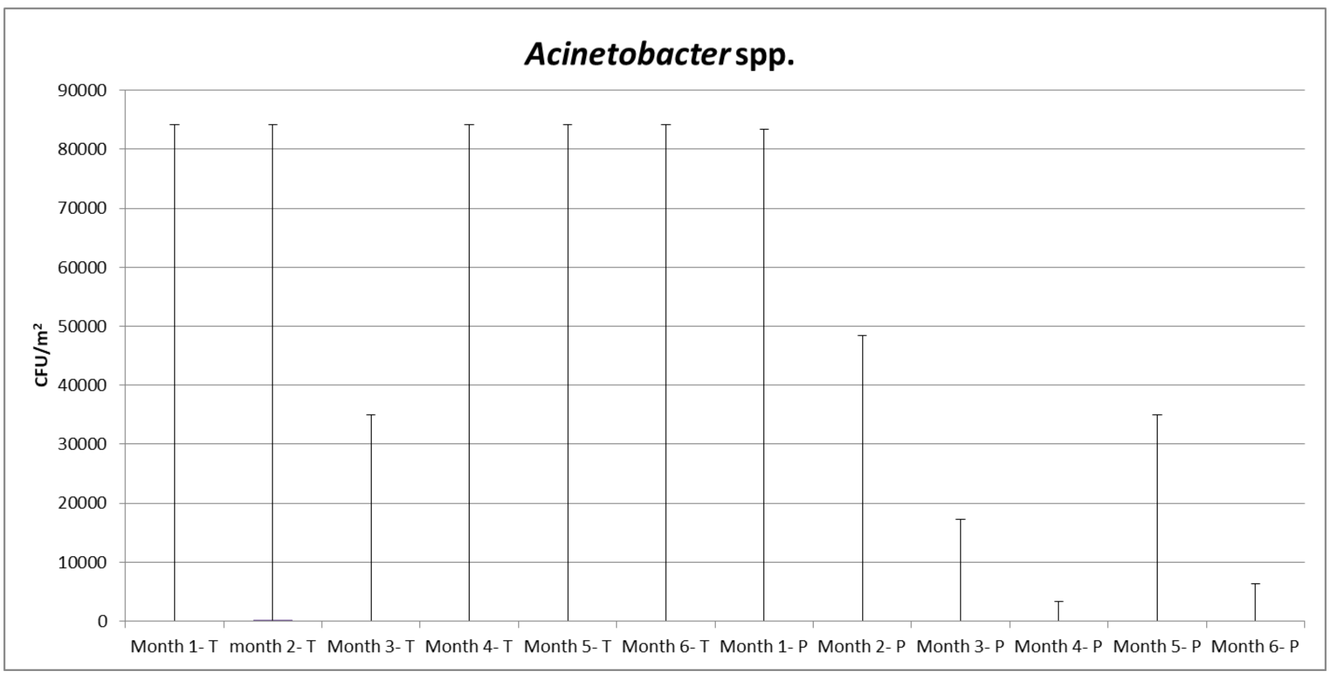


Chart 13. Representation of statistical values for *Acinetobacter* spp.

***Pseudomonas* spp.**	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	3,789	84,211	47,158	84,211	84,211	84,211	48,000	52,632	5,895	2,526	16,000	84,211
q3 (75% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 15. Median values of *Pseudomonas* spp. during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

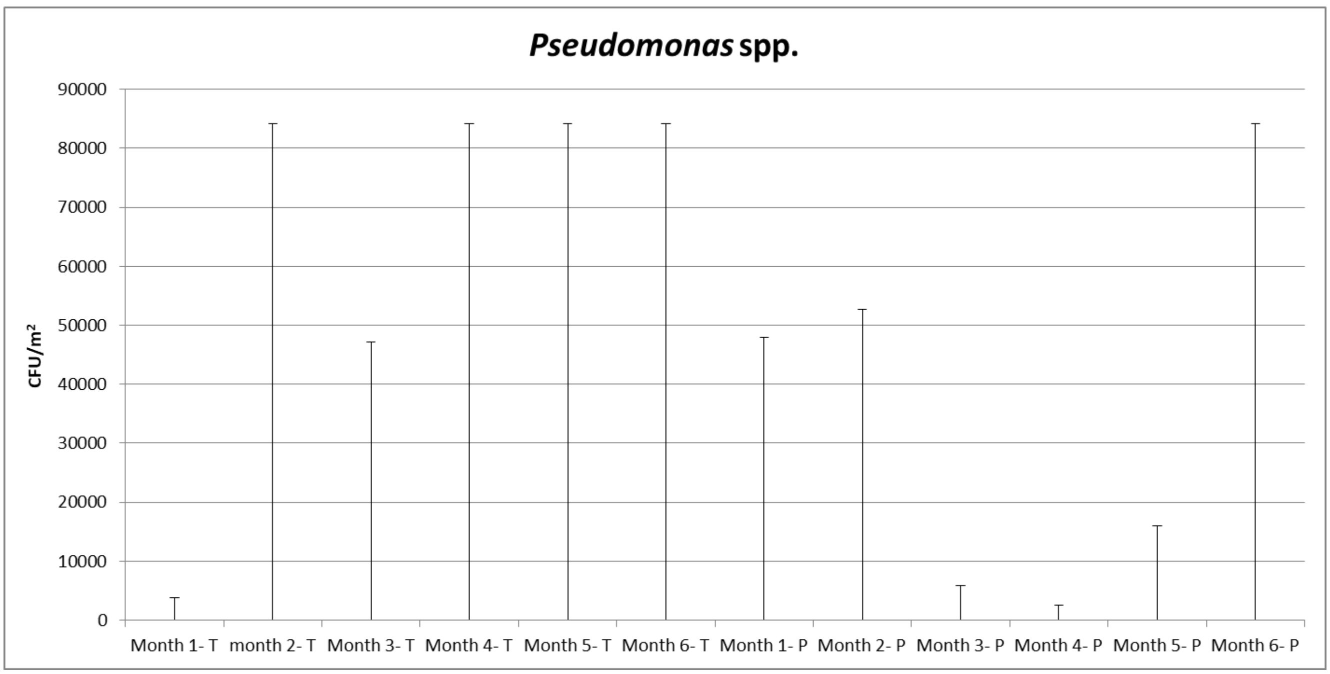


Chart 14. Representation of statistical values for *Pseudomonas* spp.

Clostridium difficile	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	48,000	8,421	13,053	14,316	24,000	7,158	18,105	4,632	7,158	1,684	842	27,368
q3 (75% of the values)	0	421	105	421	421	421	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 16. Median values of *Clostridium difficile* during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

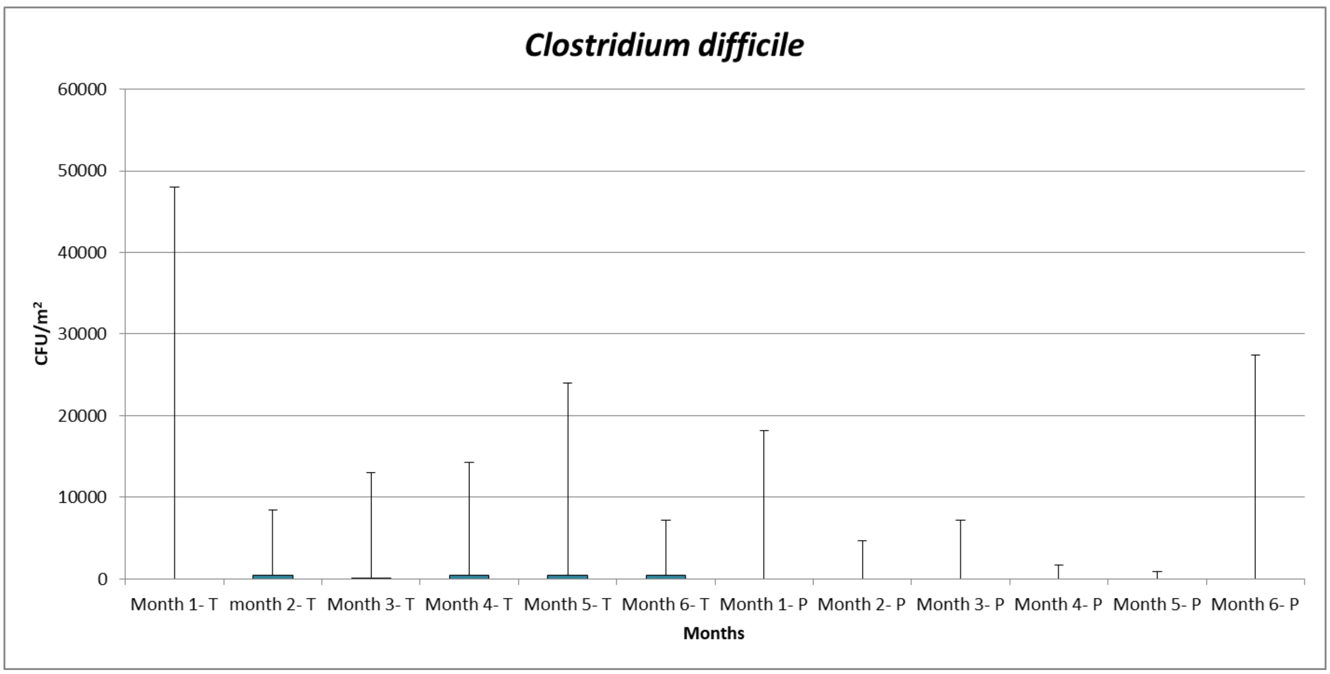


Chart 15. Representation of statistical values for *Clostridium difficile*.

***Candida* spp.**	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	84,211	84,211	84,211	84,211	84,211	84,211	84,211	84,211	48,421	55,579	84,211	24,000
q3 (75% of the values)	1,263	842	526	0	842	842	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 17. Median values of *Candida* spp. during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

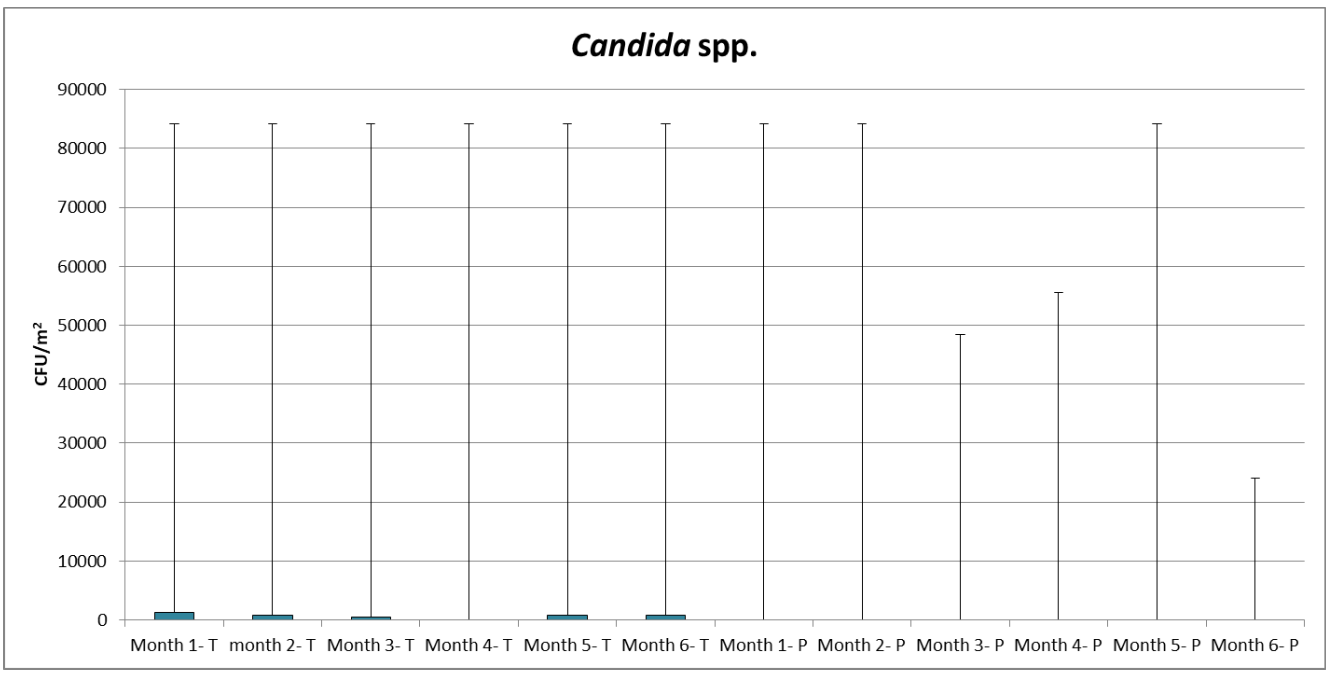


Chart 16. Representation of statistical values for *Candida* spp.

***Aspergillus* spp.**	Month 1 T	Month 2 T	Month 3 T	Month 4 T	Month 5 T	Month 6 T	Month 1 P	Month 2 P	Month 3 P	Month 4 P	Month 5 P	Month 6 T
max (maximum value)	52,632	421	1,684	1,263	10,526	842	421	842	421	421	842	421
q3 (75% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
median (50% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
q1 (25% of the values)	0	0	0	0	0	0	0	0	0	0	0	0
min (minimum value)	0	0	0	0	0	0	0	0	0	0	0	0

Table 18. Median values of *Aspergillus* spp. during the entire period of monitoring, traditional cleaning methods (from month1-T to month6-T) and PCHS (from month1-P to month6-P).

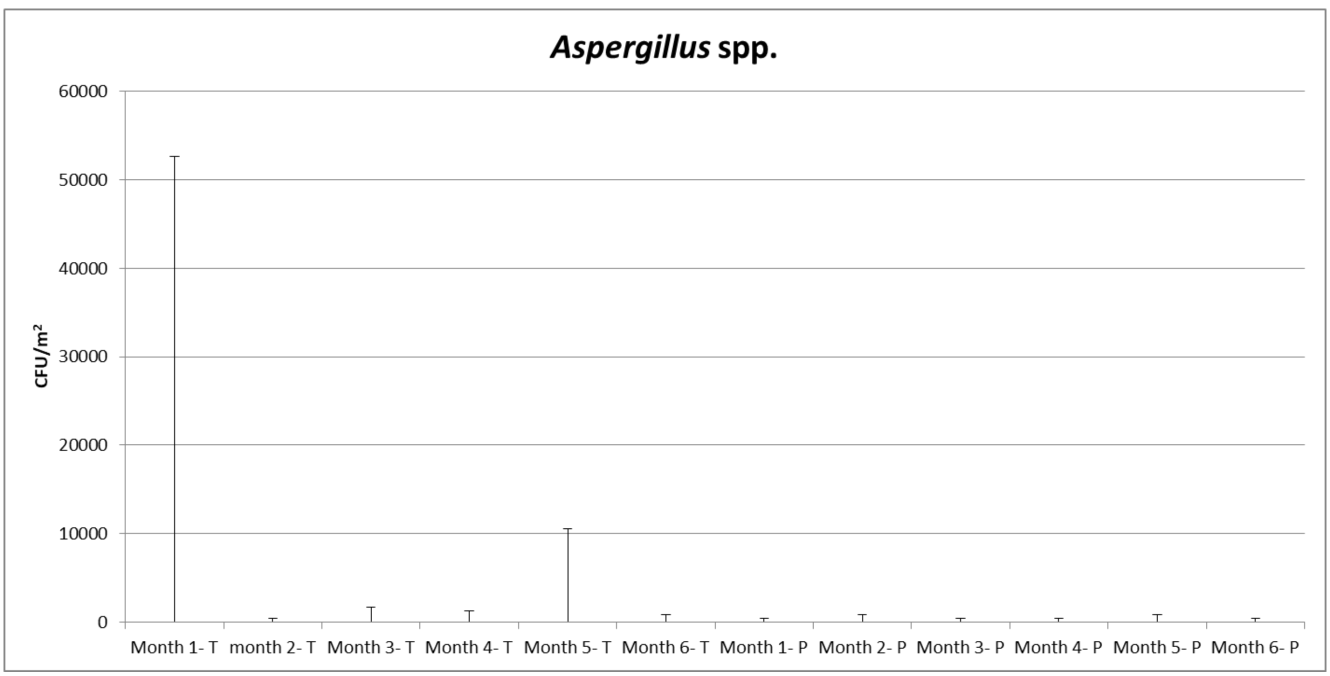


Chart 17. Representation of statistical values for *Aspergillus* spp.

This data (from Table 11/Chart 10 to Table 16/Chart 17) were further elaborated, adding up the median values (50 % of the data) of each microorganism detected in all the hospitals (control excluded) every month. The obtained value for each month(MQI) attested below the value of 10,000 CFU/m^2.

Monitoring months	*Staphylococcus spp* (CFU/m^2).	*Enterobacteriaceae* (CFU/m^2).	*Acinetobacter spp.* (CFU/m^2).	*Clostridium difficile* (CFU/m^2).	*Pseudomonas* spp (CFU/m^2)..	*Candida* spp (CFU/m^2)..	*Aspergillus* spp (CFU/m^2)..	*sum of median values of pathogens* (CFU/m^2).	*Microbial quality index (MQI).*
Month 1-T	15,158	0	0	0	0	0	0	15,158	< 10,000 CFU/m^2
Month 2-T	26,947	0	0	0	0	0	0	26,947	
Month 3-T	10,737	0	0	0	0	0	0	10,737	
Month 4-T	20,421	0	0	0	0	0	0	20,421	
Month 5-T	24,211	0	0	0	0	0	0	24,211	
Month 6-T	21,474	0	0	0	0	0	0	21,474	
Month 1-P	6,105	0	0	0	0	0	0	6,105	
Month 2-P	4,211	0	0	0	0	0	0	4,211	
Month 3-P	4,632	0	0	0	0	0	0	4,632	
Month 4-P	2,526	0	0	0	0	0	0	2,526	
Month 5-P	3,368	0	0	0	0	0	0	3,368	
Month 6-P	1,684	0	0	0	0	0	0	1,684	

Table 17. Median values of the individual microorganisms detected in all hospitals (Control excluded) every month.

Conclusions

Over the past few years the CIAS research group collected data by monitoring hospitals, clinics and operating rooms, but it is not easy to define a precise limit, just a possible range of acceptability. In fact, especially in environments such as the hospital wards, where a continuous flow of people is present: health and medical staff, patients, visitors, technicians, cleaners, etc, the phoenomena of recontamination is quite common. It is certainly indispensable to improve the hand hygiene compliance of the operators in order to reduce the transmission of pathogens associated with health care, thus the incidence of HAI (Healthcare associated infections) [37]. In addition to the hands the possible sources of infection can be: the direct contact between healthy and sick person, equipment and instruments, such as endoscopes, catheters, or food, or organic fluid like human blood. Many of the potentially pathogenic microorganisms can survive on surfaces for days and, in the form of particulate material, can be transported by the air to distant sites. So the surfaces become a reservoir for these microorganisms and as in literature, traditional methods based on chemicals, certainly effective to counteractthe pathogenic load immediately after sanitizing practices, but their action does not last over time. Futhermore the frequent use of such products, as well as having a significant environmental impact, can induce the development of drug resistance. For these reasons there is a need to find alternatives to traditional sanitisation procedures and the response could be represented by probiotic-based cleaners. PCHS cleaning system (Probiotic Cleaning Hygiene System) provides for the use of cleaners with added probiotics sporigene forms belonging to the *Bacillus* genus), which can reduce of 80-90% the contamination of hospital surfaces [36,34]. These bacteria can compete with any pathogens and, thanks to a process of biostabilisation, they remain effective over time. The Bacillus spp strains are sensitive to antibiotics and do not develop new drug resistances beyond the ones they naturally have, against penicillins and macrolides [32,33,36], or do not acquire genes from other organisms and they are not associated with infections in hospitalized patients in according to several studies conducted in different structures where they these products were employed [33,36]. The objective is to stabilize the hygienic quality of an environment over time reducing phoenomena of recolonization. As noted in the graphical elaborations, the cleaning system greatly affects the microbial load detected, with reductions between traditional and probiotic system in a range between 70-90%. The aim of to define an objective threshold limit of the surfacial contamination level was pursued.

In view of the analyzed and elaborations made as microbiological quality limit it is has been proposed the median value of 10,000 CFU/m^2 (table 1).

Microorganisms	Microbial quality index (sum of median values of pathogens)
Staphylococcus spp.	
Pseudomonas spp.	
Enterobacteriaceae	
Candida spp.	< 10.000 CFU/m^2
Clostridium difficile	
Acinetobacter spp.	
Aspergillus spp.	

Table 1. Microbial quality index scale of acceptability of MQI

The limit was defined considering the values obtained during phase 2, the period in which the probiotic-based sanitization protocol was applied, as the results showed a greater reduction in therms of contamination level. In all the structures the monitored median values were less than 10,000 CFU/m^2, the only exception is hospital 5, in which however the initial situation was more critical than other structures. The result is however significant as the pathogenic load has decreased from 60,632 CFU/m^2 to 12,632 CFU/m^2 (Chapter 3, Table 1). This script is just a brief overview of a very complex involving different research professionalism, structures, means and methods employed, with satisfactory results and that still requires additional processing of results and considerations.

The study has placed the emphasis above all on the correlation between microbial growth, infections and sanification procedures, and has involved a considerable commitment of means and people. But it would have been interesting to monitor in continuum the microclimatic parameters such as temperature and relative humidity, whose values are essential to create the optimal conditions for growth of microorganisms. Given the complexity of the project this has not been achieved, even if there are references in guidelines [26,29], in fact during the day there may be fluctuations due to the opening and closing of windows, use of the rooms, type of patient in hospital, operation of ventilation systems.

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