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Graz, am 5.9.2019 *Gernot Ribitsch eh*

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Zusammenfassung

Nosokomiale Infektionen stellen nach wie vor ein essenzielles Risiko für hospitalisierte Patientinnen und Patienten dar, jedoch haben sich in den letzten zwei Jahrzehnten unsere Möglichkeiten Mikrobiome zu beschreiben stark verändert. Mit den neuen, kosten-effektiveren molekularen Methoden hat sich das Verständnis von Mikrobiomen (dem menschlichen sowohl als dem von Krankenhäusern) insofern gewandelt, als neue Studien sich nicht mehr auf spezielle Pathogene konzentrieren sondern versuchen die gesamte mikrobielle Vielfalt zu beschreiben, mit dem Ziel zu verstehen, welche Faktoren den größten Einfluss auf diese haben. Diese Arbeit soll eine Zusammenfassung des bisherigen Wissens über Mikrobiome darstellen sowie Möglichkeiten präsentieren diese zu beeinflussen.

Abstract

Hospital acquired infections are still a major thread for the patients' security but our understanding of microbes and the ability to study them has changed over the last decade. The more cost-effective access to molecular methods to undertake microbiome-studies has led to an increase of knowledge of both, the human and the built microbiomes. Rather than being focused on certain pathogens, current research aims to describe the whole microbial community of hospitals with the goal to understand the parameters and factors, influencing it. This work aims to summarize the current knowledge of the hospital microbiome its implications for health and disease of patients as well as possible methods to capitalize of this knowledge and shape microbial communities in a beneficial way.

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Abbreviations

CDC: Center for Disease Control and Prevention

CoNS: Coagulase negative staphylococci

CRT: Cyclic reversible termination

EPS: Exopolymeric substances

HAI: Hospital acquired infection

HEPA: High efficiency particulate air

ITS: Internal transcribed spacers

MRSA: Methicillin resistant *Staphylococcus aureus*

NGS: Next generation sequencing

NICU: New-born intensive care unit

OR: Operation room

OUT: Operational taxonomic unit

PCR: Polymerase chain reaction

PMA: Propidium monoazide

SBL: Sequencing by ligation

SBS: Sequencing by synthesis

SNA*:* Single nucleotide addition

VRE: Vancomycin resistant enterococci

WGS: Whole genome sequencing

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1 Introduction

Hospital acquired infections, often involving antibiotic-resistant microbes, are one of the most serious health problems in the present day. They are the underlying cause of over 90,000 deaths per year in europe and mostly affect those that are already in weak health conditions, such as occupants of intensive care units, where the mortality rates of HAIs range from 10-25 % [1]. This issue is aggravated by the rise of antibiotic resistances of various strains. For example, methicillin resistant *Staphylococcus aureus* USA300, *E. coli* ST131 and *Klebsiella* ST258, are rapidly disseminated worldwide. Resistances against antibiotic substances that are considered backup-antibiotics for severe cases are increasing as well. While in 2001 no carbapenem-resistant strains of *Enterobacteriaceae* was known, cases rose to by 1-5% until 2010. The results of this development are an estimated 2 million cases in the USA that have led to 23,000 deaths, as reported by the Center for Disease Control [2]. Increasing costs and mortality rates have encouraged the development of new, more effective cleaning procedures, disinfectants, no-touch methods and bioactive surfaces [3]. Through the development and increasing accessibility of next generation sequencing methods that allow metagenomic studies of bacterial communities of whole areas, a new picture of the microbial diversity in human beings and the built environment, including hospitals, has been obtained. The results show that with current methods it is not possible to keep a hospital, or even specific parts, germ-free, and that there is a close relationship between the human microbiome and the microbiota of the built environment, with effects for both sides. Within hours after the arrival of a patient, the microbial community of a room changes, being dominated by microbes originating from that patient. While the focus of hygiene-interventions and infectiology departments in the past had been on a small number of pathogens, presently there is a change of perspective that broadens the view, considering the composition and interplay of all microbes in a given area (the microbiome) [4, 5]. The human microbiome project has shown links of the human microbiome to health as well as disease in the case of dysbiosis. The role of the hospital microbiome in the process of maintaining health and preventing infection is not clearly understood yet, but researchers claim, considering it is not possible to keep hospitals germ free, that a change of strategy is needed with a focus to establish a healthy microbiome [3, 6]. This could be achieved by changing the strategy of attempting to kill all the existent microbes, to seeking to understand how the abundance of beneficial microbiota can be increased while decreasing the abundance of

pathogens. Nevertheless, neither a healthy hospital microbiome nor a healthy human microbiome have been defined yet [7].

The aim of this narrative literature review is to summarize the current knowledge of the hospital microbiome, seen in the light of infections and resistances; the influencing factors; the present measures that hospitals take to maintain hygiene and cleanliness, and lastly; to show what new procedures exist or are being discussed to influence the microbiome in a way that might promote health while minimizing the risk of infection.

2 Materials and Methods

The primary method used for this narrative review was literature research in textbooks, particularly the platforms PubMed and Google scholar along with reports and guidelines from the national and international health institutions; WHO, the European Union, and the Center for Disease Control. Material was considered from the years 1995 to March 2019.
The result of the first literature research on PubMed with the MeSH terms “Hospitals” and “Microbiota” was 40 items. 10 were read fully, after screening for relevance of the titles and abstracts. 7 remained for reference research and reverse-reference research, resulting in 41 items that were considered relevant for this work, and assisted in developing an overview of the topic. Additional literature researches have been conducted on PubMed and Google Scholar with combinations of the search terms “Microbiome;” “Hospital;” “Hospital Microbiome;” “Sequencing;” “Human Microbiome;” “Hospital Cleaning,” and “Resistome.” Overall, 240 abstracts have been read, leading to the screening of 189 articles. 135 items have been considered in the final version of this thesis, consisting of 3 textbooks, 3 online resources from websites, and 129 articles from scientific magazines.

3 Findings

3.1 What is a microbiome, and how can it be studied?

In this thesis, microbiome is referred to as the community of microbiota (archaea, bacteria, protists, viruses and fungi) inhabiting a defined area such as the human body or the built environment. In literature, the term "microbiome" often refers to the sum of microbial genetic information that is present in a defined area. These definitions include organisms from all kingdoms, though research so far has been focused primarily on bacteria than viral agents, Fungi or Archaea, for example. A likely cause may be found when studying the classical culture-dependent methods, that have been used in microbiology so far. While microbiology laboratories routinely culture clinically relevant bacteria, the ability to study viruses, fungi and archaea stays limited.

Throughout literature, a difference is proposed between culture dependent and culture independent methods to study microbiota. Cultural methods and microscopy retain a large influence in clinical infectiology due to the clinically relevant phenotypical information that can be obtained through them, and the relatively low costs. Nevertheless, the use of DNA-based methods has become increasingly important over the course of recent years and is the basis for growing knowledge on the microbiome. Decreasing costs and an increasing number of applications have made them a frequently used tool in clinical practice, even more so in research [8]. Understanding the capabilities, limitations and certain pitfalls of microbiological methods that are fundamental for microbiome studies seems to be crucial. As the variety of methods in microbiology clearly outweigh the limitations of this work, it will concentrate on cultures and molecular methods, focusing on new sequencing methods; these are not as well described in literature as the classical culture-dependent procedures.

3.1.1 Cultures

In general, cultures provide phenotypical information on viable bacteria, fungi and some viruses, such as susceptibility to antimicrobial agents, morphology and metabolic capabilities, which can lead to identification and form the basis for clinical decisions. While this might suffice to identify a likely pathogen isolated from a patient, there is a great number of bacteria which are so called fastidious bacteria, requiring a complex criteria for cultivation; an estimated 90-99% of all environmental microbes are not cultivatable with

standard methods at the moment [9]. As a result, culture-based methods are at risk of providing an incomplete picture of the microbiome of the sampled area [10, 5]. Before culture can be done, a suitable medium must be chosen. There are two different classes of medias: defined media, in which the precise amounts of organic or inorganic chemicals is known, in contrast to complex media made from digests of microbial, animal or plant products existing in fluid or solid form; the definite amount of these is unknown. Further culturing media is differentiated in selective (inhibiting the growth of certain bacteria) and differential medias (containing an indicator chemical, changing color after specific metabolic reactions). The result of the culturing process allows the study of the organisms' physiological characteristics (e.g. optimal pH or temperature). The phenotypic properties can be investigated further through antibiograms, observing the growth of a colony in the presence of certain antimicrobial agent, testing for metabolic characteristics, or the presence of certain enzymes (e.g. coagulase in *Staphylococcus* spp.) [11].

3.1.2 Sequencing based methods and metagenomics

The metagenome is the sum of all genes of the organisms in a given biotope, containing bacteria archaea, viruses, fungi as well as human cells. A focus has been on metagenomic studies after the introduction of so- called next generation sequencing, allowing broad spectrum sequencing of the whole metagenome, for example, sequencing of the genome of only one organism (whole genome sequencing), or sequencing of only one target gene locus [12]. As mentioned above culture-dependent methods allow to get a picture of the viable microorganisms and their phylogenetic attributes (such as their metabolism) in a given sample-area. Nevertheless, only a little percentage of all microbes can be cultured with standard techniques and even more sophisticated methods are not able to give researchers an idea of the total abundance of microbes. Most of current microbiome-research relies on sequencing technologies, so their basic principles and applications will be discussed here briefly, since the majority of the cited studies are based on them. The development of next generation sequencing (NGS) more than a decade ago has been essential for the increased use of these molecular methods in metagenomic studies as well as studies of whole genomes.

3.1.2.1 Sequencing methods

'Next generation sequencing' is not to be understood as one certain technique or sequencing method, but rather it refers to a variety of technologies that have been developed after the so-called Sanger method which has been the golden standard of sequencing methods over

years. The basic steps of most sequencing platforms are sample taking, library preparation, sequencing and analysis of the obtained data. Sample taking is the first and maybe most critical step with the goal to extract high quality DNA, that is representative of all cells in the sample area. Depending on the sample type, which may be for example a human being or an environmental surface, different protocols exist. Investigating host-associated microbial communities require a sample that contains as little amount of the host's DNA as possible, especially when the host genome is large, because it may superimpose the information of the target-community. Selective lysis and physical fragmentation e.g. through centrifugation or filtration are examples for method in use for this purpose [13]. The goal of Library preparation is to modify the nucleic acid target, DNA or RNA, into a form that is compatible with the used sequencing platform. The basic workflow for library construction for the most common platforms (for example the Illumina platform) starts with DNA extraction and fragmentation. RNA then is converted into cDNA through reverse transcription. The so obtained DNA fragments are ligated to sequencing adapters which contain specific sequences designed to interact with the NGS device. After ligation the genetic material will mostly be amplified, as library production for most platforms requires nanograms or even micrograms of sample-DNA. Another approach is the creation of single-molecule templates without PCR to avoid the creation of mutations or other biases, such as suppression of low abundance species which cannot be amplified. In the last step actual sequences are generated via different chemical procedures for each technology [13, 14].

Preparation of templates is followed by the sequencing process, for which many different protocols and platforms exist, those are referred to either as first- or next-generation.

The term first generation mostly refers to Sanger sequencing. The basic principle of Sanger sequencing is the termination of the DNA-polymerase nucleotide addition through the incorporation of dideoxy-nucleotides. The simplified classical approach involves adding deoxy-nucleotides, DNA polymerase and a single strand DNA template into four different chambers with either one of the 4 dideoxy- nucleotides (containing one of the bases guanine, cytosine, thymine or adenosine). The addition of a dideoxy-nucleotide and consecutive termination of DNA polymerization leaves DNA fragments of different length. The different length of the resulting fragments would classically be used to perform a Western Blot to identify the DNA-sequence. Evolutions of this method would use fluorescent or radioactive die, leading to automatization and increased speed of the process. An advanced application of Sanger sequencing is shotgun sequencing, where the DNA template is first broken into short reads which can be sequenced parallelly, thus increasing the speed of the process [15].

The advantages of NGS methods compared to Sanger sequencing are that they are highly parallel and less expensive (around $500 per Mb, compared to 10- 50 cents per Mb). Additionally, some newer methods allow real-time sequencing, meaning that the elongation does not have to be stopped in order to allow detection of the base sequence which leads to a faster process. The affordability of sequencing the whole genome of different organisms has enabled the study of the genes of individual organisms, as well as broad metagenomic studies, identifying all the microbes inhabiting a given area [16]. Most next generation Sequencing platforms either work through sequencing by synthesis (SBS) using a polymerase, or sequencing by ligation (SBL) using a ligase to add the marked (for example through fluorescence) nucleotides to the corresponding template- sequence. Sequencing by synthesis (SBS) is either done in an approach called cyclic reversible termination (CRT) or single nucleotide addition (SNA). The first step of CRT is the addition of a fluorescently modified nucleotide, followed by the termination of synthesis; the remaining unincorporated nucleotides are then washed away. At this point imaging is done to identify the incorporated nucleotide. The last step is cleavage of the fluorescent dye and the terminating group; a cycle is complete, and the next one begins. Some platforms rely on four-color cycles, where all four bases are added at the same time, while in others only one base is used per cycle. A new technology has been introduced in 2014 in the form of Oxford nanopore sequencing (NPS). NPS does not require the addition of labelled nucleotides to a single stranded DNA, instead, a DNA single strand is lead through a nanopore one base at a time, leading to a voltage-shift, allowing identification of the base- sequence [17].

3.1.2.2 Sequencing Platforms

Two frequently used NGS platforms are the Illumina and the Roche 454. In the Illumina- workflow, which is a SBS based technology, random DNA fragments are immobilized on a solid surface followed by amplification, which produces clusters of identical DNA fragments. The ability to sequence from both ends of a cluster leads to reads up to 300 base pairs through so called paired-end sequencing where the process of sequencing is run first in one direction and after that in the other, producing twice the number of reads and allows more accurate alignment. The low costs of this technology (~50 US dollars per Giga base pair) and the possibility to generate draft genomes even from difficult datasets has made this platform a popular choice in metagenomics. The 454/Roche system applies epicPCR (Emulsion, Paired Isolation and Concatenation PCR), for clonally amplification of DNA fragments, which are then attached to microscopic beads. Those beads

are individually and parallelly pyrosequenced after being detached into the wells of a picotitre plate. Pyrosequencing (an example of SNA) is widely used in taxonomic studies, where single nucleotides emit a light-signal on being incorporated, the order and intensity of light peaks are recorded and through this the underlying DNA sequence is identified. The 454/Roche produces reads of lengths between 600 and 800 base pairs (a length at which only minor loss in the numbers of reads that can be annotated is created) at a cost of about 20 USD per Giga base pair. Both of the platforms allow so-called multiplexing, which is to be understood as running several samples in one lane of a flow cell saving costs and time [13]. Read length is an important factor in every NGS process and is highly dependent on the application of the produced library. De- novo assembly (the generation of an unknown gene sequence) and whole genome sequencing require a higher amount of longer reads; a range from 2 × 150 to 2 × 300 bp (the "2 ×" indicates paired-end sequencing) is recommended for Illumina platforms while for targeted sequencing (a widely used strategy for microbe-identification) lengths of about 75 bp are sufficient [18].

3.1.2.3 Applications of Next generation sequencing

Three applications for NGS should be discussed here as they have a high relevance for microbiome studies: targeted sequencing, whole genome sequencing and metagenomics. Targeted sequencing is carried out through studying only a specific gene locus of interest, for example to identify microbes. Although targeted sequencing does not lead to a picture of the whole metagenome, it can identify microbes down to species level and assess the community-diversity based on a taxonomically-informative target gene that is present in all microbes of a certain domain but differs between species. In bacteria and archaea, the 16S rRNA gene is considered the gold standard for this purpose [19]. On the one hand, 16S rRNA consists of highly conserved gene loci used as primer binding sites, on the other, it contains hypervariable regions (V1 - V9) with species-specific signature sequences used for taxonomic identification. Following amplification of the target gene, sequencing of the RNA fragments is undertaken and the resulting sequences are, as in whole genome sequencing and metagenomic sequencing, digitally compared to existing databases e.g. the NCBI genome database [8]. 16S rRNA sequencing provides an adequate resolution in assessing the members forming a bacterial community and displays their relative (but not absolute) abundance. It can also be used to track the spread of a specific taxon through a hospital, and even lead to the identification of formerly unknown species. Although 16S rRNA is broadly used for taxonomic identification, sometimes down to the strain level, phylogenetic and

functional information can only be infested by comparison to the reference genome. Even strains of the same species can be different in hundreds of genes, altering their phenotypical functions, however [20].

The identification of fungi and viruses with molecular methods differs due to their genetic features; specifically, internal transcribed spacers (ITS) are used to identify fungi. ITS are regions within the ribosomal DNA that are removed after transcription, and therefore do not exist in the finished ribosomes. The function of these genes is not known in detail, but polymorphisms make taxonomic identification possible. An example for the clinical importance of this procedure is the phenotypical similarity of some Candida species, e.g. *C. albicans* and *C. dubliensis*, which are not distinguishable only through observing morphology; the difference is clinically relevant as they have differing resistance to antifungal agents [21, 22]. Whereas the 16S rRNA gene occurs highly conserved in bacteria, viruses lack corresponding ubiquitously present gene targets, and as such this approach is not appropriate for their identification [20]. While the 16S rRNA gene provides an efficient manner for taxonomic investigation, in the recent years sequencing improved, becoming faster and more cost efficient. Novel technologies like epicPCR (Emulsion, Paired Isolation and Concatenation PCR), link the 16S rRNA gene to functional genes of the same cell in an emulsion. This allows the conservation of phylogenetic markers and functional genes throughout the sequencing process resulting in the possibility to better understand which microbes are responsible for metabolic mechanisms in the sampled community [23]. Metagenomic studies aim to display the whole genetic information of entire communities of organisms. The advantage of metagenomic analysis lies in its ability to not only provide taxonomical information, but to also show which genes exist in the studied sample, thereby allowing to infest on physiological functions of the community [13]. Nevertheless, it is not possible to match genes, for instance those coding for antimicrobial resistance, exactly to the correct bacterial species; for this purpose, whole genome sequencing (WGS) can be undertaken. WGS aims to align the obtained gene-fragments to the genome of specific organisms. The detailed image obtained like this can also be used to find the appropriate methods to culture microbes that have formerly been unculturable [24].

3.1.2.4 A new definition of species

Next generation Sequencing has changed the ability to identify microbes but it has also generated the necessity of a new (genomic) definition for species. Unlike culture-based approaches, where species have been defined based on phylogenetic properties, sequencing

methods do not allow the observation of colony formation or Gram staining. As result, the term species has been defined differently. Throughout literature, the identification threshold for organisms to be categorized as members of a species is if 97% of their gene sequences are identical, then they are also referred to as OTU (operational taxonomic unit) [22].

3.1.3 Pitfalls of sequencing-based methods

Challenges exist that limit the abilities of next generation sequencing methods to identify the microbes of a sampled community. These challenges can be found on both a microbial and technological level, or as a result of study design. While they generate a more wholesome microbial picture of the sampled area than cultures, one technological problem of culture independent methods is that without selection towards culturable microbes, the samples also contain genetic material of dead cells, not relevant for infection. The NGS platforms themselves cannot distinguish DNA from dead cells from that of living ones, thus the picture they draw can include a high number of irrelevant reads. For example, in cleanrooms viable cells account for only around 10 % of the assembled genetic material [25]. There are two common solutions for this issue; on the one hand, masking of dead cells' DNA with propidium monoazide (PMA), a photoactive DNA- binding dye; alternatively, products of viable organisms can be detected through proteomic and metabolomic approaches. This means to infest from metabolism products, and the produced proteins on which of the detected bacteria are vivid [20]. The principle of PMA-treatment is based on the notion that dead cells lose their membrane integrity, leaving their DNA "naked," enabling the covalent binding of leaving those molecules non-amplifiable [25]. In addition, removal of DNA from dead eukaryotic cells results in a higher likelihood for low abundance viruses to be detected in metagenomic analysis after the procedure. This is important, as although viruses are clinically relevant, relatively little is known about the human or built environment virome, which is not only formed by viruses but also includes a vast amount of bacteriophages, using bacterial cells of the human microbiome as their hosts [26]. There are many differing amplification methods that have their own limitations; for instance, PCR is primer-dependent, and so far even the universal ones cover only approximately 85% of the known taxa, thus leading to an incomplete picture of the metagenome. One must keep in mind that through sequencing it is possible to only infest the relative abundance of different taxa not their absolute numbers [27]. Disadvantages of sequence-based methods can be found in each work-step beginning with DNA extraction, where different techniques lead differing access to parts of metagenomes. Challenges that the microbes provide are owed to their ability to

rapidly acquire new mutation. This limits the power of WGS to recreate a taxon's spread in outbreaks. For this purpose a solution at strain level is needed, and some authors note that even results with sufficient resolution need to be seen critically, as strains can mutate within one patient, therefore providing a false picture [28]. Tagini et al. observed that sequencing-based identification varies between species; metagenomic sequencing for the detection of shiga toxin positive *E. coli* in theirs study only reached a sensitivity of 67 % compared to conventional culture methods, for example [29].

A lack of global study protocols, different methods, platforms, and sample sizes make direct comparison of results difficult, with some researchers suggesting that the technical differences in microbiome studies of the built environment may overshadow biological variability [30]. Lastly, the enormity of data produced by NGS is to be mentioned; sequencing data generated worldwide exceeded 15 petabytes in 2013[17].

3.2 The built environment in general and the influence on human health

Next generation sequencing methods do not only reshape the perception of the human microbiome, but also of the built-environment microbiome. This chapter will provide a summary of the general composition of this microbiological habitat, environmental as well as human influence and implications for health and disease; following this, the hospital microbiome will be discussed specifically.

Indoor environments are not a homogenous ecosystem. Buildings and rooms within them show different characteristics and are used for separate purposes. Restrooms and offices for instance are examples for the high contrasts found in the built environment. They show not only a different mode of use, but also have a relative difference in the amount of dry and wet surfaces as well as ventilation modes. In hospitals, differences in environment can be extreme; intensive care units' and general wards, are required to meet stricter criteria of hygiene than office buildings or homes. This criteria often require confinement and protective clothing only worn within the given area [27, 31].
The composition of the indoor microbiome is dependent on four fundamental factors: architecture and building design, the mode of ventilation, geographical factors, and human occupancy [32]. The influence of architecture and building design is best described as the functional intention of rooms and how they are used. Research of Kembel et al. show the existence of signature-taxa in nearly every indoor space of a studied building, most abundantly Proteobacteria and Firmicutes [33]. An example of a microenvironment, restrooms contain relatively more microbes associated with the human gut microbiome, while offices and classrooms have a relatively high abundance of human skin microbes and, depending on the mode of ventilation, variable microorganisms that originate from the exterior environment [27, 30]. Similar to the human microbiome, studies on viruses of the built environment microbiome are rare [34].

The functional environment is not the only influencing factor on the microbial community, but also their exposure to light; dust in dark rooms contains more microbes than in rooms that are exposed to sunlight through windows [35]. In addition, geographic factors such as the landscape, meteorology, and climate effect the microbiome by influencing how microbes are aerosolized, transported, and dispersed. Fungal occupation for instance is highly

dependent on geography, and typical microorganisms naturally living in humid environments (e.g.: members of the phylum Cyanobacteria and of the genus *Spirochaeta* and *Proteobacteria*) inhabit buildings located near water, while they are absent in buildings situated in more arid locations [36, 32]. In addition, outside flora can be considered another geographic factor; as chloroplast sequences of trees in the close environment of buildings can be found inside buildings, pollen are to be considered a possible vector for plant associated microbiota [5].

As outdoor air is one of the major influences for the composition of indoor air, geography and ventilation modes are connected factors in window-ventilated buildings. With the help of mechanical ventilation systems, environmental factors like humidity, temperature, and the type of air flow can be controlled, while the entry of microbes can be avoided through the application of high-efficiency particulate air (HEPA) filters. Mechanical ventilation leads to a less diverse microbial community, where human- associated microbes account for a larger percentage [33].

After outdoor air and unknown sources, human skin is acknowledged as the third most common source of microbes found in indoor air samples. A single human being sheds approximately 10^7 bacteria per hour originating from skin, the oral cavity, and the gastrointestinal and urinary tract, thereby shaping the indoor microbiome [27]. Samples of dust in homes show that it contains up to 112,000 phylotypes of bacteria, some, such as *Staphylococcus* and *Corynebacterium*, can be found in the human microbiome, suggesting this as their origin. On the one hand, humans are considered a source of microbes found in dust, while on the other they resuspend dust, and thus contribute to particle emissions [30]. Surfaces, influenced not only by random shedding, but by physical contact such as work surfaces, for instance, are further examples of the human impact on the built environment's microbiome. These sites predominantly carry microbiota associated with the human skin [32]. Given the interactions between human occupants and their home-microbiome, it is possible to consequently predict the family living in a house by comparing their skin microbiomes to the microbial community of their homes. Other humans inhabiting the same home have similar microbial communities, regardless if they are members of the same family or not [37]. Besides the aforementioned factors, literature discusses pets and plants, evidencing that they increase the diversity of the indoor microbiome, thereby dispersing characteristic microbes [38].

Knowledge of the indoor microbiome shows that the microbial composition of our homes have an impact on certain, especially atopic, diseases. The hygiene-hypothesis for the

development of asthma exists as an example of the interaction between the human immune system and the environment. Specifically, childhood-asthma prevalence could be shown to be lesser in children that are exposed to a more diverse microbiome (e. g. when growing up on farms); living with a dog in early childhood, for example, shows a negative relation to asthma prevalence. Despite these findings, no healthy indoor microbiome has been defined yet [39, 40].

3.3 Hospital acquired infections

Hospital acquired infection is defined as, “an infection occurring in a patient during the process of care in a hospital or other health-care facility which was not present or incubating at the time of admission. This includes infections acquired in the hospital, but appearing after discharge, and also occupational infections among staff of the facility” [41]. HAIs are a major problem of health care, becoming more serious with the increasing rate of antibiotic resistance. With the information of metagenomic studies, a shift of interest occurred in the discourse concerning nosocomial infections and hygiene, away from only investigating bacterial species or other isolated causative agents towards assessing how the known pathogens and their ability to cause infections is modulated by the composition of the hospital microbiome [22]. This chapter will first discuss the epidemiology and consequences of nosocomial infections, and then provide a brief overview of the most common causative agents and some of their characteristics.

Each year, an estimated 4 million hospitalized patients in Europe and an estimated 1.7 million in the USA develop a HAI. The most common are pneumonia, surgical site, urinary tract, gastrointestinal and bloodstream infections. The risk of acquiring an HAI increases with hospital size and differs with departments within the hospital environment; ICUs, especially NICUs, and surgical departments show the highest prevalence of nosocomial infections [42, 43]. Table 1 lists the key risk factors for HAIs that Vincent et al. defined in their work: they are related to the underlying health impairment, to the acute disease process, or to invasive procedures and those related to other treatment [44]. There is evidence that environmental contamination with pathogens also increases the risk of developing an HAI [3].

The consequences of nosocomial infections include increased mortality, extended hospital stay, and a rise in health care expenses. In the United States HAIs are estimated to cost the economy between $28bn and $45bn a year [45]. There is a geographical difference of ICU-mortality in patients with HAIs across Europe. In particular, rates are higher in the southern countries than in the northern ones, Switzerland and Scandinavia report rates of below 10%, while in Greece it is more than 25%. This may have many causes, but one could be that ICUs in southern Europe are generally smaller and the conditions of patients are more severe; as such, they are more likely to die during their stay in an ICU [44].

In low income countries, of which data is available, the percentage of HAIs reported is higher than in developed nations. SSIs appear to be a particular burden in these regions, with more than 10% of the patients developing an infection after undergoing surgery; the rate of infection reaches up to 30% in certain hospitals [46].

When comparing the studies of nosocomial infections it is important to note that the rate of infections is dependent on study quality, and the rate is typically higher in studies that are considered to have a higher quality- level [46].

Table 1 Risk factors for HAIs [47, 44].

Key Risk factors for HAIs	
Related to the general health status	**Related to Invasive Procedures**
Advanced age;	Endotracheal or nasal intubation;
Malnutrition;	Central venous catheterization;
Alcoholism;	Extracorporeal renal support;
Heavy smoking;	Surgical drains;
Chronic lung disease;	Nasogastric tube;
Diabetes.	Tracheostomy;
	Urinary catheter.
Related to acute processes and injury	**Related to treatment**
Surgery;	Blood transfusions;
Trauma;	Recent antimicrobial therapy;
Burns.	Immunosuppressive treatment
	e.g. corticosteroids;
	Stress-ulcer prophylaxis;
	Recumbent position;
	Parenteral nutrition.

3.3.1.1 Pathogens of HAIs

Staphylococcus aureus (30%), *Pseudomonas aeruginosa* (29%), coagulase negative staphylococci (19%), yeasts (17%), *Escherichia coli* (13%), enterococci (12%), *Acinetobacter* spp. (9%), and *Klebsiella* spp. (8%) were found to be the most commonly isolated ICU-pathogens in the frequently cited EPIC study conducted in Europe in 1995 [47]. A more recent paper studying acute care hospital in the USA, showed a broader spectrum of pathogens, and identified C. difficile as the most common cause of infection. Table 2 shows the relative distribution of pathogens found in patients with HAI in US hospitals [42].

Table 2 Pathogens of HAIs and example habitats [42, 47].

Relative distribution of HAI Pathogens in the USA, common infections and habitat.			
Pathogen	**Percentage (all HAIs)**	**Common Infections**	**Most common Habitat**
Clostridium difficile	12.1	Pseudomembranous colitis.	Spores in patient environment; human gastrointestinal tract.
Staphylococcus aureus	10,7	SSIs, Osteomyelitis; Skin infections.	Skin; nostrils; biofilms on invasive devices.
Enterobacteriaceae	19,2	UTI; Meningitis; Bronchitis.	Oropharynx; lower gastrointestinal tract.
Enterococcus spp.	8,7	BSI; UTI; Meningitis.	Gastrointestinal tract.
Pseudomonas aeruginosa	7,1	Pneumonia; SSI; Burn wound infections.	Humid areas; Assisted ventilation devices
Candida species	6,3	Pharyngitis; Colitis;	Commensals of multiple body sites.
Streptococcus spp.	5,0	SSIs, Pneumonia, UTIs	Oropharynx; gastrointestinal tract.
Coagulase negative staphylococcus	4,8	SSIs; BSI	Skin; biofilms on invasive devices.
Enterobacter spp.	3,2	UTI; Meningitis	Gastrointestinal tract.
Acinetobacter baumannii	1,6	BSI; SSI; UTI	Environment.
Proteus mirabilis	1,6	UTI;	Gastrointestinal tract
Stentrophomonas maltophilia	1,6	UTI; Pneumonia	Biofilms; hemodialysis fluids.
Yeast, unspecified	1,6	-	-
Viruses	0,6	Pneumonia; Hepatitis	All kinds of cells of the human organism.
Others	15,9	-	-

Pathogens that cause infections in the hospital environment can fall into one of two groups: either the patient acquires them from external sources in the hospital, so called exogenous infections, or they are opportunistic pathogens that are often part of the patient's microbiome; changed immune situations as well invasive procedures enable them to enter the bloodstream and cause diseases. As mentioned, whole genome sequencing might help distinguish one from the other. Generally, a microbe needs to have two properties to be the cause of nosocomial infections. First, they cause disease, second, they are able to survive in the hospital environment for a certain amount of time, sometimes up to weeks [11]. Although environmental contamination is assumed to be a risk factor for infection, literature shows that there is little evidence linking a certain strain in the patient's environment to the same patient's illness. Nor is there proof showing that environmental-dysbiosis was the underlying cause. There are some pathogens, however, for which environmental contamination is accepted as a risk factor, such as *Clostridium difficile* [48].

3.3.1.2 Gram- positive bacteria

Common gram-positive bacteria that frequently lead to HAIs are Staphylococci, Streptococci and *Clostridium difficile.* Clinically, *Staphylococcus* spp. are divided into coagulase negative and positive staphylococci. coagulase negative staphylococci (CoNS) are one of the most abundant microbes of the human skin microbiome; Staphylococcus Epidermidis is the most common clinically encountered species of the group and the most common cause of primary bacteraemia. Transmission often occurs through invasive devices, such as intravascular catheters or prosthetic joints where they can form biofilms, making them more resistant to antibiotic therapy. Common antimicrobial resistance and the high abundance of CoNS make them a potent pathogen [49, 50].

The clinically most important representative of coagulase positive staphylococci is *Staphylococcus aureus,* which inhabits the nostrils of 20-40 % of the normal population, as well as the rest of the human skin. In the hospital environment it can survive for days, and the hands of health care workers are twice as likely to be contaminated with MRSA from environmental sources than by having contact with infected patients. The conditions that MRSA causes range from benign skin infections to pneumonia, osteomyelitis, and sepsis. It takes a special place among the Staphylococci as being the most virulent, and a major cause of health care associated bloodstream infections. Due to its high number of mobile genetic elements, multidrug resistance is common among *S. aureus* strains [49, 51].

Enterococcus spp. belong to the genus *Staphylococcus* and are among the leading causes of hospital acquired infections in the United States. The two species that are responsible for many infections are *E. faecalis* and *E. faecium*. *E. gallinarum* is the only other species that is known to be a nosocomial pathogen. The natural habitat of this family is the human gut where they form a minor population, though they tend to dominate the gut microbiome of patients receiving broad spectrum antibiotics. *Enterococcus* spp. can cause blood stream infections alongside urinary tract infections, as well as meningitis, mainly in patients with a suppressed immune system. They are found in the hospital environment and spread usually via the hands of health care professionals [49].

Clostridium difficile is held accountable for around 25% of all cases of antibiotic-associated diarrhea. *C. difficile* in its viable for hours on dry surfaces, further its ability to form spores allows it to survive for more than five months on hospital floors, thus making it difficult to eradicate and therefore environmental contamination in hospitals is widely accepted to play a role in transmission. Physiologically present gut microbes normally protect individuals against colonization of *C. difficile*, except when disruption of the normal composition occurs, for example during antibiotic therapy. Especialy new-borns, who lack colonization-resistance, are susceptible to infection with *C. difficile*. The presentation of infected patients may range from asymptomatic carrier state over diarrhea to toxic megacolon [52].

3.3.1.3 Gram negative bacteria

Gram negative bacteria that will be named here are different *Enterobacter* spp. *Pseudomonas aeruginosa* and *Acinetobacter* spp.

P. aeruginosa is an opportunistic pathogen with a broad antibiotic resistance spectrum that normally inhabits humid areas, found in bathrooms and shower hoses. It is known to cause infections in patients with a weak immune system and is a feared infection in patients with burn injuries. Besides open wounds and burn injuries, infection occurs through invasive procedures such as urinary catheters and lumbar punctures [50].

Acinetobacter baumannii, *A. pittii,* and *A. nosocomialis* are typical health care associated pathogens, responsible for a high percentage of infections worldwide. Due to high resistance against environmental factors, they are commonly found in hospitals where they cause a variety of infections associated with mechanical ventilation; they can also be found as the cause of blood stream, surgical site, and urinary tract infections. Transmission occurs mostly after noncompliance with hand hygiene and failure to disinfect mobile hospital equipment.

Acinetobacter spp. are known for their multiple resistance mechanisms and association in many outbreaks [53, 52].

The Family of *Enterobacteriaceae* contains the nosocomial pathogens *E. coli*, *Proteus* spp. and *Klebsiella* spp. Their principle habitat is the lower gastrointestinal tract, but they also colonize the oropharynx of many hospitalized patients. *Enterobacteriaceae* are the most common gram-negative isolates in microbiology laboratories, causing a variety of infections [49]. As facultative pathogens, they lead to HAIs in patients with poor health conditions and a reduced immune competency. The percentage of resistances to beta-lactam antibiotics (including carbapenems), and fluoroquinolones is high among this family's species [54].

In addition to the named specific germs, causes of disease can be found in the composition of the whole microbial community. Dysbiosis of the gut, after antibiotic treatment for example, can lead to *Clostridium difficile* infections. A wider range of conditions is linked to the gut microbiome, for instance inflammatory bowel disease and obesity [55].

3.3.1.4 Viruses and fungi

Bacteria account for a high percentage of HAIs, though viruses and fungi are also common pathogens in infections of hospitalized patients. Data on viruses causing HAIs is contradictory; while some studies found viruses to be the cause in a small percentage of nosocomial infections (0.6%), as mentioned above, others assess that it is 5% or more [56]. Even when displaying a small percentage of HAIs, viral infections can lead to severe nosocomial problems for healthcare workers due to their ease of spread and long term survival rates on dry inanimate surfaces ranging from days to months [56]. The group of viruses that have been found to be the cause of HAIs is large, and environmental contamination does not play a role in all of them; for example, HIV or Hepatitis B and C which are transferred through contact with the infected persons blood, and although preventive measures are taken, cases of infection still occur through unsafe syringe (for example use in multiple patients or recapping) and blood transfusions [57]. Viruses are the cause of a variety of infections in hospitalized patients, particularly respiratory (SARS, RSV, Influenza), blood stream, and gastrointestinal infections (Norovirus, Rotavirus) [58].

Infection with Norovirus for example can lead to gastrointestinal symptoms and loss of fluid - life-threatening in patients with poor health conditions. Norovirus is typically spread by contaminated food or water, person to person contact or aerosols, but has been observed to survive for weeks on dry surfaces; environmental contamination has led to outbreaks in

the past, for example in a theatre, where people sitting in the same row where a person, infected with norovirus had vomited the previous day, were infected. Outbreaks in hospitals have led to the closure of entire wards in the past [59, 60].

Candida spp. are the most health relevant fungi for nosocomial infections. They are commonly isolated from the gastrointestinal tract and skin, as well as from the genital tract of women or people with urinary vesicular catheters; they also inhabit the hospital environment. *Candida* spp. are yeasts that live as commensals within the human body, though can also be a potent pathogen. Risk factors for invasive candidiasis are immunosuppressive diseases, hematopoietic stem cell or solid organ transplantation and others that are associated with a compromised immune system [61]. Mortality due to invasive candidemia is high, with rates between approximately 30-60 % [62]. This high rate might also be associated with the poor general health conditions of patients at risk for infection. Most of these infections are endogenous, but patient to patient transmission can occur [49].

3.4 The hospital microbiome

All the aforementioned factors that influence the microbiome of built environments in general have an effect in hospitals, too. But when discussing health care facilities, more factors need to be considered, given the existence of more and less confined areas, with differing cleaning procedures with the aim to keep patients and hospital staff safe from infections. Studies indicate that these confined areas and patient rooms after terminal cleaning are not free from common pathogens, leading to nosocomial infections in around 4 million patients in Europe's hospitals yearly [63, 43].

In the past, the focus of infectiological studies was set on single pathogens causing infections in patients, as it is still routine in clinical practice. High throughput sequencing in combination with cultures and other classical methods makes it possible to obtain a more detailed picture of the hospital microbiome, and the hospital environment as a contributing factor to HAIs and its influence on the human microbiome. This influence might be especially important in new-born intensive care units (NICUs), where patients have not developed a stable microbiome, and therefore are highly susceptible to colonization by environmental microbiota. Tests that were completed after cleaning procedures have been applied show that with current measures it is not possible to keep a hospital (or parts of it) germ-free, or even eliminate all of the most common pathogens from the hospital environment. Studies also show that human occupancy as well as other factors contribute to a variable microbial colonization, with a selective pressure towards human associated microbes that can survive routine cleaning procedures [64]. Knowing this leads to some questions: Which microbes are forming the hospital microbiome? In which way does environmental contamination contribute to nosocomial infections? Is it possible to beneficially influence the hospital microbiome rather than killing whatever microbes are susceptible to current cleaning agents (hypochlorite, UV- light etc.)? Would this lead to the establishment of a healthy microbiome-composition and a decrease in the number of HAIs? And lastly, what might be such as beneficial microbiome?
Prior to the discussion of what could contribute to a beneficial hospital microbiome, this chapter will summarize current knowledge of the general composition of microbiomes of different hospital sites, the factors that have an influence on it, and the means of microbes to travel across this environment. Vectors that need to be considered are equipment; endoscopes and surgery equipment come into contact with the mucosa and body cavities;

stethoscopes have direct contact with the patient's skin, and as healthcare workers hands can contribute to the transmission of pathogens, things that are touched by them, such as keyboards and faucet handles, could also play a role in microbial spread and transmission.

3.4.1 Composition of the hospital microbiome

Differing sections of hospitals have unique microbiomes that underlie alterations over the course of time. They show seasonal changes as well as the influence of other factors, like humidity and temperature, airflow, and human occupancy. The areas of a hospital range from patient rooms, to waiting halls, where depending on the hospital size, over a hundred people may be present over the course of one day, along with confined areas [65].

While inter-hospital varieties in microbial abundance are significant, each area (and even surfaces within them), characterized by differing functions, cleaning methods and architectural design, are likely to harbor a unique community of microbes. Due to these variations, it seems more important to discuss general trends and influences rather than providing detailed information on each area that has been sampled in the past [66].

Studies that have been conducted on the hospital microbiome typically lead to the conclusion that microbes commonly associated with humans, and which are most likely originating from the human microbiome, account for the highest relative abundance. Overall, eight phyla are dominant in hospital environments, with individual relative abundance: Proteobacteria, Fusobacteria, Firmicutes, Deinococcus- Thermus, Cyanobacteria, Candidate division TM7, Bacteroidetes and Actinobacteria. In three individual studies, either Proteobacteria or Firmicutes were the most abundant phylum [66, 36, 65]. A comparison of four hospitals in Taiwan showed that even on a genus level, a small number of genera account for more than half of the bacterial community. Nine genera could be found in all four hospitals, making up for 58.03% of all the present microbes; *Acinetobacter, Chroococcidiopsis, Corynebacterium Dysgonomonas, Enterobacter, Massilia, Propionibacterium, Pseudomonas* and *Staphylococcus.* The results show that overall diversity is limited, and only a small amount of mostly human-related bacteria account for the highest percentage of present microbes. Nevertheless, relative abundance varies significantly between hospitals; the percentage of *Dysgonomonas*, for example, ranged between 1,15% and 53.13%. While *Dysgonomonas* was the dominant genus in three central condition-ventilated hospitals, in the fourth hospital assessed in the study, *Staphylococcus* (19%) was the dominant genus; the hospital was naturally ventilated and was not situated in an urban, but rather a rural area [66]. Even within

a room, surfaces show variations in their microbial structure depending on their use, and how frequently they are used. Studies conducted in other hospitals report that *Streptococci* shows a relative overrepresentation on high touch surfaces in ICUs [65, 37, 36]. Characterization of microbes on a species level, with culture-dependent methods, confirms the presence of common nosocomial pathogens, for instance: S. *aureus, K. Pneumoniae, P. aeruginosa, A. baumannii* and *S. epidermidis* on environmental surfaces [67]. The predominance in relative abundance of these human associated microbes (and pathogens) is shown to persist even after routine cleaning procedures, indicating that despite the implementation of infection control measures, pathogens persist that could be transferred from environmental surfaces to patients and thereby cause infection [66].

The finding that the human-related microbes are dominant in the hospital environment is consistent through literature. While knowledge of the human microbiome has increased over the last decade thanks to cheaper sequencing methods and initiatives like the human microbiome project, many questions remain unanswered mostly due to its high variability and diversity. It is composed of 10-100 trillion microbial cells, differing significantly between body sites and is not stable over the course of time. The human core microbiome, referred to as OTUs that can be found in samples of all body areas of a human being, does only account for 5–10%. While it is possible to determine the origin of a sample, being for instance the skin or the oral cavity, microbial abundance in a specific area changes over time, and thus we can distinguish between a permanent and a transient human microbiome. Most microbes that belong to the resident microbiome have been defined as commensals, although this definition and its implication may not be completely suitable according to current knowledge [68]. Per definition, a commensal relationship is only beneficial for one organism (the commensal), while the host is neither harmed nor influenced in a beneficial way. Nevertheless, latest research shows that so-called commensals can cause harm as well as benefit. Although the underlying mechanisms are not fully understood, the microbiome contributes to the host's immune system and at the same time common commensals (e.g. *S. epidermidis*) can cause severe infections in their hosts [69].

As discussed, other microenvironments with differing conditions can be observed in the general wards of a hospital; bathrooms for example, that include wet areas like taps, showers and basins which can be a source of aerosols containing *Pseudomonas aeruginosa*, an opportunistic pathogen. While this is interesting when looking at the hospital microbiome

and diversity in different areas, the clinical implications and impact on patients health of these aerosolised microbes is not yet understood [70].

3.4.2 Factors that influence the hospital microbiome

The microbiomes of the confined and unconfined hospital areas (patient rooms, nursing stations) show some parallels. Microbes originating from patients and health care workers are over-present in both, still the microbial community in general wards is more diverse and the influence of the environment is greater in these areas. In some ICUs, the reported dominant organisms are reduced to 4 genera (*Staphylococcus* spp*., Streptococcus* spp*. Corynebacterium* spp*.* and *Acinetobacter* spp.). This relatively low diversity might be explained through stricter cleaning protocols and a higher percentage of mechanical ventilation allowing only filtered air to enter the inside environment [71].

Apart from the cleaning protocols and the importing of pathogens through infected patients, factors influencing the composition of microbial communities of hospitals are similar to those influencing the built-environment microbiome in general: the impact of human occupancy, temperature, humidity, ventilation, floor- levels, seasonal changes, and light exposure have been investigated. Results show that human occupancy is the largest influencing factor [71]. A demonstration of human impact can be found in the results of continuous investigation and characterization of the microbiome in a newly opened hospital over the course of one year, displaying the influence of humans on the microbial community's composition. Sampling began before the hospital had been opened. Prior to opening, *Acinetobacter* spp. and *Pseudomonas* spp. Dominated, and once the hospital was operational, floors and nursing-station surfaces started to be colonized by the human-skin associated genera *Corynebacterium*, *Staphylococcus*, and *Streptococcus*, with the relative *Acinetobacter* and *Pseudomonas* abundance decreasing significantly [37].

Two examples for the ambivalence of the relationship between human occupants and the hospital microbiome are: the colonization of skin and gut of patients in NICUs with microbes from their environment and the association between infections of prior room occupants and the acquisition of pathogens of patients inhabiting the same room later.

A recent review concludes that most evidence suggests a higher risk of infection with certain pathogens (influenza and Group A streptococci), while data from studies on VRE, MRSA and C. *difficile*, are thus far inconclusive, and no connection could be made for extended-spectrum beta-lactamase–producing organisms [72].

Not only human occupants but also the outdoor environment around a hospital are shown to influence the indoor microbiome through different climate and flora. An example of the influence of climate is a study conducted in a hospital in Spain. The named hospital is situated in proximity to the sea, and samples of the general hall showed that it is colonized by microorganisms typically living in humid environments that have not been found in other hospitals (e.g. *Cyanobacteria, Spirochaeta* or *Thiobacillus*) [36].
In an ICU in Graz, Austria, that has been partly ventilated by outdoor air bacteria that are related to plants, pine had been found on surfaces along with pollen from pine trees, forming a substantial part of the flora around this unit, thereby suggesting them as a source of those microbes [5]. Another environmental factor on hospital air is the presence of renovation or construction works, with the potential increase of suspended dust and associated microbes such as *Aspergillus* spp. [73].

3.4.3 The human microbiome

Tthe abundance of human related bacteria in the hospital environment is high, and most of them seem to have their origin in the human skin, due to shedding of cells and physical contact to environmental surfaces, while microbes that are normally found in environmental samples, from soil or plants for example, are less abundant due to the performed cleaning procedures. While knowledge of the human microbiome has increased over the last decade thanks to cheaper sequencing methods and initiatives like the human microbiome project, many questions remain unanswered, mostly due to its high variability and diversity. It is composed by 10-100 trillion microbial cells, differs significantly between body sites, and is not stable over the course of time [11].
The human microbiome shows two important characteristics. First, the different conditions that can be found throughout different body parts, lead to big differences of their microbial community. As consequence, OTUs that can be found in samples of all body areas of a human being, the so called “core microbiome”, only account for 5-10 per cent. Given these differences between body sites it is possible to determine the origin of a sample. Second, microbial abundance in a specific area is not stable over time, thus we should distinguish between a permanent and a transient human microbiome [74].
Most microbes that belong to the resident community have been defined as commensals, although this definition and its implication might not be completely suitable according to current knowledge. Per definition a commensal relationship is only beneficial for one organism (the commensal), while the host is neither harmed nor influenced in a beneficial

way. Nevertheless, latest research shows, that so-called commensals can be the cause of harm as well as benefit. Although the underlying mechanisms are not fully understood, the microbiome contributes to the host's immune system and at the same time common commensals (e.g. *S. epidermidis*) can cause severe infections in their hosts [69].

The skin displays the inhomogeneity of the human body as a biotope. Different humidity temperature and salt levels on different skin sites lead to the formation of micro environments, showing one of three properties: moist, humid and sebaceous. Figure 1 shows a topographic map of the different sites of the human skin microbiome [75]. Although different, there is a predictable pattern for each of them; the sebaceous sites are generally poorer in bacterial diversity, and the dry skin areas are generally the most diverse being more susceptible to temporal differences. Collectively, over 20 phyla can be found on human skin, but most microbes belong to a small number of them. Most phylotypes originate from one of only 4 groups: Actinobacteria (~51.8%), Firmicutes (~24.4%), Proteobacteria (~16.5%), and Bacteroidetes (~6.3%); others account for around 1%. On a genus level, *Propionibacteria, Corynebacterium* (both Actinobacteria) and *Staphylococcus* (Firmicutes) are most abundant with differing percentages, followed by *Anaerococcus*, *Streptococcus*, and others including fungi (most commonly *Malassezia*) and Archaea. The microbiome is considered to contribute to the skin's role in the immune system; *S. epidermidis* can inhibit skin inflammation as well as it inhibits skin pathogens like *S. aureus* and Group A streptococcus, for example, through phenol-soluble modulins. But *S. epidermidis* is also the most frequent cause of hospital acquired infections. Other examples of skin disorders that are influenced by the microbiome are seborrheic dermatitis, where *Malassezia* spp. are likely to play an important role, and acne vulgaris, caused by the common commensal *P. acnes* [75, 11].

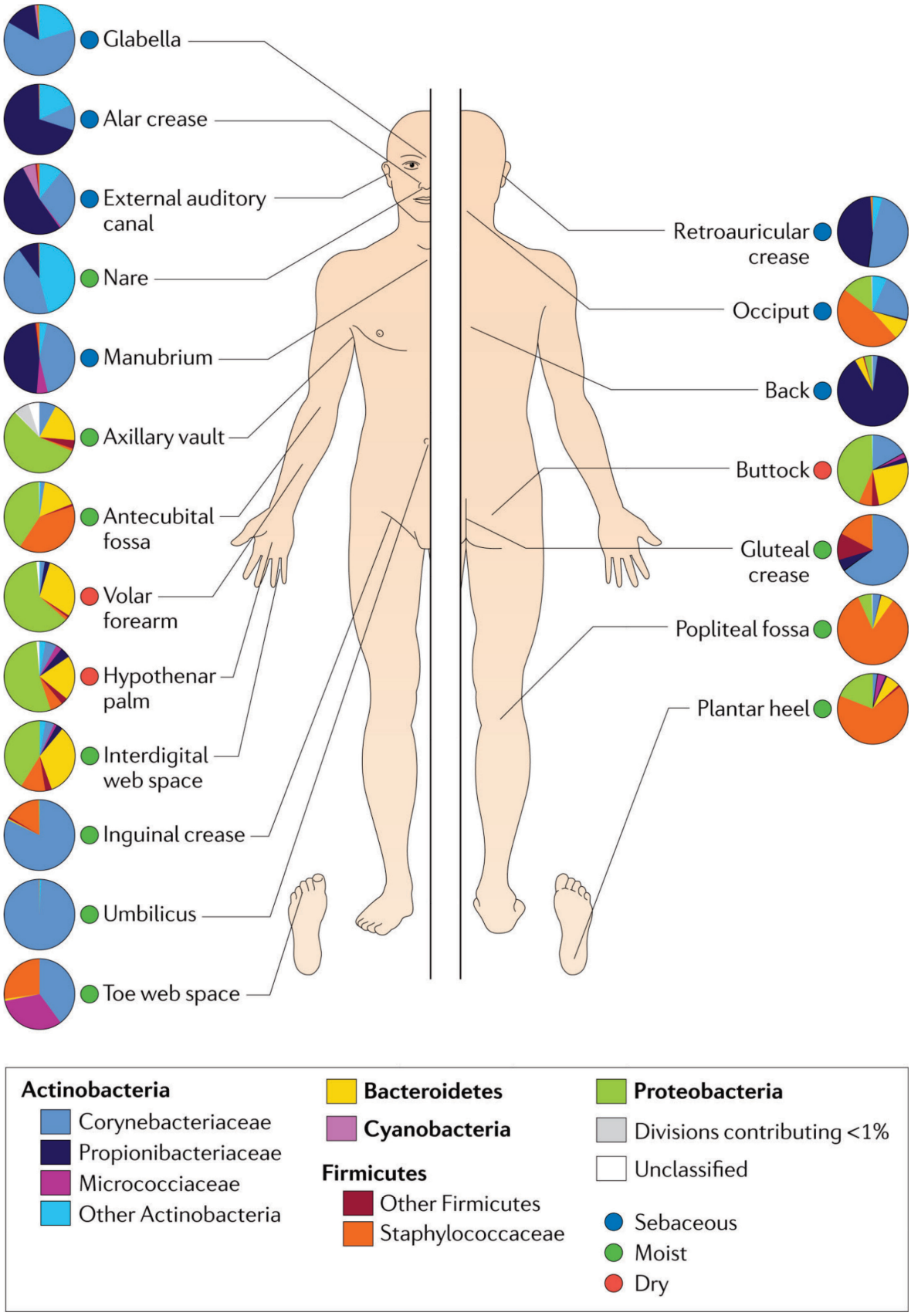

Figure 1 Topographical map of the relative distribution of microbiota on different human skin sites [75].

The microorganisms normally colonize the human gastrointestinal tract are found mostly in the close patient environment often on bedsheets and pillow covers, as well as on elevator buttons in the general halls where Bacteroidetes suggest fecal contamination [65, 76]. Bed making by healthcare workers has the potential to disperse these microbes and promote their spread throughout a patient's room [70]. While the gut harbors the highest number of microbes, it is dominated by only three phyla: Bacteroidetes, Firmicutes and Proteobacteria, containing 98% of all the phylotypes. The relative abundance of these phyla shows big differences, however, and can reach from 90% Bacteroidetes to 90% Firmicutes. Of course, the different properties of gut sections, for example the acid stomach, lead to different microbial communities. Bacteria of the gut microbiome produce vitamin K and B12 on the one hand, while on the other hand the immune system is shown to not develop properly in the absence of microbial stimulation. Mice models showed that *B. fragilis* plays an important part in this process. The oral cavity, like the skin microbiome, is formed of microenvironments; the bacterial diversity of the subgingival plaque is not the same as in saliva, where Firmicutes, Bacteroidetes, and Proteobacteria are dominant. Although the skin and gastrointestinal tract, are considered the dominant sources of human microbiota in the built environment the oral cavity and urogenital area should be mentioned here, too. The oral cavity itself is formed by multiple micro environments; saliva does not show the same pattern as the subgingival plaque. Overall, the oral microenvironments genera *Streptococcus*, *Pasteurellaceae*, *Prevotella*, *Vellonella*, *Fusobacterium, Neisseria, Actinomyces, and Porphyromonas* are the most abundant and inhabit the oral cavity in different compositions [11]. Although the relationship is not fully understood yet, the acquisition of caries is influenced by the oral microbial community. Firmicutes, Bacteroidetes Actinobacteria, Tenericutes, Proteobacteria, and Fusobacteria are the dominant phyla of the urogenital tract. The most abundant genera found here include *Lactobacillus* (dominating the vaginal microbiome of healthy females*), Prevotella, Gardnerella, Atopobium, Sneathia, Bifidobacterium, Megasphaera,* and *Anaerococcus* [11]. Although metagenomics can detect virus DNA and RNA, complementary to the built virome, information on the human virome is sparse, in part because they lack targeting genes such as the 16S rRNA gene. A lot is known about viral influence on diseases, but little seems to be known about the existence and the nature of interactions of the virome and their human hosts in physiological settings While a different mixture of animal viruses inhabit the human body, and viral DNA is incorporated in 5-8% of human DNA, the most abundant viruses are bacteriophages, which are estimated to outnumber bacteria 20 to 1. Mucosal bacteriophages

are shown to play a role in the human immune response, as they can kill individual pathogens before they can pass the mucosal barrier [11].

3.4.3.1 NICUs an example of the interplay between the environmental and the human microbiome

Patients in new-born intensive care units, among them low birthweight and very low birthweight infants, are very susceptible to all kinds of diseases because of their underdeveloped state, or other conditions, compromising their immune system. As such, nosocomial infections lead to an especially high mortality in these facilities. Low birthweight infants have underdeveloped skin, and resultantly their resistance towards pathogens is weak. Devices such as stethoscopes and ECG-electrodes that come into contact with infants may harbor pathogens. Since it is not possible to keep NICUs or other parts of the hospital germ-free, their occupants may benefit from a healthy hospital microbiome. NICUs are especially interesting because the human microbiome is acquired over time. For example, the gut microbiome reaches an adult state at after approximately 2.5 years [77]. Therefore, watching the process of this microbiome-acquisition in new-borns might lead to a better understanding of the interaction between the environment and the human microbiome.

Mothers are a major influence on infants' microbial colonization, this influence begins with delivery mode. While natural delivery leads to the presence of microbes of the mother's genital tract in infants, caesarean section changes the way infants are colonized. After delivery, not only does close skin-contact and breast feeding contribute to shaping the microbial community, but also the environment, which is shown to play its part. New-born intensive care units facilitate a microbiome that is, similar to other hospital areas, dominated by human-associated organisms, most of them having their origin in human skin [78].

Surfaces that are in contact with infants' skin are colonized with high relative abundances of *Streptococcus, Staphylococcus, Neisseria,* and *Enterobacteriaceae* [79].

Metagenomic analysis of NICU-rooms and 50 infants over three years showed that subspecies (defined with 99% genomic similarity) found on surfaces can also be detected in fecal samples of multiple infants. *E. faecalis, S. epidermidis, K. pneumoniae, Propionibacterium avidum, Escherichia coli,* and *P. aeruginosa,* the most common among them [77].

Brooks et al. point out that to evaluate which microorganisms in new-borns are obtained via the environment, observation on strain-level (99.999 % genetic identity), might lead to more reliable results. Following analysis that showed although environmental influence is not the dominating pathway for the formation of the infants' gut microbiome, and there are other contributing factors like diet and host genotype, infants share strains with their environment and with other children in the same NICU. This strain-sharing was not only detected in patients co-inhabiting a unit, but also those that were living in the same unit months apart from each other [80, 77].

Another finding supports the theory that the intensive care unit's environment influences new born microbiomes, that inhabitants of different units share different microbiota, and that the mode of delivery also has an impact on the later colonization of the human gut. The gut microbiome of infants that have been born vaginally resemble the vaginal tract, while babies that were delivered through C-section have a gut microbiome more similar to the environment [81].

The incubator is a key characteristic of the preterm-infant care areas and is a crucial tool for the care of these patients, having a beneficial effect on skin integrity and temperature regulation. Hartz et al. found that the colder areas of those devices can be a biotope for *Staphylococci* and gram-negative bacteria. In the same paper, they state that so far, little is known about what steps to take to keep infection risk in incubated infants at a minimum [81].

It is not known to what extent microbial colonization (and its environmental influence factors) influence infants' health. Differences can be observed comparing the microbiome of children with necrotizing enterocolitis (NEC) to healthy infants. A review comparing two studies on the impact of breast feeding on NEC, showed that infants that had developed NEC prior had a relatively high percentage of Proteobacteria and Actinobacteria in their gut microbiome, compared to a healthy control group. In the other study, the NEC-group showed a lower relative abundance of Firmicutes. Healthy infants were more likely to receive breast feeding than others. Although these studies are not addressing the influence of the environmental microbiome regarding the health of the studied groups, and the manner in which the microbiome influences the development of NEC is still unknown, they show that there may be a connection [81].

Interactions between the microbiomes of humans and the built environment have also been suggested. As described earlier, it is possible to match families to their home by comparing microbial communities.

3.4.3.2 Temperature and Air quality

Tests on the impact of temperature and relative humidity on survivability of infectious agents and HAIs show that it is difficult to make general propositions for these two qualities. Data on relative humidity and temperature's influence on the microbiome is considered inconclusive, as even members of the same kingdom react differently to conditions and while some might survive better in environments with high temperatures and relative humidity, others react contrarily [82].

Viruses with lipid envelopes (most respiratory viruses) tend to survive longer when relative humidity is lower, while viruses with non-lipid envelopes react in the opposite way, different bacterial species also react differently to these conditions [82]. A recent analysis of Mahnert et al. did also not support the theory of a big influence of temperature and humidity. They found higher correlation of the microbial community with longitude, latitude and sea-level than with the parameters temperature and humidity [83].

Mechanical ventilation itself is shown to decrease indoor-fungal elements, but the influence of temperature and relative humidity upon them remains unclear [82].

Knowledge, that air quality, plays a role in HAI- transmission, goes back as far as the 1850s, when Florence Nightingale, the English founder of modern nursing, could show that inmates of a military hospital in Scutari, Turkey, discovered that the opening the hospitals windows lead to better patients' health outcomes. Generally, indoor air contains a mixture of particles, including organisms from all kingdoms from outdoor and indoor sources. Besides the sources of air, other factors influence indoor air composition, including the amount of people in a room; shedding particles themselves, while also dispersing them from surfaces and floors through movements. Others are the use of different filters, airflow-types, and airflow-rates [73].

Ventilation modes vary between hospitals and departments. On the one hand, there are window ventilated rooms and mechanical ventilation with or without the access of outdoor air. On the other hand, there are different kinds of filters, air flow and pressure gradients.

Ventilation with outdoor air contributes to the hospital microbiome, by increasing microbial diversity compared to rooms only ventilated with indoor air. The rooms that were ventilated with indoor air showed increases, especially of the relative abundance of human associated airborne microbes and potential pathogens. Outdoor air enriches the microbial community with organisms commonly found in aquatic, soil habitats, or from trees in the close environment [22, 5, 66, 84].

One example for the composition of hospital air can be the taxonomic classification through shotgun metagenomics of ducts in different parts of a mechanically ventilated hospital, with high relative abundances of eight orders belonging to three phyla making up for 54-81% of the microbes. Two of them belonged to the Firmicutes: Bacillales, Lactobacillales, four were orders of Actinobacteria: Propionibacteriales, Micrococcales, Actinomycetales, Corynebacteriales, and two were Gammaproteobacteria of the orders Pseudomonadale and Xanthomonadales. The named Firmicutes and Actinobacteria are common commensals of the human microbiome, while the last two are likely to originate from ecological habitats. Pseudomonadales are common in soil samples, while Xanthomonadales are associated with plants [84].

While mechanical ventilation is used to control air flow directly, building design plays a role as well. Influencing factors are windows, in addition to room size, and the floor on which rooms are located. Sections that are located on higher levels have lower air pressure.

The different air flow types in hospitals contain turbulent flow, increasing the effectivity of air exchange, but also allowing broader microbial dispersion; laminar flow (also called unidirectional) where contaminants are carried away parallel flow-lines, and mixed flow, a combination of both. Laminar flow minimizes the spread of microbes and leads to lower air-particle counts. Mixed flow allows the selective application of laminar flow around critical areas such as operation zones in surgery theaters, while others are being ventilated with turbulent flow. When used with HEPA-filters, mechanical ventilation reaches an efficiency of 99.97% in removing airborne particles that are bigger than 0.3µm [85].

Furthermore Kembel et al. discovered that an effective way to decrease the relative abundance of airborne pathogens is to increase air flow (air changes per hour) and relative humidity [33].

But there is also a downside to mechanical ventilation. Installation is expansive, as well as maintenance. Badly maintained ventilation systems lead to less air changes, along with higher loads of bacterial-and fungal pathogens. Escombe et al. demonstrated that rooms with high ceilings and large windows allowed an average of 40 air changes per hour in a tuberculosis ward in a hospital in Lima. A poorly maintained mechanical ventilation system in the same institution failed to reach the intended 12 air changes per hour, thus increasing the risk of Tuberculosis-transmission. Nevertheless, they also point out that in window ventilated rooms, it is difficult to maintain negative pressure, which can be controlled through mechanical systems. Negative pressure is often applied to avoid spread of particles

from patient rooms into other areas of the hospital might occur, especially on completely still days [86]. This also highlights one of the advantages of mechanical ventilation: the controlling of room air pressure, widely used in operation rooms, alongside laminar airflow to minimize particle count around the operation tables [85].
Relative humidity can be controlled through mechanical ventilation devices. Isolation rooms are another common use of differing pressure-zones, where negative pressure can be sustained to avoid air passing into other parts of the building [87].

A range of study methods are used to describe the microbes of indoor air samples. Some of them are culture-based, and others use molecular methods, or sheer particle count, to study the influences on the microbial composition of hospital air. Yet, there is no broad consensus on transmission of certain pathogens via air and consequential measures.
For example, in the Netherlands, isolation rooms for patients with MRSA have an antechamber with negative pressure to avoid airflow towards general parts of the hospital; in the UK, no such measures are recommended on a national level. A second example is the recirculation of indoor air, permitted in the USA, while UK guidelines prohibit recirculation. Still, little is known about recirculation and bacterial infections, but recirculated fungal particles might lead to infection, especially in immunocompromised patients [70].
Further studies have investigated the influence of seasonal changes on hospital air quality, but results so far were unable to show a clear trend [73, 88].

3.4.4 Confined habitats

Confined habitats in hospitals include ICUs and operation rooms, where a high level of cleanliness is required, and the risk of infection is higher than in general wards. This is due to the nature of invasive procedures, or the poor health conditions that patients are in when they are transmitted to these areas. Furthermore, they are more likely to have artificial ventilation assistance, drainages or central lines.; all of them are risk factors for nosocomial infection. This is displayed in numbers, where the risk of contracting a nosocomial infection in the ICU is higher than in the general clinic wards [87]. In the OECD health Report for 2016, the percentage of patients that acquired an infection in the ICUs of Europe was assessed as 19.5 % compared to 5.2 % for other wards [43].
In addition to patient's health conditions, enhanced cleaning procedures lead to higher selection pressure, and the microbial abundance in these sections is less diverse than in unconfined indoor environments. As such, the microorganisms that survive under these

circumstances acquire resistance mechanisms, allowing them to cope with antibiotic treatment and adapt to low nutrition content, or dry surfaces [87]. Often the entrance of microorganisms from outdoors is limited, through air conditioning and clothing that is only worn inside these areas, so the relative abundance of human related microorganism is higher, while the microbial community is less diverse compared to unconfined areas. This was demonstrated, for example in a hospital in Spain, comparing the ICU to the general hall [5]. The general hall samples in this study examined 1,636 OTUs, while 744 were detected in the ICU. Samples were performed at a cluster-distance of 0.05, meaning, that an OTU is formed by DNA samples with 95% corresponding base pairs. So, the ICU harbored less taxa, which were relatively more abundant, most likely due to confinement. They also observed an overrepresentation of pathogens in the ICU compared to the general area one of them was *Streptococcus,* a versatile genus, that causes a broad range of diseases [36].

The human relatedness of microorganisms in the ICU seems to be clear throughout literature, although the relative abundance of microbes may differ throughout studies. In general, confinement leads to an overrepresentation of gram-negative bacteria, compared to unconfined habitats where Gram positive bacteria dominate the microbiome. Variations on super-kingdom level can already lead to differentiation between controlled and uncontrolled environments. The rate of bacteria compared to eukaryotes is significantly decreased from uncontrolled areas (99% bacteria, 1% eukaryotes) to ICUs (55% bacteria) [83].

Differences are more significant on phylum-level. The number of phyla detected in ICU ranges from 7 to 15; in different studies normally Proteobacteria, Actinobaceteria, Firmicutes and Bacterioidetes are overrepresented with a different relative composition, with either Proteobacteria or Actinobaceteria showing the highest abundance. Others that could be detected frequently were Acidobacteria and Nitrospira [5, 36, 87].

On a genus level, the composition of the microbial community also depends on the sampling sites. Air samples for instance show coagulase negative staphylococci accounting for 94.3% of all detected bacterial strains, followed by *Micrococcus* spp. and *Bacillus* spp.

16S rRNA pyrosequencing of samples from surfaces, devices and work spaces shows that skin-associated bacteria are highly abundant, e.g. *Propionibacterium* and *Pseudomonas* (as mentioned earlier a common nosocomial pathogen). The floor samples presents different findings, containing genera that typically originate form environmental sources such as soil or water, with *Acinetobacter* being the dominant genus [5].

Figure 2 shows an overview of bacterial phyla and genus found in different sites.

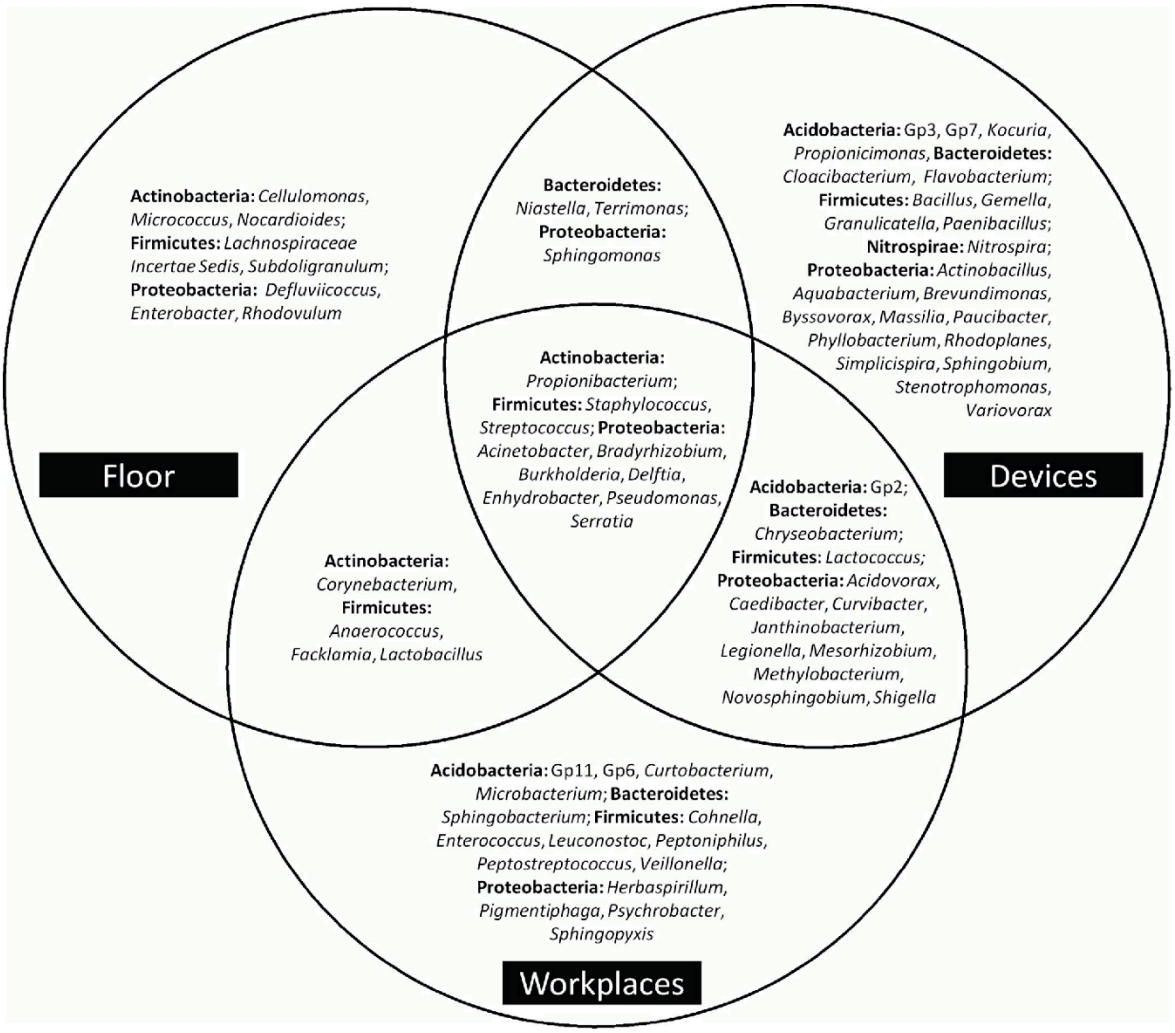

Figure 2 Microorganisms on different hospital-surfaces [5].

While most of these microbes do not normally cause infections e.g. *Micrococcaceae*, *Corynebacteriaceae* or *Sphingomonadaceae,* or may have a beneficial effect, other species that can cause infections were found. *Staphylococcus aureus*, various *Enterococcus* spp., *Escherichia coli*, *Pseudomonas* aeruginosa, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Enterobacter* spp. *Acinetobacter baumannii,* and other opportunistic pathogens have been identified, despite terminal cleaning (cleaning procedures that are done after a patient's discharge) [87, 36].

One study matching the findings in general wards could show that the relative presence of microbiota, related to humans, is significantly higher in intensive care units compared to unconfined indoor environments such as restrooms [65].

The microbiome formation of hospital surfaces is not only dependent on the type of surface, but as well on the distance to the inhabiting patient, meaning it is higher in areas close to the inhabitant, like bed tables than in areas that are situated at more distance. A relatively high

number of bacteria normally found in the human gut and skin are present on bedsheets and pillows, namely *Faecalibacterium prausnitzii, Massilia timonae, S. aureus,* coagulase negative staphylococci*, Pseudomonas species* and *Propionibacterium acne.* Multi-drug resistant organisms and MRSA could be found as well. On the listed textiles and surfaces, they form dry-surface biofilms, which persisted for as long as 12 months after cleaning with a hypochlorite solution. A biofilm is a structure where a community of organisms is encased by exopolymeric substances (EPS), accounting for more than 90% of their material. This finding is of concern, as microbes in biofilms are shown to have increased resistance towards environmental influences and disinfection agents. Especially when those agents are used in sub-lethal concentrations, they can cause microorganisms to undergo phenotypic adaption, promoting increased lateral gene transfer and mutation. Consequently, resistance of organisms in biofilms is up to 1000x higher compared to their planktonic form. These effects are stronger in multi-species biofilms [76, 89, 90].

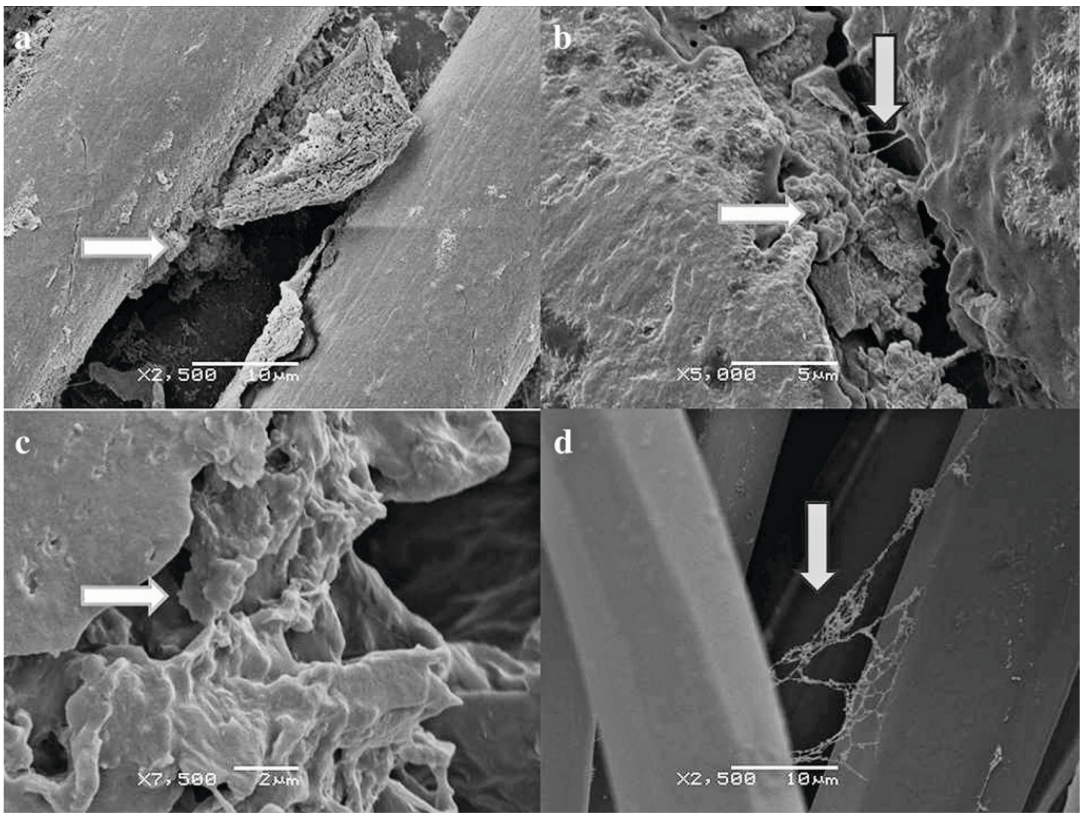

Figure 3 Electron microscopy of Biofilms on different surfaces. (a) blind cord (magnification x2500); (b) ward door (magnification x5000); (c) reagent box (magnification x7500); (d) curtain (magnification 2500). Vertical arrows: residual EPS after processing. Horizontal arrows: coccoid bacteria embedded in EPS [76].

Operation rooms are a further example of confined environments in hospitals, where surgical site infections (SSIs) are a feared outcome of procedures. SSIs are infections that affect either the incision or the deeper tissue at the procedure-site, occurring up to thirty days after the procedure, or up to one year when patients have received implants [85].

Multiple factors can lead to SSIs, but they can be divided into three primary categories: patient-related characteristics (e.g. age, obesity and comorbidities), characteristics of the surgical procedure itself (e.g. duration, type and personnel behavior), and third, the OR environment. As such, most hospitals have strict protocols to minimize the risk of infection ins patients that undergo surgical procedures. They are normally organized into areas with a decreasing microbial burden, from the reception towards the operation theatre, with an increasing positive air pressure gradient preventing the influx of unfiltered air. Airflow is often kept laminar or mixed, with laminar airflow around operating tables; while in theory this should decrease microbial load, there is a lack of meta studies confirming this [91].

The two most common descriptions of OR cleanliness has historically been cultures and air particle count; both approaches are in question to be appropriate tools to assess microbial communities. Findings of other studies show that particle count does not correlate with the number of colony-forming units. Further, they only identify a minority of bacteria, while the majority of causative agents (mostly anaerobia, dominated by Bacteroides) of SSIs stay undetected [91]. Nevertheless, there have only been few studies on ORs using molecular methods. Evidence of the few that exist show that the ORs show similar patterns as other hospital areas: human associated microbes are a major source of OR-surface microbiomes. The majority has been reported to belong to the phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Cyanobacteria. Common species are *Staphylococcus aureus* (leading to mostly endogenous infections from the patient's skin)*, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis,* and *Enterococcus faecalis* [87].

An example for the indirect influence of geopolitical situations onto the hospital microbiome is the relative high contamination rate of ORs found in a study in Palestine, showing that 45.31 % of the OR's surfaces and equipment are contaminated with *S. aureus.* The authors correlate this to struggles in the Gaza strip that only had ceased shortly before the study was conducted, leading to deficiencies in disinfectant quality and sterilization techniques, and a shortage of spare parts [92].

The implications for HAIs resulting from the microbial composition of hospital surfaces and air are still a matter of debate, and cleaning guidelines instructed by organizations such as the CDC (Centre for Disease Control and Prevention) classify surfaces as areas of low risk

for infections, but findings of recent studies oppose, which will be discussed further in the next chapters [93, 3]. Over 20% of HAIs are considered to be transmitted through healthcare worker's hands, which are in constant contact with the environment; nevertheless, evidence linking infections to environmental contamination is sparse [3]. Oberauner et al. compared the microbes that they found on different ICU surfaces, suggesting that the infections that were reported during the time of their investigation matched with their findings [5].

3.4.5 Potential Transmission pathways for microbes in the hospital

Equipment in use by healthcare workers also forms a distinct habitat in the hospital microbiome. Frequent use and contact with multiple patients a day could play a role in the transmission of microbes, especially onto healthcare worker's hands. Research shows that white coats, keyboards, faucets, mobile phones, writing pens, case notes, medical charts, and wrist watches as well as personnel attire could be a source of nosocomial infections, although their role in outbreaks is not completely clear. What is known, is that they are capable of harboring potential pathogens [94]. This chapter will discuss these item's microbial colonization and their potential role in the transmission of microbes throughout the hospital.

3.4.5.1 Diagnostic tools and devices

Medical charts are normally handled by a high number of health care workers before and after patient contact and are often stored in plastic covers. Cultures performed on samples of these covers show that they are contaminated to a high degree with potential pathogens, with differing rates of contamination across general wards (over 60%), ICUs (over 80%) and surgical wards [95, 94, 96]. The composition of the bacterial community on the charts differs between the studies, which may be the result of the different protocols of these studies.
Table 3 shows the cultured bacteria of studies undertaken in two different hospitals. Within the species range of the swabs taken from charts was MRSA (6.8-9.3%), one of the leading causes of nosocomial infections within ICUs, and a severe health care problem due to its antimicrobial resistance pattern.

Table 3 Bacterial contamination of patient files [94, 96].

Bacteria found on patient files	
Gram positive species	**Gram negative species**
S. epidermidis	*Sphingomonas paucimobilis*
Staphylococcus aureus	*Pseudomonas aeruginosa*
MRSA	*Escherichia coli*
Enterococcus faecalis	*Klebsiella pneumoniae*
Streptococcus viridans	*Pantoea* spp.
Corynebacterium spp.	*Acinetobacter baumannii*
Bacillus spp.	*Serratia marcascens*
Others	Others

While CoNS, *Bacillus* spp. and *Corynebacterium* spp. are members of the common skin microbiome and considered mostly avirulent, they can be pathogenic for certain individuals, such as those with suppressed immune systems. CoNS are often found to be multi-drug resistant. One study comparing the different sections of a hospital (general ward, surgical ward, and obstetric ICU) concluded that the lowest amount of contaminated charts was found in obstetric gynecologic general wards and obstetric ICU (50.0%), which may be explained by the fact that patients in these environments are generally younger and healthier, and a decreased use of endotracheal as well as gastric tubes, which are known to harbor a variety of bacteria [94]. The studies showed no direct causation between the medical chart's contamination and infections. It should be noted that the antibiotic resistance patterns of the bacteria on the files, matched those of patients with nosocomial infection in the same critical care unit, however [96].

Mobile phones are the most-used non-medical devices in the hospital, acting as tools for research on medication, calculations and many more purposes. Thus, they are being used in all parts of the hospital, including toilets, at home, and in private contexts. A review in 2015 found 39 studies on the contamination of mobile phones between 2005 and 2013, becoming a contemporary field of interest. No studies based on genetical identification methods could be found on PubMed or Google Scholar. The most common isolates to be found were *Staph. aureus* (22.81 %), CoNS (16.67 %), the most common gram-negative isolates were *Acinetobacter* spp., *P. aeruginosa*, and *K. pneumoniae*. *E. coli*, and MRSA were also isolated in some studies [97].

Further it could be shown that the microbes detected on the phones of health care workers can also be isolated from their hands. While most mobile phones are contaminated by non-pathogenic microbiota of the normal human skin microbes, mostly *S. aureus*, CoNS and

Micrococcus, literature shows that the prevalence of nosocomial infection agents among them ranges from 10-100% [97].

Despite harboring a lot of bacteria, mobile phones are rarely cleaned. Heyba et al. learned through a questionnaire, that only 33.5 % of the personal working in the studied hospital had ever disinfected their phone [98].

Normally patient files and mobile phones are used by hospital staff, therefore contaminating their hands, but do not come into direct contact with patients and their environment. In contrast, diagnostic devices as well as attire have direct patient-contact, and bed sheets are part of the areas closest to the patients. Stethoscopes and ultrasound probes are example diagnostic tools that are used many times per day, on multiple patients, and are shown to be inhabited by various bacterial species. Analysis of stethoscopes in 1997 showed that they are carriers of potential pathogens such as *Staphylococcus aureus*, *Acinetobacter* spp. and *Enterobacter agglomerans*, and 45% of health care workers clean their stethoscopes only yearly, or never [99].

Although the given study is from 1997, and the problem of HAIs might not have been considered in the same way it is today, which could have changed the habits of stethoscope disinfection, the results still indicate that stethoscopes are to be considered as a vector for bacterial transmission. Ultrasound devices are a further example of a diagnostical tool used on several patients a day; whereas probes come into direct contact with the patients and are cleaned after every procedure, the mechanism of the machine (keyboards, gel bottles, etc.) can be potential habitats for bacteria; they are shown to be contaminated by several types of pathogens including, most commonly, methicillin sensible *S. aureus*, MRSA, coagulase negative Staphylococci, *P. aeruginosa*, *Corynebacterium* spp., *Acinetobacter* spp. and *Bacillus* spp. During ultrasound diagnostics, healthcare staff touch the patients skin directly with their hands, providing a potential transmission pathway from the machine onto the patient and vice versa [95].

3.4.5.2 Hospital textiles

Whitecoats and nursing uniforms worn by healthcare staff are in contact with patient surroundings every day. Cultural identification methods show that most textiles contain bacteria that can be linked to common skin bacteria such as coagulase negative staphylococcus; common pathogens can be found as well, but the rate of contamination varies. For example, the range of contamination with *S. aureus* ranges from 6-32%, for MRSA from 0-79% (the highest percentage was measured during an MRS outbreak), and for gram-negative pathogens from 11-23% [100]. Factors that contribute to the risk of contamination are frequency of use (uniforms, that are changed daily are less likely to be inhabited by resistant pathogens than those that are changed every second day), and duration of use (contamination rates increase over the course of a day) and recent patient contact [53, 101]. The profession within the hospital is not shown to have an impact on the uniform contamination rate, it was found to be the same for example in doctors and nurses [101]. The extent of contamination also includes personal clothing worn in a hospital setting; doctors neckties have been shown to carry *S. aureus* (32 %) and *Bacillus* spp. [53].

Pyrosequencing showed that pillows and matrasses harbor bacteria from patient's skin and gut microbiome, as well as hospital environmental microorganisms, known for their pathogenicy and survivability [76]. Over all the evidence of infections through hospital textiles is sparse, but there are some case studies in which textiles are considered a possible source of infection, as they are shown to be contaminated with the same bacterial species as patients [102]. From 1972 to 2015, only 12 outbreaks have been linked to hospital textiles, most of them caused by *Bacillus cereus*. Often, they could be linked to problems within the cleaning cycle such as bad storage conditions, deficiency during the laundering process (inadequate temperature), or dust contamination of the clean textiles. In four cases, hospital laundry workers are reported to possibly have been infected by unclean hospital linen or bedsheets. Nevertheless, literature suggests that health care worker's attire and hospital textiles are not likely to cause nosocomial infection [103].

While there are no globally used guidelines for the laundry process of textiles, literature suggests that due to the low rates of infection, the applied methods are sufficient [103]. Still textiles, even after cleaning, are considered as a potential vector for pathogen transmission by some researchers, but there is little evidence linking them to nosocomial infections, and no study has demonstrated cross transmission of pathogens by apparel so far [53]. Data on this matter is inconclusive and contradictory; comparing studies is difficult as there is no standard protocol to study textiles, while the abundance of microorganisms varies between

different sampling sites and times [100]. Of course, the devices and textiles discussed here do not display all that are used in the hospital environment, but they can be an example of materials in contact with multiple persons and their close surroundings, over the course of one day.

3.4.5.3 Health care workers hands

As mentioned, a major source of nosocomial infections is a person's endogenous microbiome, where pathogens exist that would be harmless under normal circumstances, but in situations of impaired health and immune system or attributed to surgery, these opportunistic pathogens like CoNS can lead to major infections [75].

When considering the transmission of microbes throughout the hospital, and endogenous microbes from different body parts of the same person, healthcare personnel hands are estimated to contribute to 20-40% of hospital acquired infections. Contamination can occur through contact to patients' skin or body fluids, devices and hospital surface [52]. In fact, environmental microbial load is one of the best predictors of the contamination of hands with pathogens [104, 76]. The most isolated microbiota on hands of nurses and doctors are skin bacteria, but 10% are contaminated with pathogens that are capable of causing HAIs (e.g. *Enterococcus* spp*., S. aureus and Acinetobacter* spp.). One study revealed that secretary's hands showed the highest contamination rate among the ICU staff of one hospital; over 45% of them were contaminated, suggesting that hand hygiene interventions should not only address doctors and nurses [67].

In order to use hands as a transmission pathway, microorganisms need to survive on them for a certain amount of time. The survival time varies between species, and ranges from minutes in the case of *E. coli,* to more than an hour as shown in Table 4 [105].

Table 4 Survival time of hospital pathogens on hands [105].

Survival time of microorganisms on hands	
Microorganism	**Survival time**
Acinetobacter calcoaceticus	60 min.
E. coli	50 % survival after 6 min.
Enterococcus faecalis an faecium	60 min.
Shigella dysenteriae	Up to 60 min.
Pseudomonas aeruginosa	30 min.
Rotavirus	16 % after 20 min.; 1-8 % after 60 min.

3.5 The hospital resistome, mechanisms, sources and human influence

The aggravation of the issue of hospital acquired infections is owed in part to antibiotic resistance (often to multiple substances) to pathogens such as VRE, MRSA, and coagulase negative staphylococci. Modern molecular methods help localize resistant genes in the environment and show how they might spread to hospitals. Understanding the hospital resistome and the factors influencing it is important, as an estimated 25,000 deaths in Europe are attributed to resistant microbes [2].

Antibiotic resistance can be divided into two categories. The first category is inherent to a certain species due to its natural characteristics, for example the resistance of most gram-negative organisms against penicillin G. Their bacterial outer membrane prohibits the access of the substance to its target structure, the peptidoglycan layer. From this natural resistance an acquired resistance can be distinguished, when species that formerly have not been resistant towards an antimicrobial substance develop resistance, either vertically via mutation, or horizontally via conjugation, transformation and transduction. This can happen across species as well as kingdoms [11]. The foundation for horizontal gene transfer is that bacteria possess a number of mobile genetic elements, all together forming the mobilome. The most frequent pathway of horizontal gene transfer which is considered to be conjugation; this mechanism requires cell to cell contact, so that plasmids and integrative conjugative elements (transposons) can be transferred from a donor cell to a receiving organism, thus enabling a transfer of genetic elements that normally cannot be mobilized.

The genome of *Staphylococcus* spp. for example is formed to 25% of mobile genetic elements, making this pathogen very versatile towards external influence [106].

Table 5 Horizontal gene transfer of microorganisms [106].

Mechanisms of horizontal gene transfer	
Transduction	The process of introducing foreign DNA into a cell by a vector like a bacteriophage.
Transformation	Genetic alteration after direct uptake of exogenous genetic material form the environment.
Conjugation	Transmission of antibiotic resistance genes via plasmids or transposons.

The most common resistance strategies are illustrated in Figure 4. One of them is inactivation through enzyme degradation, such as that by tetracycline inactivating enzymes, or beta-lactamases. Protection, alteration or overexpression of the drug targets leads to infectivity in VRE, where peptidoglycans are modified, thereby decreasing the affinity of vancomycin. MRSA expresses variants of the normal penicillin-binding target proteins that are insensitive towards the drug. Decreasing the concentration of antimicrobial substances through efflux pumps can be effective against multiple chemicals. Others are specific to certain antibiotics like tetracycline efflux pumps. Cell-wall permeability can be reduced through expressing more selective porin variants; bacteria can use one of those mechanisms or have a combination of methods at hand to survive contact with antimicrobial substances [106].

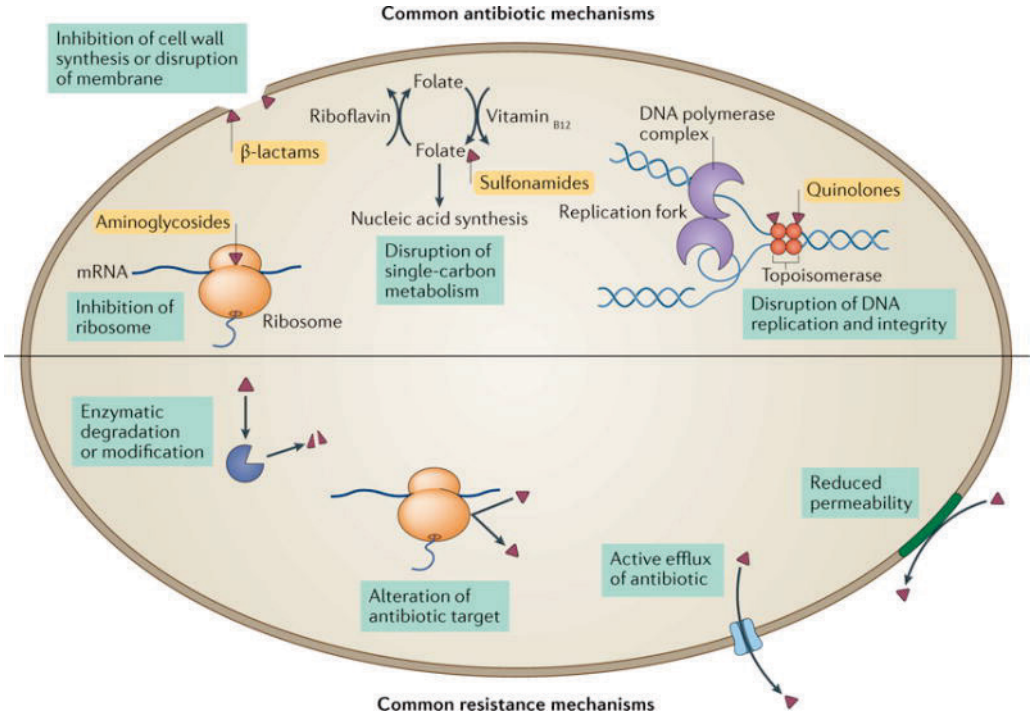

Figure 4 Common antibiotic mechanisms and common resistance mechanisms [106].

The resistome is formed by all the genes of a metagenome that directly or indirectly contribute to antimicrobiotic resistance. Antibiotic resistance genes of pathogens that lead to phenotypic resistance only comprise a small formation of this collection of genetic information, non-pathogenic commensals and environmental microorganisms also contribute, although the relationship between commensals and pathogens in the hospital setting is not fully understood. Additionally to the genes, that code for proteins contributing to the bacterial defenses against antibiotics, the resistome contains genes, that code for proteins leading to moderate resistance. These might evolve to become more potent; they are called silent resistance genes. Research has shown that these silent genes can lead to

resistance when their mode of expression changes, for instance due to selective pressure in hospital [107].

Proto-resistance is associated with other functions than resistance in their original context (some trigger genetic effects including modulation of gene transcription, apart from resistance), but have the potential to become potent resistant genes due to mutation. Structural studies have demonstrated a close relationship between ancient nucleotide polymerases and aminoglycoside nucleotide transferases (causing resistance against aminoglycosides), this suggests that these nucleotide polymerases are proto-resistant genes that evolved into these drug-modifying genes. Even genes that make an organism insusceptible towards one substance-class can be a proto-resistance gene for the resistance towards another. An example is aminoglycoside acetyltransferase transferase; it typically leads to resistance against aminoglycoside through mutation the enzyme's spectrum can be widened to some quinolones [107, 108].

Resistance genes do not only exist in hospitals but are found in the genomes of most bacteria in differing environments, and although their influence on human health is best visible in the hospital setting, it seems that the hospital resistome is closely related to the resistances in other environments. Metagenomics have already revealed multiple environmental sources for resistance mechanisms that are relevant in clinical context. A lot of them are associated with environmental actinobacteria, commonly found in soil. One gram of soil contains around 580 species of actinobacteria showing resistance mechanisms, similar to clinical samples. *Kluyvera* spp. are common environmental bacteria, and most likely the source of a gene coding the extended beta lactamase CTX-M, that is carried by major global pathogens. Waterborne species are suggested as being the source of a quinolone gene (qnr) that leads to quinolone resistance in *Klebsiella pneumoniae* [107].

Environmental samples show that resistance genes are older than the discovery of antibiotics by humans; a sample of 30,000-year-old soil from permafrost revealed glycopeptide resistance genes [108]. This might not be surprising, as antibiotics are ancient and the organisms that are producing them build resistance to shield themselves from their own products [107].

The question how the hospital and the environmental resistomes are connected stays unanswered at present. A theoretical mechanism for environmental horizontal gene transfer is transduction via phages. They persist in an environment over a long period of time and therefore might possess the potency to lift the requirement that bacteria need to be present at the same time in the same place for gene transfer [109].

Even with next generation metagenomic methods, it is yet not possible to identify all the genes that might induce resistance in an organism, as genes might not be present as resistant genes in their native context. Often, they are expressed with low numbers of copies, thus not promoting resistance; however, in a new genetic context, that induces induction of expression, they might lead to the production of proteins making their host resistant towards antibiotics. An example for this are efflux pumps belonging to the resistance/nodulation/cell division, which are found in organisms of all kingdoms. Their innate function is to rid those organisms from toxic substances, but when they are mobilized and later expressed at high levels in pathogens like *E. coli, Campylobacter jejuni* or *Pseudomonas aeruginosa*, they lead to efficient efflux of multiple classes of antibiotics [107].

While environmental sources may play a role in the acquisition of resistance mechanisms in hospitals, human action is shown to contribute largely in shaping the hospital resistome. Microbiota in confined habitats with frequent use of antimicrobial agents not only show a shift from Gram positive towards Gram negative bacteria, but also a difference in resistance mechanisms. Findings of Mahnert et al. show that compared to environments like office buildings and homes, a relative reduction of diversity of around 50% in confined habitats is accompanied by a 20% relative increase of resistances, suggesting that biodiversity and resistance are somewhat negatively correlated. Not only overall difference was significant in this study but also the different rates of mechanisms against specific antibiotic agents. Confined habitats showed an increased resistance towards fluoroquinolones and triclosan, while offices and homes have relatively more resistance mechanisms against macrolides, aminoglycosides and diaminopyrimidines [83].

While increasing microbial diversity might lead to a decrease in the sum of resistances in the built environment, it is not the only suggested method. Crofts et al. proposed several measures connected to antibiotic use to fight antibiotic resistance that include surveillance (e.g. through functional metagenomics), synergistic use of drugs, and the use of adjuvant substances that inhibit antibiotic resistance enzymes e.g. beta-lactamase-inhibitors. A synergy of meropenem, piperacillin and tazobactam was shown to be effective against MRSA, but the combination of synergistic substances has more beneficial effects, placing microbes under selective pressure, causing them to discard their resistance genes [106].

Our limited knowledge of the resistome is linked to the limitations of metagenomic analyses. While they offer a culture independent approach to resistance gene detection those disadvantages must be considered. Translation of a gene may or may not produce a

functional product depending on minimal base changes in its sequence, leading to different amino acid residues in the protein, thus altering its substrate preferences, binding sites or overall function. Even if 100% similarity of a target gene and the reference gene in a database are given, the reads do not account for the whole gene sequence, but only a part of it, hence in the microorganism the gene might be truncated or otherwise altered and in consequence be non-functional. After identification of a resistance gene, it is hardly possible to determine from which bacterium of the sample it originates, so knowledge of their genomic context is sparse [110].

A method to overcome issues of functionality of genes can be functional metagenomics, a tool unbiased from culture and sequence. In this approach, DNA is extracted from a microbial community, sheared into target sizes and cloned into screening vectors. These genes can then be transferred into hosts and selected for a phenotype of interest; one that survives in the presence of a certain antibiotic substance, for instance. Through this, one can obtain the information if these genes are functional, as they must be to induce resistance in the host [106].

There are still some limitations to functional metagenomics that need to be overcome, given that the genes must be functional in the host. *E. coli* for instance, is a common host, but the function rates of genes from gram-positive organisms are relatively low in *E. coli.* Further it likely leads to an overestimation of resistance-rates, as genes that are expressed and lead to resistance in a studied host may be unable to disseminate in their environment, or not cause resistance in the original organism [106]. Another limitation lies within the reference databases that are used in order to identify resistant genes. A variety of databases exist and consulting one or the other leads to different result, leading to both, over or underestimation of the resistances of a community [110].

3.6 Maintenance and cleaning in the hospital environment

As already mentioned, various factors make hospitals different from other general built environments regarding microbial communities, and how they are controlled; one major impact factor is cleaning and maintenance.

According to Dancer et al. cleaning has two main functions; the first is restoration of appearance and maintaining of function, while preventing deterioration; the second is microbiological, reducing the number of microbes and substances that might help them grow or interfere with following disinfection and sterilization to promote a healthy environment [111]. It is unclear what 'clean' means, however, as there is no commonly accepted threshold for the number of microbes. Different countries have different guidelines for cleaning procedures and protocols, and there is no consensus on how the efficiency of the conducted measures should be monitored. Most studies concentrate on detecting the presence or absence of certain microbes that are known for their potency to cause HAIs. But with the evolution of metagenomics and the growing ability to display the microbial community, some researchers claim that there has been a change of perspective, from the concentration on certain pathogens towards observing the microbial ecology, to trying to show how microbes interact with their environment [4]. According to some, the goal of cleaning should not only be to eradicate those pathogens from the hospital environment, but to influence the microbial community in a more specific way, promoting the growth of beneficial or harmless microbes [87, 112].

Before discussing new approaches in establishing a healthier hospital environment, this section will summarize current cleaning methods, their potency, limitations, and ways to improve hospital cleaning.

Although the last years of research have provided more evidence that environmental contamination plays a role in hospital acquired infections and their rate correlates with the abundance of pathogens, it is hard to prove that a microbe causing infection in a patient is originating from the hospital environment, and there is still little evidence to link a clean hospital to reduced infections. Many studies show that cleaning leads to a reduction of microbial load, and although observations of enhanced or improved cleaning interventions show a decrease in the number of infections, most of them have been carried out in outbreak situations, where a bundle of measures had been taken and it is not completely clear which impact cleaning had by itself. To date, there is still a lack of evidence linking bacterial load

to the number of infections as an independent factor, nor is there a consensus about the frequency or method of cleaning [113, 7, 114, 3, 111, 48]. This is owed to some factors: firstly, as mentioned, cleaning is not easily investigated independently, as many known risks contribute to infections in hospitalized patients (e.g. antimicrobial consumption, insufficient isolation rooms, or poor hand hygiene). Secondly, Dancer et al. suggest that cleaning has never been seen investigated in an evidence based scientific approach. This opinion is partly supported by Han et al. who found a lack of comparative studies comparing different cleaning methods. While finding results on the contribution of cleaning to infection-prevention is difficult, due to a lack of risk- based standards to verify if a hospital is really clean, the focus in most of the existing studies lies on surface contamination rather than patient outcomes [115, 48].

3.6.1 Detergent based Cleaning

When considering cleaning methods, one must distinguish between detergent based cleaning, disinfection and sterilization.

Detergents are surfactants, used to keep surfaces optically clean and dirt free. One frequently used example is the phenolic quaternary ammonium, which possesses disinfectant properties as well. Often detergents are used together with disinfectants [116]. Detergents are capable of removing microbes, but not necessarily killing them, thus when used alone, may promote the spread of microbes from one hospital site to another [48].

Disinfectants are a group of chemicals that kill microorganisms and are primarily used on surfaces. Nevertheless, they do not necessarily kill endospores. Most commonly used are chlorine-based products, for example sodium hypochlorite also referred to as “bleach” or microbiocidal phenolics. Negatively, they are more expensive compared to detergents, and some are environmentally unfriendly with the potential to persist in water courses under towns. To unfold their bactericidal power, they need to be applied according to the manufacturer’s recommendations regarding their contact-time, concentration, and the amount per square meter; otherwise they fail to kill certain microorganisms. This causes them to be dependent on proper use, which has been shown to not always be achieved in the past. Therefore, other less user-directed decontamination procedures are being investigated, or are already in use (e.g. hydrogen peroxide vapor, or UV light) [11, 48, 7].

Sterilization is a process that destroys all microorganisms as well as their endospores, conducted through heat in so called ‘autoclaves,’ where steam under pressure reaches temperatures of around 121°C. Sterility can also be achieved through radiation. Sterilants

are used for decontamination in settings where heat, or radiation cannot be used or are unpractical. For example, polyethylene tubes, and other instruments require this approach. Common chemicals for this so called “cold sterilization” are formaldehyde or glutaraldehyde [11].
When considering cleaning, one must also differentiate between daily procedures that are carried out (sometimes more than once a day) while rooms are occupied, and so called “terminal cleaning” after patients have been dismissed. This is also dependent on the region; while the Centre for Disease Control and Prevention and the WHO recommend daily routine-cleaning of patients rooms, the national guidelines in Taiwan only demand terminal cleaning while daily maintenance is carried out hospital-dependent, for example [117].

It is a matter of debate which sites should be cleaned with detergents only and which require disinfectants. Clearly the latter are favored in outbreak situations, and it has been argued that that they should be recommended for routine cleaning of frequent hand-touch sites close to the patient, as they could be a source of cross transmission [7].
As mentioned in the section above, little evidence comparing differing cleaning procedures exists, especially on a patient outcome level. As such, the capabilities and disadvantages of some of the most used chemical and physical procedures shall be discussed separately, starting with chlorine-based chemicals. hypochlorite (bleach) is the most widely-used chlorine disinfectant. Its broad-spectrum antimicrobial potency is due to the release of free chloride, although the exact mechanism is not yet completely understood, and may be a combination of numerous factors including the oxidation of sulfhydryl enzymes and amino acids, ring chlorination of amino acids, loss of intracellular contents, decreased nutrition uptake, breaks in DNA or DNA-synthesis depression and others. Chlorine products have been used widely for water treatment where they act against *Legionella,* but it is also used on surfaces, effective against viruses, bacteria and fungi. Namely it has been shown to be biocidal against *C. difficile* spores*, S. aureus, Salmonella cholerasuis, M. tuberculosis* and other pathogens.
Disadvantages include their dependence on correct use, their toxicity is potentially causing symptoms like ocular irritations, and the production of a carcinogen bis-chloromethyl ether when it comes into contact with formaldehyde [93]. Quaternary ammonium compounds are detergents as well as disinfectants that disrupt cell membranes and denature essential proteins, in addition to inactivate energy-producing enzymes. They are widely used for surfaces that are uncritical, according to the CDC. They show effects on coated viruses,

bacteria (not on spores) and fungi, and are generally ineffective against *M. tuberculosis* or nonenveloped viruses. Other liquid chemical disinfectants are alcohol, which is mostly used for equipment or instruments: formaldehyde and glutaraldehyde [93].

3.6.2 User independent Cleaning

Some newer cleaning methods that are not user-dependent are UV light, Hydrogen peroxide vapor and ozone.

UV radiation (emitted for example from mercury vapor lamps) generates alterations in DNA and RNA structures such as formation of pyrimidine dimers from thymine and cytosine, which are incompatible with life. Since they are not only acting against microorganisms but also dangerous for human beings, they can only be used when no one is in the room, and thus are more suitable for terminal rather than daily cleaning. Its maximum bactericidal effectiveness occurs at 240- 280 nm. UV light is shown to be effective against MRSA, VRE and *C. difficile*. Unfortunately, it is dependent on many parameters; wavelength, exposure time (45 minutes are better for *C. difficile* than 20 minutes), and distance to the light source, which affects intensity. Shadowed areas of a room are unaffected by UV radiation [63].

Hydrogen peroxide (H_2O_2) damages DNA, lipid membranes of microorganisms as well as organelles through oxidative action. Clinical studies have focused on MRSA, VRE (against VRE it is most effective), *Acinetobacter* spp. *Klebsiella pneumonia* and *C. difficile* spores, and showed that H_2O_2 is effective against them and others, including fungi, and viruses. Catalase positive bacteria are more resistant towards its effects [63].

Passaretti et al. could show decreased acquisition of multidrug resistant organisms in patients of an ICU, after terminal hydrogen peroxide vapor has been used in addition to routine terminal cleaning with quaternary ammonium and liquid hydrogen peroxide (this was only used in rooms of patients that had *C. difficile*) [118, 48, 116]. A device that is best put in the center of a room releases the vapor, in a process that is completely automated, however precautions need be taken; window ventilation and air con of a room need to be closed or deactivated to ensure that no H_2O_2 can escape the room [116].

There has been research on decontamination procedures that so far have been found not suitable for the use on environmental hospital-surfaces e.g. ozone and steam. Ozone has bactericidal, fungicidal and viricidal potency as well, and is widely used in the food industry. The underlying mechanisms are not well known but is seems to work through oxidation of cellular walls and membrane components. It is cheap, but at the concentrations needed for antimicrobial activity it is toxic and corrosive, therefore it is not suitable for surface

decontamination but can be used for sterilizing instruments. Steam, although microbicidal at certain pressures, condenses ambient air and may result in the promotion of biofilms; is not compatible with electronic devices, and leads to aerosolization of microbes [63].

Aside from chemicals, discussion also exists concerning textiles used for cleaning. Microfibers are positively charged, so they remove particles by static attraction and capillary actions. Nevertheless, they have not been shown to perform better than conventional cleaning cloths. Ultramicrofibers, characterized through thinner fibers, seem to be more potent at removing certain pathogens. Both share the disadvantage that they are incompatible with chlorine based products, and lose their cleaning potency over time, thereby potentially promoting microbial spread after that period [63].

Even when cleaning has been performed correctly, recontamination can occur within hours. More recently the idea of "self-cleaning" materials has emerged, referring to surfaces that are coated with bioactive materials, including metals like zinc, copper, silver, or titanium dioxide. This could prevent the re-contamination of surfaces, or prevent primary contamination altogether [116, 119, 48]. Copper produces reactive oxygen radicals that damage DNA and RNA, as does titanium dioxide in the presence of UV light. While research on titanium dioxide is in an early stage, copper coated surfaces have been shown to reduce microbial burden in multiple studies, although the number of these studies conducted is a limiting factor. Salgado et al. showed a decrease of 83% in the microbial burden of copper-coated surfaces in patient rooms of 3 ICUs compared to conventional surfaces in a randomized control trial. They also point out that additional studies are needed to determine the clinical effect of copper alloys, however [120].

While these coatings may decrease microbial burden without the risk of failure misapplication, they share the disadvantage of being relatively expensive [48]. The microbiocidal surface coatings could be applied in the form of nanoparticles. The decrease of particle-size to the nanometre-range would lead to an increase in overall contact-surface. Magnesium nanoparticles have been observed to act against *E. coli* and *S. aureus* not only in their planktonic form, but in biofilms as well [121]. Shark-skin like surface micropatterns have been shown to reduce the abundance of MSSA and MRSA [122].

Table 6 Common and new cleaning procedures, and surface coatings [48, 116, 120]

Advantages and disadvantages of substances and materials for hospital surfaces			
Substance/ Material	**Advantages**	**Disadvantages**	**Use**
Hypochlorite	Microbiocidal; cheap; sporicidal	Dependent on correct use; not sporicidal	Routine surface-disinfection; enhanced cleaning on high touch surfaces
Hydrogen peroxide vapor	User independent; sporicidal	Toxic, may not be used in occupied rooms, nor get out of them;	Effective in addition to routine terminal cleaning
Quaternary ammonium	Cheap; detergent and disinfectant;	Not sporicidal; no effect on *M. Tuberculosis;* user dependent	Disinfection of uncritical surfaces
UV light	User independent; sporicidal at longer exposure times;	Depending on multiple factors (e.g. wavelength, distance, time); some areas might not be reached at all; not for occupied rooms	Addition to routine terminal cleaning;
Ozone		Corrosive at microbiocidal concentration; toxic for humans; so far not recommended for surface cleaning;	Sterilization of instruments
Copper coating	microbiocidal without need of further action;	Expensive; more data needed;	So far not in routine use

Besides the named methods, air filters are in use ensuring air renewal and the reduction of contamination more effectively than open windows, when maintained correctly. HEPA remove airborne particles greater than 0.3m in diameter, with an efficiency of 99.97%, including bacteria and spores [63].

Research conducted on the outcome of cleaning procedures highlights the importance of monitoring efficiency, not only in outbreak situations but regularly. In the absence of a clear picture as to what 'clean' defines, this may be difficult to achieve, however. Existing monitoring practices include visual assessment, ATP bioluminescence detection, or the detection of indicator organisms (e.g. coagulase positive Staphylococci). There is a consensus that visual assessment alone does not provide sufficient information on the microbial burden of an area, given that visually clean objects may still be contaminated with pathogens [48, 123, 64]. Molecular based assessments have shown that even after terminal cleaning has been done, that pathogens survive these procedures, and may even be positively selected through the absence of non-harmful or beneficial organisms. If the microbial abundance is assessed by molecular methods (e.g. metagenomics), the detection of genetic

material from dead organisms may provide a misleading picture, as has been shown by metagenomics of a cleanroom and the common (not confined) room outside of it: Comparison without prior PMA-treatment showed only little difference in microbial abundance between those areas, while PMA revealed that over 90% of the reads had come from dead cells. Overall, the abundance of microorganisms was smaller in the named cleanroom, but the diversity remained almost constant [124]. The importance of constant monitoring is highlighted by the fact that 50-60% of the surfaces that should be cleaned by housekeeping staff stay untreated, thus showing the important role of staff in the cleaning process [123].

Failure to achieve cleanliness is often linked to a lack of staff, leading to limited time resources. In response, the introduction of additional or better trained staff has been attributed to lower pathogen presence. In a UK-hospital introduction of one extra cleaner for high touch surfaces lead to a 26.6 % decrease of MRSA infection rates. considering the resources that would have been used for these patients, Dancer et al. concluded that the additional cleaner lead to cost reduction of at least 30,000 pounds in one year, already considering the salary of the additional cleaner [64].

Havill et al. proposed a program for best practice of surface cleaning in the hospital. It contains the formation of a multidisciplinary taskforce to define the cleaning process and the substances to be used; education of the environmental service personnel, to ensure the correct distribution and contact times of disinfectants; followed by monitoring and feedback. They also point out that a clear distribution of responsibility is needed regarding which areas are to be cleaned by household staff or nurses , thus preventing the complete lack of cleaning of certain areas [125].

3.7 New Methods to influence the hospital Microbiome

The section above evidences that cleaning is a matter of debate, with many regional differences in practices and protocols and it is often not carried out satisfactory. Most of the studies on microbes in the hospital environment and the effects of cleaning are focused on certain pathogens, and mostly carried out during outbreak situations rather than during hospital routine. Additionally, most either use cultures or sheer particle count to assess the effect of cleaning within the hospital microbiome. NGS-based approaches that display the composition of the microbial community better than those depending on cultures, have shown that with the existing cleaning methods, even when carried out properly, it is impossible to keep the areas of a hospital germ free, neither in general wards, nor in confined habitats [126].

The selective pressure that is imposed on microbes induces the acquisition of resistance mechanisms and adapt to the present conditions [36]. A possible explanation might be the lack of a rich microbial community leading to less competition, so that microbes that can survive under these harsh conditions are selected and can spread easily [22].

It has been observed in soil probes that the survival of alien *E. coli* correlates inverse to the richness of the present microbiome, suggesting that higher competition leads to less accessibility of nutrition for invading species. This negative correlation was especially driven by the amount of Actinobacteria [127]. More efficient cleaning has even been associated with an increase of fatal infections [4]. These findings suggest, that germ-free might not be equal to healthy.

The introduction of plants, probiotic cleaning detergents, or bacteriophages and how those might lead to a reduction in abundance of common pathogens are is discussed. Plants, like humans, have their own specific microbiome, consisting mainly of organisms that are either beneficial or neutral towards humans, but they also give home to human pathogens such as. *Burkholderia cepacia, Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia*. Nevertheless, current knowledge of the interplay between the microbial community of plants and their surroundings is sparse. Pollen has been suggested to act as a shuttle for microbes to enter the built environment from the outside, or to move around the inside. It is shown that typical plant microbes are often found in the microbiome of buildings. They share numerous microbes with the human skin microbiome such as archaea like Thaumarchaeota, alongside of bacteria like *Methylobacterium* spp., *Delftia acidovorans*, *Caulobacter* spp. and others. Experiments have shown, that plants lead to an increase of diversity of the microbial

community in their surroundings; they do not exclusively add microbes that have no, or beneficial effects on humans, but also with the above-mentioned pathogens. Further, they carry fungi (*Aspergillus ochraceus* or *Penicillium* spp.) that might have allergic potential [38, 128] .

In 2009 a paper by Falagas and Makris summarized the results of 10 in vitro studies where probiotics led to a decrease in the number of surface-microbial pathogens. Bacteria used in these studies contained *Lactococcus lactis*, *Lactobacillus* spp., *Streptococcus mitis*, *Streptococcus thermophilus* A and B. From this, they hypothesized that probiotics could be used for cleaning purposes in the future; they also assessed that it is not certain if bacteria, that survived on agar-plates would be able to germinate on environmental surfaces [129].
Later, a Bacillus-based detergent consisting of spores of *B. subtilis, B. pumilus* and *B. megaterium* (all of those are apathogenic bacteria) was tested in multiple hospitals in Italy, Belgium and a dental clinic in the USA.
In a study conducted in two clinics in Italy and one in Belgium, this cleaning approach led to a decrease in the number of all the tested pathogens. Coliforms were reduced by an average of 74% (±21%), S. aureus by 78 % (±15%) after six weeks. *C. difficile* was only detected regularly in one hospital, where it showed a wide variety of counts dependent on surface and time points leading to a high standard deviation, but a reduction could be achieved. The effect on *C. albicans* was only monitored in the two Italian hospitals, resulting in a reduction by 82% (±19%).The mentioned effects resulted from comparing the probiotic cleaning detergent with prior cleaning practices, which consisted of a detergent and chlorine-based disinfection in the Italian hospitals, and is not further described for the one in Belgium [130].
The active introduction of certain bacteria into the hospital environment might risk increasing the antibiotic-resistance of the present pathogens through horizontal gene transfer. Another trial in a private clinic in Italy focused on resistance-mechanisms, showing a decrease in resistance genes in the pathogens S*taphylococcus* spp., *S. aureus*, *Enterobacteriaceae* family, *Acinetobacter*, *Pseudomonas* spp., *Clostridium* spp., *Candida* spp. and *Aspergillus* spp. Nevertheless, the MRSA-gene (which is common in *Bacillus* spp.) that is associated to resistance against erythromycin was detected more often after the invention [131, 132].
The effect on the incidence of hospital acquired infections using the same Bacillus-based detergent has been tested in six Italian hospitals, replacing the prior used chlorine products.

Before the intervention, the HAI-incidence of those hospitals was 4.8%. In the six months following the intervention, the incidence of all kinds of HAI dropped to 2.3%, although it should be noted that patients were not matched in this study, and therefore their condition is a possible confounder [133].

Bacteriophages, viruses that infect bacteria and archaea, are considered the most abundant organism in the biosphere and are easy to isolate. Infection with virulent phage's leads to rapid multiplication and ultimately lysis of the host organism, while they are nontoxic to eukaryotic cells. While some are specific for one bacterial species, others can use a variety of species as their hosts. Mixed therapeutic results, poor understanding and broad-spectrum antibiotics so far have prevented phage-therapy usage modern medicine, but their effect on certain bacteria without harming eukaryotic cells might be used for hospital-decontamination as well as in patients with infections of antibiotic resistant drugs. In mice-models, phages have been shown effective against. *P. aeruginosa* infected lungs and burn wounds, as well as against *S. aureus.* Studies on in vitro surfaces have been undertaken with *Staphylococcus* spp*., Streptococcus* spp*., Proteus* spp*., E. coli, Acetobacter baumannii* and *Pseudomonas aeruginosa,* showing that phages led to a reduction of these bacteria [134-136].

D'Accolti et al. used a combination of a probiotic detergent and phages on surfaces (plastic, glass, and ceramic) that had been inoculated with *S. aeruginosa*, *S. aureus* and *E. coli*, with density of 4×10^4 CFU/m², mimicking the bacterial load on hospital surfaces. They state that prior in vitro studies often used prolonged contact times or higher bacterial densities compared to the conditions found in hospital. They observed that the phages were stable over a period of 7 days in the solution of probiotic and sterile water, leading to a bacterial reduction of up to 90% (compared to mock-treated surfaces) [136].

The use of phage's needs to be conducted carefully, as some are known to mobilize or encode bacterial virulence factors and in their hosts so that improper selection could lead to infections with more virulent bacteria [134]. As discussed in the section about the resistome, phages can also lead to the transfer of antibiotic resistance genes.

3.8 Discussion and Conclusion

With improving technology and the growing ability to sequence genomes faster and cheaper, knowledge of microbiomes grows. Compared to the human microbiome and its implications for health and disease, research on the hospital microbiome seems to be less advanced. Most studies on the theme seem to be explorative, concentrating on taxonomically identifying the microbes throughout different hospital-areas. Although there are many hypotheses, concrete facts on the impact of the hospital microbiome on health outcomes and the consequences of dysbiosis of the built environment are sparse. The relatively high abundance of human-associated microbes is consistent throughout literature as well as the increase of this relative abundance in confined areas, for instance, ICUs. In addition, multiple studies have shown that human-influence through confinement and daily cleaning procedures leads to a reduction of the absolute number of microbes, but also reduced overall diversity by up to 50%. The result is a higher relative presence of human-related bacteria, and a decrease in microbes from environmental sources. Lower diversity due to confinement also leads to higher resistance rates.

Discussion of environmental parameters, temperature and relative humidity, and their potential influence have shown that pathogens react in different ways. The distinct behavior of microbes belonging to the same kingdom towards these parameters does not show a pattern that would allow a general statement on how room temperature and relative humidity could be regulated in a beneficial way. Studies conducted on mode of ventilation have shown that mechanical ventilation combined with HEPA-filters are effective in reducing the amount of airborne microbial particles, especially from outdoor sources, while in window ventilated rooms diversity is larger. While mechanical ventilation and different flow types (laminar, turbulence, mixed) can be effective in special settings, for instance operation theaters, or to establish negative pressure in isolation rooms, the largest impact was shown for air changes. Particularly in settings where hospitals do not have the money to either install a mechanical ventilation system, or to ensure adequate maintenance, natural ventilation through windows is superior.

The role of microbial load on hospital-surfaces for nosocomial infections and what actually ‘clean’ defines is a matter of debate. Cleaning has been shown to be effective in reducing microbial load, but its actual role in the prevention of infection is not completely understood due to a lack of randomized controlled trials. What can be shown though, is that standard cleaning protocols mostly fail to eliminate pathogens from hospital surfaces. In fact, these

protocols are often not carried out in the proposed way, leading to whole areas that stay uncleaned, mostly due to a lack of staff.

Health care workers hands provide an important pathway for microorganisms to be moved from contaminated areas throughout the hospital, and potentially lead to infections. Considering this, the high percentage of pathogen-contaminated items of clinical work that sometimes pass multiple professionals' hands (patient files, phones, sonography machines), might be of concern, even if at present there is insufficient evidence linking the contamination of these tools to nosocomial infections in patients.

The limited potency of keeping hospitals germ free has led to a change in perspective, and methods to influence the microbiome are being developed. Solutions of detergents containing a mix of probiotics have led to a reduction of pathogen load on hospital surfaces through colonization, with *Bacillus* spp. considered to be harmless for human occupants. This method has already been effectively tested in multiple health care centers. At present, results rely on pre-post studies (without matching of patients), and not randomized controlled trials which could lead to more reliable results in the future. In situations where a reduction of germs is inevitable, metal-coated "self-cleaning" surfaces could be used to prevent recontamination of surfaces after routine procedures. A disadvantage of these surfaces is their relatively high price.

There exist important limitations of this study that should be mentioned. First, there is a high inhomogeneity throughout methods used in the described studies and while the aim has been to describe their findings, comparability might be limited. Still evidence on the topic seems to be mostly consistent and where this is not the case, it has been openly addressed.

Second, this review might not display all the evidence that is available, as only articles published in the English language from 1995 to March 2019 have been considered, and there has been no contact to authors to consider new, unpublished findings.

This review aims to display the current status of research on the hospital microbiome and methods to influence it. Overall, it can be said, that there is a lot of research-activities in this area leading to considerably differing results.

While the picture of the hospital microbiome becomes more detailed, future research would benefit from the implementation of standard procedures for metagenomic studies to guarantee comparability of these studies. Focus should be directed towards the development of procedures to beneficially influence the microbiome, and these should be tested in randomized controlled studies. The fact that existing procedures are often not carried out is

correctly should lead to actions, employing enough maintenance-staff, along with considering better education housekeeping personnel about the importance of their work.
Lastly the question: “What is a beneficial hospital microbiome?” remains mostly unanswered, and most of the suggestions rely on theories that will need to be tested in the future.

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