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CHARACTERIZATION OF THE INDOOR MICROBIOME IN A NEWLY BUILT HOSPITAL

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Graz, 04.02.2020

DECLARATION

I herewith declare that I have written this thesis independently and without outside help, did not use any other sources than the ones indicated, and that I have identified the sources used, either literally or in terms of content, as such.

Graz am 04.02.2020 Polina Mantaj eh.

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Дорогие родители, благодарю вас за вашу поддержку и помощь на протяжении всей моей жизни и особенно моего образования. Я вас очень люблю.

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List of Abbreviations and Acronyms

Abbreviation	Explanation
s	Second(s)
µl	Microliter(s)
rpm	Rotations per minute
g (gravity)	Gravity of Earth (9.8 $\frac{m}{s^2}$)
g (mass)	Gramm(s)
°C	Degrees Celsius
min	Minutes
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
PMA	Propidium monoazide $C_{27}H_{32}N_6^{2+}$
ml	Milliliter(s)
W	Watt
kHz	Kilohertz (10^3 Hz)
RSV	Ribosomal sequence variant
LDA	Linear discriminant analysis
bp	Base pairs
CFU	Colony forming unit

ZUSAMMENFASSUNG

Heutzutage verbringen wir durchschnittlich 87% unserer Zeit in Innenräumen, aber es gibt nur wenig Kenntnisse über die mikrobiellen Gemeinschaften dieser Innenräume.[1] Gerade im Krankenhausumfeld sind diese Mikroorganismen für unsere Gesundheit und unser Wohlbefinden von besonderer Bedeutung.

Hier untersuchen wir das Mikrobiom der neu gebauten chirurgischen Abteilung am Universitätsklinikum Graz, Österreich, indem wir Bakterien und Archaeen identifizieren und charakterisieren. Über einen Zeitraum von einem Jahr wurden an sieben verschiedenen Tagen (vor und in zunehmenden Abständen nach der Erstbelegung) Proben in drei Patientenzimmern der Abteilung für Allgemeine Chirurgie entnommen. Beispiele für Oberflächen sind Fußböden, Toiletten, Türgriffe und Kissen sowie selten berührte Oberflächen wie Lichtleisten. Nach DNA-Extraktion direkt aus der Probe und Amplifikation der hypervariablen Region V4 des 16s-rRNA-Gens wurde das gewonnene Material auf einer Illumina MiSeq-Plattform sequenziert. Die Sequenzierungsdaten wurden dann gefiltert, bereinigt und mit spezieller bioinformatischer Software (Qiime2, Calypso) analysiert. Neben der 16s-rRNA-Analyse wurden Abklatschtests an denselben Stellen durchgeführt.

Insgesamt werden die untersuchten Oberflächen von humanassoziierten Gattungen wie *Corynebacterium*, *Staphylococcus*, *Streptococcus* und *Acinetobacter* dominiert. Boden- und Türgriffproben weisen im Vergleich zu anderen Standorten die signifikant höchste Diversität auf, während Proben von Toiletten und Wasserhähnen einen signifikant geringen Reichtum an Mikroorganismen aufweisen. Es wurden außerdem saisonale Unterschiede festgestellt und gleichzeitig vorkommende Bakterienspezies konnten in Bezug auf die Oberflächentypen, auf denen sie gefunden wurden, gebündelt werden.

Diese Erkenntnisse könnten dazu beitragen, die Dynamik von Mikrobiomen in Krankenhäusern zu verstehen und operative Merkmale zu identifizieren, die diese Gemeinschaften beeinflussen, um beispielsweise die Ausbreitung von nosokomialen Krankheitserregern zu verhindern.

ABSTRACT

Nowadays, we spend an average of 87% of our time indoors but lack important knowledge about the microbial communities of these indoor spaces.[1] Especially in the hospital environment, these microorganisms are of particular importance for our health and well-being, since nosocomial infections are the fifth leading cause of death in acute-care hospitals.[2]

Here we study the microbiome of the newly built surgical department at the hospital of the Medical University of Graz, Austria by identifying and characterizing bacteria and archaea. Samples were taken in three patient rooms of the department of General Surgery on seven different days (before and in increasing intervals after they had been first occupied) over a period of one year. Sampled surfaces include floors, toilets, door handles and pillows and rarely touched surfaces like lightbars. After direct DNA extraction from the sample and amplification of a marker in the 16s rRNA gene, the gained material was sequenced on an Illumina MiSeq platform. The sequencing data was then filtered, denoised and analysed through specialised bioinformatical software (Qiime2, Calypso). Apart from the 16s rRNA analysis, direct contact tests were performed on the same locations.

Overall, the surfaces are dominated by human-associated genera like *Corynebacterium*, *Staphylococcus*, S*treptococc*us and *Acinetobacter*. Floor and door handle samples have the significantly highest diversities compared to other locations, while toilet and water tap samples have significantly low richness. We also found seasonal differences and were able to cluster co-occurring bacterial species regarding the surface types they were found on. Although harmless and beneficial species were in the majority, several pathogens and their spread on the studied floor were identified.

These findings are helping to understand the dynamics of hospital microbiomes and identify operational characteristics that influence these communities, thereby for instance finding ways to leave harmless or beneficial bacteria in their environment while specifically targeting and reducing harmful species.

1 INTRODUCTION

Microorganisms spread across all environments on Earth, natural environments and indoor spaces alike; from extremely cold or dark habitats to extremely hot or dry spaces, in geysers, the deep sea and the poles; being central to Earth's ecosystems and cycles, constantly adapting to changes of external factors and influencing human health and well-being in a much greater extent than we thought possible a few decades ago. Microorganisms also make up the microbiota found in and on all multicellular organisms and date back to at least 3.45-billion-years ago as a study from 2017 on Australian rocks that once contained microorganisms shows.[3]

Microbes are also a crucial part of human life and health, as they serve in food production, in sewage works, produce fuel, enzymes and other bioactive substances. They are indispensable tools in biology and medicine but have also been used for biological warfare and bioterrorism. They live in and on the human body and build the human microbiota including the important gut microbiome. They are also the target of hygiene procedures, because they can act as pathogens responsible for countless and sometimes life-threatening diseases.

Apart from their own bodies' microbiota humans constantly get in contact with external microorganisms. Nowadays, most of these contacts occur in an inside environment where humans live and work. Data from the 2001 National Human Activity Pattern Survey (NHAPS), showed that Americans spent an average of 87% of their time in enclosed buildings.[1] Although this data is relatively old, one can assume that it still reflects the ratio of time people in developed countries spend indoors compared to outdoors. Therefore, increasing our understanding of the microbiota of the indoor environments, particularly in hospitals, and how it affects humans could contribute to new models of future health and well-being.

1.1 The microbiota and the microbiome

The diverse and dynamic microorganisms surrounding humans comprise of ever-changing combinations. They are also found in altering phases like proliferating, dormant, inactive or dying. All these cells, fragments of cells, their excretions, and also free DNA encircle and affect us constantly. But we do not fully understand this microscopical world surrounding us everywhere yet. We only begin to fathom this complex system and its entanglement with our own life and health.

Today two different terms are in use to describe these communities of microorganisms. The term microbiota includes archaea, bacteria, eukaryotes (e.g. fungi) and viruses, while micro-animals (e.g. *Demodex folliculorum* and *D. brevis*) living on the human body are excluded. The term microbiome describes "the collective genomes of the microorganisms found in an environmental niche or the microorganisms themselves".[4] It was first defined as "a characteristic microbial community occupying a reasonably well defined habitat which has distinct physio-chemical properties" by Burge in 1988 but was made popular by J. Lederberg beginning in 2001.[5] In this thesis, "microbiota" refers to symbiotic microorganisms and "microbiome" refers to their total genomes.[6]

Since microorganisms were discovered by two Fellows of The Royal Society, Robert Hooke and Antoni van Leeuwenhoek between 1665-83, studies have relied on microscopy and culture. The first link between microorganisms and disease was found by Agostino Bassi between 1808 and 1813. He discovered a fungus pathogenic to insects called *Beauveria bassiana*. Few decades later in 1847 Ignaz Semmelweis, a Hungarian obstetrician working at the Vienna General Hospital, investigated the high maternal mortality from puerperal fever following births assisted by doctors and medical students. Assuming that puerperal fever was a contagious disease, Semmelweis had doctors wash their hands with chlorinated lime water before examining pregnant women and obtained a reduction in the mortality rate.[7] Later the famous works of Louis Pasteur and Robert Koch shaped our understanding of infectious diseases caused by microorganisms called pathogens – also known as the germ theory. Science then science and medicine focused on the pathogenic microbes for a long time. Although most bacteria are harmless or even

beneficial, as fewer than a hundred are estimated to be pathogenic to humans.[8] In contrast, thousands of usually harmless species live in the human digestive system.

For quite a long time it was assumed that the number of microbial cells in and on the human body exceeds the number of human cells by 10 fold,[9] but it turned out to be an overestimation as a more recent study showed that the ratio is closer to 1:1.[10] Nevertheless large parts of the human-associated microbiome are uncultivable, which has possibly led to an underestimation of its diversity.[11-13] Conventional sequencing uses a culture of identical cells to produce DNA. Studies that compared conventional sequencing and sequencing of ribosomal RNA (rRNA) genes in samples taken directly from the environment, showed that cultivation based methods often find less than 1% of the bacterial and archaeal species in a sample.[14], with extensive effort and targeted culturing it is possible to cultivate and characterise more species, as Browne and Colleagues showed for the gut microbiota.[15] Advances in research tools like the ability to perform genomic and gene expression analyses of single cells and of entire microbial communities in the disciplines of metagenomics and metatranscriptomics, extensive databases available to researchers across multiple disciplines and new methods of mathematical analysis suitable for complex data sets made the recent progress in this field possible.[16] studies use these new techniques to sequence the DNA of microbial communities found in and on the human body, on surfaces indoors or from the environment, and try to characterise their complexity, functions and abundance, trying to grasp the interaction with and impact on humans.

Many studies investigated the invisible micro-world in and on the human body. The microbiota inhabiting our gastrointestinal tract is well known to play an important role in many host processes. Much is already known about the gut microbiota in health and disease: The gut microbiota help breaking down certain polysaccharides[17], influence inflammatory bowel diseases[18] and even play a role in central nervous system disorders[19]. Our knowledge of these diverse communities continues to advance quickly. Recent attention focuses on understanding how the gut microbiota contribute to protection against disease, aiming to translate these findings into clinical applications.

It is easy to imagine, that the microbiota living inside our body are having an impact on our well-being, but the microbial communities we get in contact with every day in our living spaces and workplaces might influence us in similar ways. As Pekkanen et al. showed

asthma risk may be lowered in a Finnish cohort of 182 children not living on farms by making their home microbiota composition more similar to a farm home like microbiota.[20] Recently their findings were confirmed in a cross-sectional study of 1031 German children.[21] This demonstrates that the indoor microbiota affect human health in more ways than we knew, and more research is needed to fully understand the interplay of indoor microbiota and human inhabitants.

1.2 Microbiota of the built environment and hospitals

Research on microorganisms in built environments is not new. Many studies investigated the spread of the microscopic roommates surrounding us everywhere we go. But until recently this research was undertaken with limited tools. As already mentioned above, cultivation-based methods can only reveal part of what really survives on indoor surfaces. Although rRNA sequencing of cultured isolates became popular in the 1990s, the first study to apply molecular amplicon sequencing approaches to the actual microbiome of the built environment was conducted in 2004. Kelley and colleagues investigated the microbial communities of shower curtains.[22] The microbiota that were found by them involved many alphaproteobacterial genera, such as *Sphingomonas* and *Methylobacterium*.

Before building design and operation of public buildings can be changed in a way to help human health and well-being, it is necessary to define the bacterial communities, where they are found indoors, and which factors influence them most. Several studies already investigated indoor microbiomes in different building types. Examples for novel research on the microbiota of the built environment include investigations of public restrooms[23], homes and kitchens[24-26], indoor air[27], university classrooms and office buildings[28,29], the Louvre Museum[30], metropolitan subways[31] and even the ISS[32]. Researchers interest also lays on the microbiome of human-associated objects like phones and shoes.[33] Another interesting emerging field is the influence of plants to the indoor microbiome.[34,35]

Mostly, we design, build and operate our buildings to be unfriendly environments for microbial life, which can result in selective pressures that allow only a small part of microorganisms to survive.[36] In a meta-analysis the top ten taxa associated with indoor

environments were identifiable as human-associated microbes (e.g., *Corynebacterium, Streptococcus, Enterobacteriaceae, Staphylococcus, Propionibacterium, Lactococcus*) and outdoor habitats (e.g., *Streptophyta*, *Pseudomonas, Acinetobacter*, and *Sphingomonas*). The influencing factors known to date to have an impact on indoor microbiota are summarized and adapted to the hospital in Figure 1. These influencing factors begin with the buildings construction since architecture and ventilation were found to have a strong effect on the microbiota of patient rooms.[37] Consistent sources for indoor microbes are outdoor air and human skin, due to the high emission rate of up to 10^6 bacteria per person-hour.[26,37,38] Humans have been clearly identified as an important source of the microbial diversity in many indoor environments and are also its primary vector.[23,29,39] Human skin-associated and gastrointestinal or urogenital-associated bacteria were found to be dominant

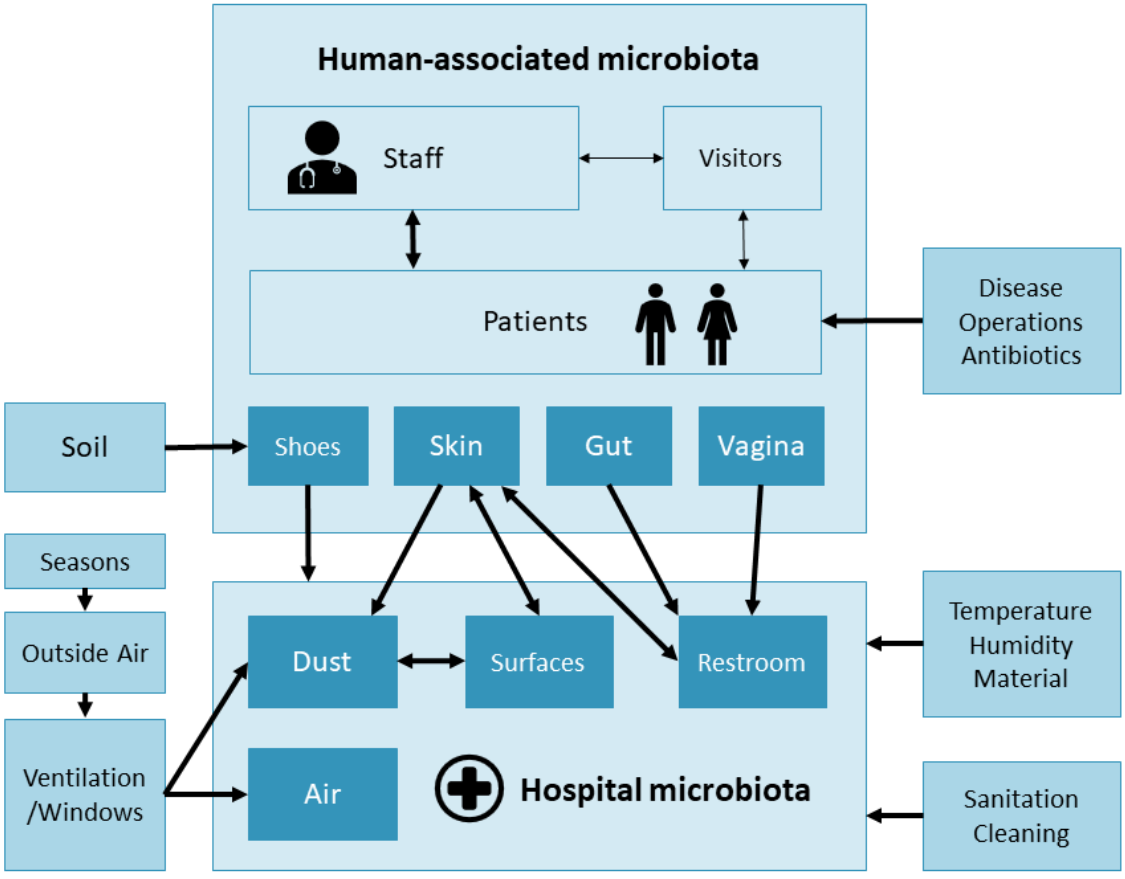

Figure 1 Hospital microbiota and its relation to other microbial communities (inspired by "Studying the microbiology of the indoor environment" Scott T Kelley & Jack A Gilbert)

on many indoor surfaces.[26,40] Furthermore, the direct transmission of skin microbiota through surface contact was found to have a significant and area-specific effect. Skin contact with surfaces can transfer millions of microbial cells, which results in recurrent colonization of frequently touched surfaces, like door handles, keyboards and toilet seats.[23,29,39]

Moreover, a meta-analysis from 2015 showed that far-reaching factors, such as geography and building type, have high impact on the composition of indoor microbiomes.[11] So although some taxa seem to be found regularly in indoor environments, differences between buildings still can be great as a study from Finland by Rintala et al. shows.[40] They also found that gram-positive species dominate indoor dust and suspect this dust might originate from users of the building. The microbial communities of dust and indoor air have been investigated in several studies.[41,42] Microorganisms from outside air enter build environments through the ventilation system or simply through open windows. However, Tringe et al. suggest that indoor bacteria are mostly indoor-generated and not accidental transients from outdoor environments.[41] Similarly, Lax et al. investigated the microbial communities of family homes and found that microbiota in each family's home were identifiable by family.[43] A study on public restroom surfaces by Flores et al. found that restroom surfaces are dominated by human-associated microbes and they presume that bacterial pathogens are spread between people by touching of surfaces. Apart from human skin also other areas of the human body can contribute to the indoor microbiome. The gut and vaginal microbiome are important distributors of microorganisms and are associated with surfaces in restrooms.[23]

Another essential contributor to indoor microbiota are external environments. Dust samples in offices revealed bacteria associated with soil and plants.[29] Their types and spread appear to be dependent on the ventilation[37] of the corresponding building, as well as local climate[29] and season[40].

All of these findings are important regarding pathogen transmission in a hospital environment, not only from patient to patient, but also from staff and visitors. Much remains unknown about the interplay between microbiota of built environments and human inhabitants, especially in the context of hospital hygiene and possible beneficial effects on human health.

In contrast to most indoor environments, hospital rooms and surfaces are routinely monitored.[44] Typical cultivation techniques, for instance contact plates, are regularly used to monitor the microbial burden. As already mentioned above, these standard techniques can miss a great part of the actual microorganisms and microbial diversity present. Nevertheless, a global threat to many patients in hospitals are hospital-acquired infections (HAI). They remain to be one of the leading causes of death in hospitals around the world.[45] HAI are associated with increasing economic burden, length of stay, complication rates, and worsening overall morbidity and mortality.[46,47] A multicenter study concerning data from 2012 found overall healthcare-associated infections prevalence was 6.2 % in Austria. The highest rate of 20.9 % was found in intensive care departments.[48]

However, hospitals are not only a reservoir for nosocomial pathogens but also home to non-harmful microorganisms. Hospital surfaces are regularly being cleaned and disinfected with chemical substances, wiping out not only harmful species but also potentially beneficial microbes.[49] Thus newer approaches to hospital hygiene focus on specifically targeting the harmful species while leaving the beneficial microbes unharmed. Recently D'accolti et al suggested a bacteriophage-probiotic sanitation for hospitals, where specific pathogens like *Staphylococcus* spp. are successfully targeted with significant decrease rates of up to 97%.[50] The remodulation of the hospital microbiome is associated with up to 99% decrease of the antimicrobial-resistance (AMR) genes found on hospital surfaces[51] and a reduction in HAIs compared to conventional chemical cleaning. They further propose microbiome-based monitoring to control AMR and HAIs in hospitals.[52]

1.3 The human microbiome and its members

As mentioned above, human-associated microorganisms are a crucial part of indoor microbiomes. Therefore, this chapter summarizes recent knowledge about important members of human microbiome regarding the most important body locations for indoor environments.

1.3.1 Bacterial habitats of the human body

Although most of the time we are not able to see or feel it, we share many of our bodies' surfaces and cavities with an unimaginably large number of microorganisms. The Human Microbiome Project found that humans are hosts to thousands of bacterial species with each body site having a differential composition of microbial communities.

While in general the skin and vagina show smaller diversity, mouth and gut have the greatest richness of different species. The bacterial community for a specific site on a body differs in each individual, not only in kind, but also in abundance.[53]

1.3.1.1 The skin bacteriome

Around 1000 species of bacteria from nineteen phyla inhabit the human skin.[54] Most colonize the uppermost layers of the epidermis and parts of hair follicles. These microorganisms are usually harmless non-pathogenic or even commensal and mutualistic inhabitants. Beneficial bacteria can help prevent transient pathogenic microorganisms from colonizing the skin by active secretion of chemicals, competition for nutrients or the stimulation of the immune system. However, some members of the skin microbiota are potential pathogens which can cause life-threatening illness, when their numbers rise above typical limits, or they get into generally sterile areas (such as the blood or the peritoneal cavity). Nevertheless, these potential pathogens are counted as members of the normal human microbiome.[55]

For a long time *Staphylococcus epidermidis* and *Staphylococcus aureus* were thought to be the dominant species of the skin, but 16S rRNA gene research found that although these species are common they only make up 5% of skin bacteria.[54] However, skin diversity provides a rich and diverse environment for bacteria. Most of which come from four phyla: *Actinobacteria* (52%), *Firmicutes* (24%), *Proteobacteria* (16%), and *Bacteroidetes* (6%). A study from 2009 showed that the spread and composition of the skin microbiota also differs regarding whether the skin area is oily, moist or dry. Further there are also areas of the skin that have similar and less similar microbiotas between people. Areas with least

similarity between individuals were the spaces between fingers, the spaces between toes, axillae, and umbilical cord stump. Most similarly were beside the nostril, nares (inside the nostril), and on the back.[54]

1.3.1.2 The gut microbiome

The human gut microbiota is a well-investigated part of the human microbiota. It has crucial functions and is linked to several diseases and disorders. Compared to other parts of the human microbiota, the gut microbiota has the greatest numbers of bacteria and the highest number of species.[56] The configuration of the gut microbiota changes over time, due to diet changes, and general health changes.[18,56] Furthermore, each anatomical region has its own distinctive microbial composition. The stomach and small intestine in general harbor less species of bacteria, while the colon contains a much more divers and higher populated ecosystem, which is represented by up to 1000 different species.[57,58] However, 99% of the bacteria are embodied by only 30 or 40 species.[59] Most people seem to share a small number of core species, while the overall composition of microbes can be very different between individuals.[60] Within an individual, the gut microbiota mostly stays constant, although some changes can occur with changes in lifestyle, diet or age.[57,61]

The four central bacterial phyla of the gut microbiota are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*.[62] The dominant bacterial genera are *Bacteroides*, *Clostridium*, *Faecalibacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus,* and *Bifidobacterium*.[57,59]

1.3.1.3 Vaginal microbiota

The vaginal microbiota was first discovered by the German gynecologist Albert Döderlein in 1892.[63] It is a crucial part of the overall human microbiota, due to the significant impact it has on women's overall health.[64] The main inhabiting bacteria of a healthy individual are of the genus *Lactobacillus*.[65] These species produce lactic acid, which is thought to protect the female host from infection by pathogens.[66] However, several studies have proven that

a noteworthy proportion (7–33%) of healthy asymptomatic women lack considerable numbers of *Lactobacillus* species in the vagina,[67,68] instead harbor a vaginal microbiota consisting of other lactic acid-producing bacteria, i.e. species from the genera *Atopobium*, *Leptotrichia*, *Leuconostoc, Megasphaera, Pediococcus, Streptococcus* and *Weissella*.[68-70] Other bacterial species frequently found in the vagina are Gram positive cocci: *Atopobium vaginae, Peptostreptococcus spp., Staphylococcus spp., Streptococcus spp., and Bacteroides spp., Fusobacterium spp., Gardnerella vaginalis, Mobiluncus, Prevotella spp.,* and Gram-negative enteric organisms, such as *Escherichia coli., Mycoplasma* and *Ureaplasma*.[71,72]

1.3.2 Archaea

Archaea are found in the human gut, but the numbers of species are much lower compared to the great variety of bacteria in this body site. The most abundant group are the methanogens, represented by *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*.[73] Besides the gut archaea can be found in the vagina, oral cavity (tooth microbiota) the navel, skin.[74] Currently there is no clear evidence for pathogenicity of any archaean species, although this has been suggested many times.[75] However, a correlation between disease and the number of methanogenic archaea was detected: the severity of a periodontitis correlated with the amount of present archaea in (inflamed) gums. There are similar findings for colorectal cancer and diverticulosis. Still, these archaea only contribute indirectly to the disease by helping the growth of actually pathogenic bacteria - the archaea are not.[75]

1.3.3 Fungi

Most common form of human-associated fungi are yeasts in the human gut.[76-78] Because of its pathogenic ability in immunocompromised patients *Candida* is a well-known species.[79] However, yeasts are also commonly found on the skin, where they live in the uppermost layer of the epidermis (stratum corneum). Fungal skin infections are caused by

yeasts such as *Candida* or *Malassezia furfur* or dermatophytes like *Epidermophyton*, *Microsporum* and *Trichophyton*.[80,81]

1.3.4 Viruses

Viruses, in particular bacterial viruses (bacteriophages), inhabit several body sites which include the oral cavity,[82] gut,[83] lungs[84] and the skin.[85] They have been associated with periodontal disease,[86] HIV-AIDS,[87] and inflammatory bowel disease.[88]

1.4 Tools in microbiome research

1.4.1 16S rRNA gene sequencing

While conventional sequencing begins with a culture of identical cells as a source of DNA, metagenomic analyses take DNA samples directly from the environment. A lot of interest in metagenomics comes from the findings that showed that an immense part of microorganisms present had previously gone undetected. Studies often focus on specific marker genes like the 16S rRNA, 23S rRNA, rpoB or gyrB.[89] The 16S rRNA is the main molecular component of the small subunit of the prokaryotic ribosomes (Figure 2).[90] The encoding gene is located in the ribosomal DNA (16S rDNA) and comprises of approximately 1550 base pairs.[91] This gene contains both highly conserved, identical in almost all known bacteria, and variable segments, each characteristic for a specific cluster of bacteria. Sequencing of the 16S rDNA region allows identification and classification of bacteria and archaea.[92] There are different primers available to amplify different regions of

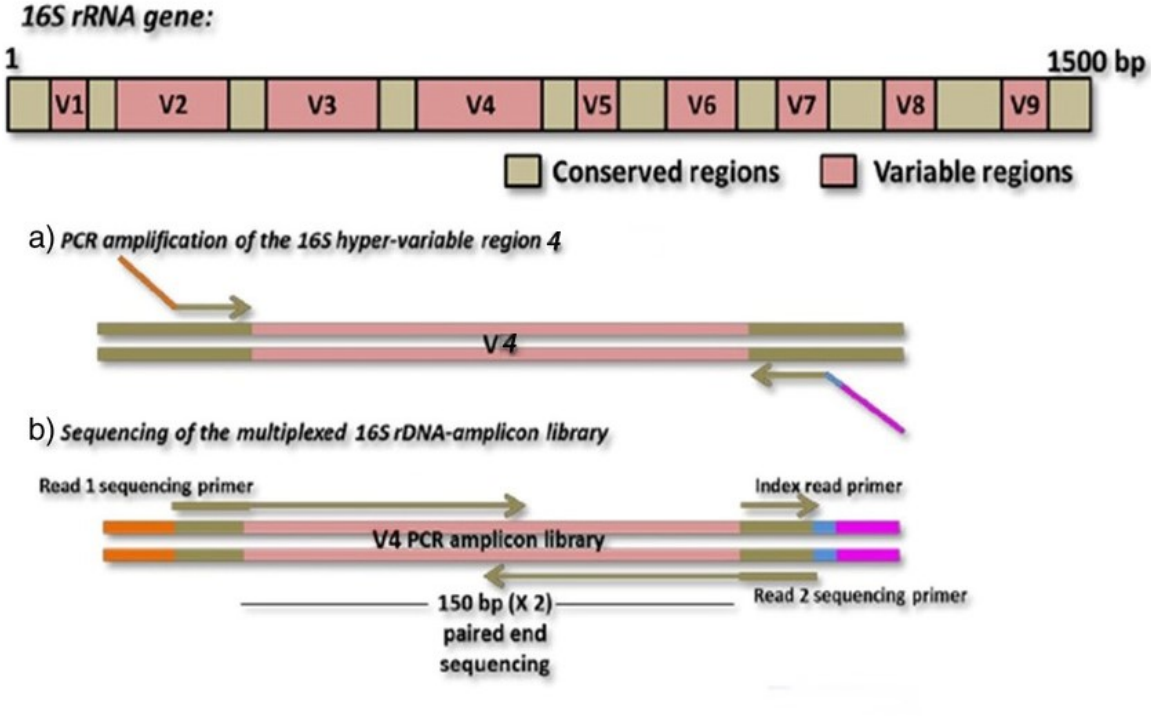

Figure 2 16S rRNA Sequencing

the 16S rRNA gene via PCR. To identify the microorganisms, sequencing results need to be compared to databases, which store a large number of sequences.[93] Recent publication reports that 16S rRNA gene sequencing of the V4 region was highly successful at family level (99.71% correctly classified). At genus level 48.18% were correctly classified, while 51.79% were reported to be unclassified and misclassification was at 0.03%.[94] Difficulties with the exact identification of microorganisms using 16S rRNA gene sequencing occur for two main reasons: inaccurate or not enough stored sequences in databases, and some species have similar or identical nucleotide sequences in regions of interest.[94]

Nevertheless, this method has proven to be useful for the investigation and detection of rarely isolated or insufficient described bacteria. 16S rRNA gene sequencing has been used in important projects like The Earth Microbiome Project and The Human Microbiome Project.[95,96] There have been found many 16s rRNA sequences which belonged to species

that have not been described yet, indicating that there are still lots of non-described organisms.

Originally Norman R. Pace and colleagues began to work in this field by using PCR to explore the diversity of rRNA sequences.[97] The understandings gained from these studies led Pace et al. to suggest the idea of replicating DNA directly from environmental samples in 1985. In 1991 the first isolation and cloning of bulk DNA from an environmental sample was published by Pace and colleagues.[98] Extensive efforts guaranteed that these were not PCR false positives and confirmed the existence of a variety of previously unknown species. In 1995 Healy reported the metagenomic isolation of functional genes from "zoolibraries" created from a culture of environmental organisms grown in the laboratory on dried grasses.[99] Soon after that Edward DeLong et al. began with the construction of environmental phylogeny libraries based on signature 16S sequences, gained from marine samples.[100]

Subsequent studies showed that the clear majority of microorganisms had previously gone unnoticed. For example, Mya Breitbart and colleagues showed that 200 litres of seawater contain over 5000 different viruses by using environmental shotgun sequencing, in 2002.[101] Gene Tyson and colleagues extracted and sequenced DNA from an acid mine drainage system. They found complete, or nearly complete, genomes for a few bacteria and archaea that were previously uncultivable.[102]

In 2005 the first sequences of an environmental sample, found with high-throughput sequencing (massively parallel pyrosequencing developed by 454 Life Sciences), were published by Schuster et al.[103] The use of next-generation sequencing technologies to characterize microbiomes of outdoor and built environments is revealing new insight regarding microbial colonization and distribution. These methodologies allow extensive screening of microorganisms, including those that are uncultivable in laboratory conditions, in less time than before and quickened progress in this field.

1.4.2 Single cell sequencing

Single cell sequencing examines the sequence information from individual cells, delivering precise information about the function of an individual cell and its microenvironment.[104] Currently there are more than 100 known methods of single cell sequencing.[105] Microbiomes are one of the main targets of single cell genomics because of the difficulties in culturing many microorganisms in most environments. Single cell sequencing is a powerful tool to gain microbial genome sequences without the struggle of cultivation. For example, single-cell transcriptomics allow to find genetical cell-to-cell variability and epigenetic modifications in a population of the same microbial species. This helps to understand the populations rapid adaption to environmental changes.[106] Apart from microbial research, single cell RNA-Seq is becoming broadly used across biomedical research including neurology[107], oncology[108], autoimmune disease[109], and infectious disease[110].

Similar to other NGS research, single cell sequencing commonly needs the following steps: isolation of a single cell, extraction and amplification of nucleic acid, sequencing and bioinformatic data analysis. But the performance of single cell sequencing is comparably more challenging. Difficulties arise from the small amount of starting material, which make degradation, sample loss and contamination more likely. Also due to the heavy amplification needed, uneven coverage, noise and inaccurate quantification may occur.[111]

1.4.3 epicPCR

In 2015 Manu Tamminen and Eric Alm from the Massachusetts Institute of Technology presented a new method called epicPCR (Emulsion, Paired Isolation, and Concatenation PCR). This high-throughput technique allows to link gene function and phylogenetic markers in uncultured single cells at comparably low cost. [112] As interactions between microbial cells are difficult to investigate in single-cell resolution, epicPCR can help to find the complete interaction network in a specific environment.[113] For example epicPCR was used to examine Wastewater treatment plants (WWTPs) as sources of antibiotic resistant

bacteria. Hultman, Tamminen et al. utilized epicPCR to detect antibiotic resistance genes in bacterial hosts. Four resistance-associated genes were found in influent and effluent. The bacterial carriers of these genes were different in influent and effluent, with a generally decreasing variety in the effluent.[114]

1.4.4 Cultivation and the "microbial dark matter"

Microbial cultures are elementary diagnostic and research methods in molecular biology and medicine. There are many different types of cultures available - general categories are broth cultures, agar plates, solid plate culture or stab cultures. The selection of the right cultivation method depends on the targeted organism(s), their growing condition and downstream use.[115] Further it is often necessary to isolate a certain microorganism in a pure culture, meaning that cells can grow in absence of other species or types. These cells can be genetic clones if they originate from a single cell or single organism.

But as already mentioned, only a small part of all known microorganisms is culturable with standard techniques. This is partially due to lack of knowledge or ability to maintain required growth conditions. This massive part of unculturable microorganisms is sometimes called the "microbial dark matter" because of the difficulty in studying these microorganisms.[116] Although recent advances in cultivation methods made it possible to increase microbial recovery to 300-fold, the greatest part of environmental microorganisms remain undescribed and uncultured.[117,118]

1.5 Aims of this project

As explained above, the microbiota of indoor environments is just beginning to be described and explored in its full extent. Especially the knowledge about hospital microbiota and its interaction impact on humans is not fully understood yet, although it might have major implications for hospital hygiene. However, as more knowledge emerges about the indoor microbiota, the question arises as to whether this knowledge might be useful for us. There are still several open questions in this field. Which part of the found

organisms is biologically active and alive? Can they encourage disease, or do they have a role in protecting us from it? Are they dynamically adapting to our efforts to control or reduce them? How can built environments, in this case hospitals, be optimized by taking into consideration what we already know?

This study is part of a bigger project, where samples from several wards of the same building, including the new ICU ward, were taken and partially also analysed in the same manner as described in this thesis. The goal is to describe the hospitals microbiota in general and to find possible differences between areas, especially in regard to how frequently certain areas are touched. It is assumed that the microbiome of the hospital is mostly influenced by human-associated microorganisms and that the microbiome is significantly different in certain types of locations. Three different sizes of patient rooms were chosen, because it was assumed that the number of patients in a room might influence the diversity of its microbiota. Due to the possibility of this new opening of a hospital department, not only can the “mature” microbiota of a running hospital ward be observed and described, but possibly also the genesis of this special microbial community.

All these findings may help to modify hygiene and cleaning routines in a positive way or help to improve future construction of public buildings, especially hospitals in regard to preventing pathogen transmission.

2 MATERIAL AND METHODS

2.1 Sampling design

The construction of the new department of Surgery at the Country Hospital Graz (Landeskrankenhaus Graz) and the Medical University of Graz began in July 2013. A graphic representation of the building complex is visible in Figure 3. Staff and patients began to move in starting on 7 June 2017. A total of 273 beds are distributed in several department - 190 beds in general wards, 25 beds in IMC, 40 beds in the ICU wards and 18 in the day clinic.[119] For this study, rooms of the General Surgery ward on the fifth floor were studied.

Before sampling several different sites were chosen inside the new patient rooms of the General Surgery's ward floor (see Table 1). These sites stayed the same over the course of the whole study and are found in all three of the selected patient rooms on this floor. One

Figure 3 The newly built Department of Surgery

room is a single-bed-room, another is a two-bed-room and the third is a four-bed-room**.** Although the number of beds is different, every room has the same equipment and standardised furnishing (see Figure 4 A representative patient room, in this case a 2-bed-room (second bed not visible).). These three rooms are marked on the floor plan (Figure 5).

Four different forms of sampling were used: Sampling with untreated swabs, PMA-treated swabs, agar plates and wipes. In Table 1 the forms of sampling for each location is visible.

Most of the chosen nine sampling locations are being frequently touched, others are places on which dust supposedly accumulates and which are more rarely touched by staff or patients. Also, the floor under patients' beds was chosen as a sampling location. Altogether a number of 293 samples and controls was taken over the period of one year.

Figure 4 A representative patient room, in this case a 2-bed-room (second bed not visible).

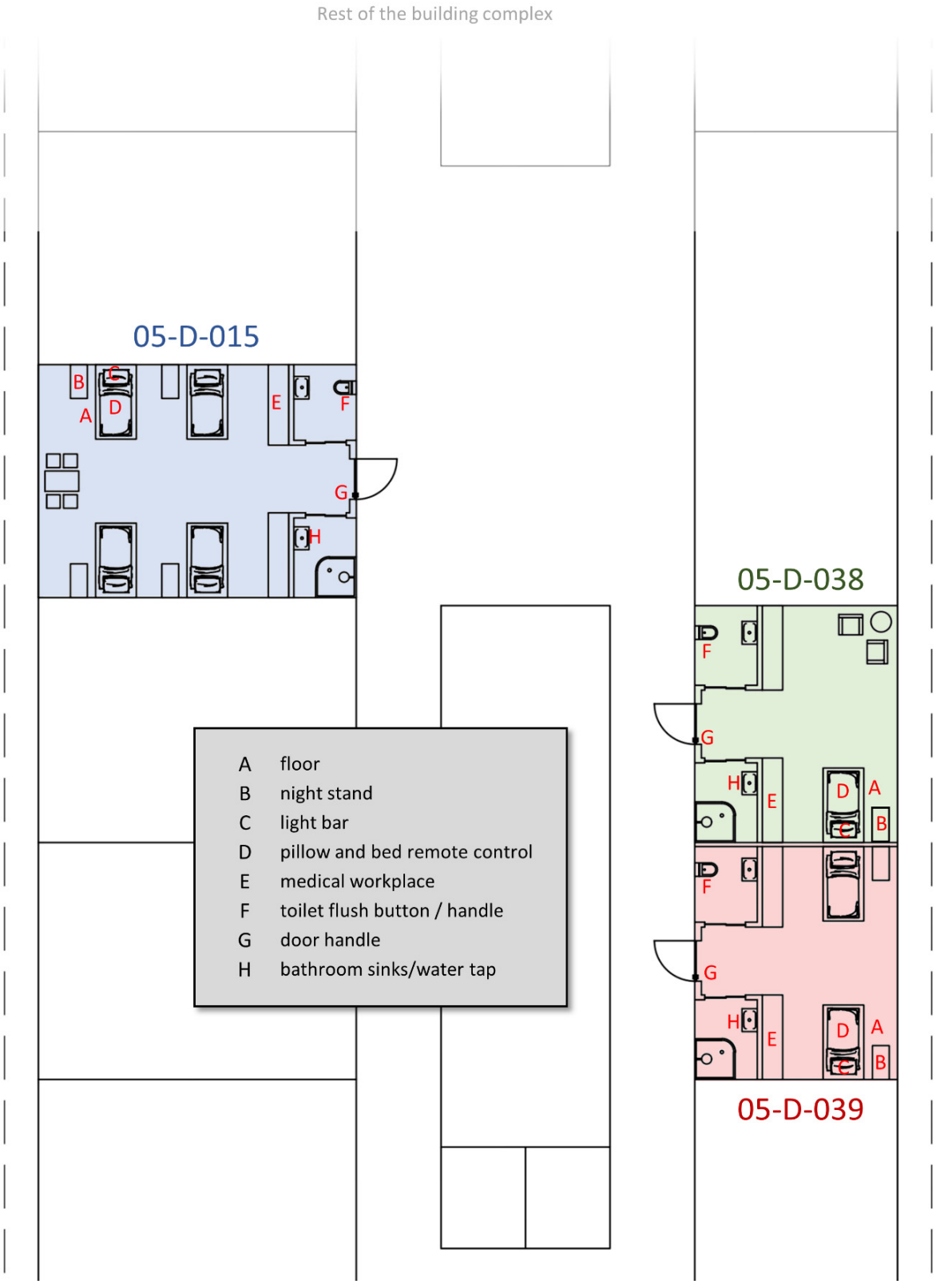

Figure 5 Simplified floor plan. The three sampled rooms are highlighted in colour. A-H indicate the sampling locations.

In total the rooms were sampled seven times over the period of one year. First sampling (t0) took place one day before the new building was officially opened and staff and patients moved in on 7 June 2017. The next sampling (t1) underwent one day after the room was first occupied. The following samplings took place at intervals of one week (t2), four weeks (t3), 12 weeks (t4), 26 weeks (t5) and 52 weeks / one year (t6) after moving. Also, one corresponding set of samples were taken from the old building.

Due to unforeseeable circumstances sometimes, rooms were not accessible, or the sampling could not be done in the same day for other reasons. In these cases, the sampling was caught up as soon as possible. The exact dates of sampling can be seen in the List of Samples in the Appendix on page 76).

Table 1 Forms of sampling for each sampling location

Sampling location	**Swab**	**PMA Swab**	**DCT**	**Wipe**
lightbar above beds	X	X	X	
medical workplaces	X		X	
bathroom sinks	X	X	X	
pillows	X		X	
water taps	X		X	
door handles	X		X	
bed remote controls	X	X	X	
toilet flush buttons / handles	X	X	X	
Floor under bed			X	X

For the 16S rRNA investigation all surface samples (except for floor samples, see below) were taken with sterile nylon-flocked swabs (Swabs: BD BBL CultureSwab EZ, Collection

& Transport System Copan FLOQSwab 552C, COPAN, Italy) from the following surfaces from a 5 × 5 cm area: lightbar above beds, medical workplaces, bathroom sinks and frequently touched items such as pillows, water taps, door handles, remote controls and toilet flush buttons or handles. Samples from the lightbars, sinks, toilet and remote controls were taken twice. The second sample was taken with the same type of swab but treated with PMA (see page 33). As the toilet button itself was not large enough to take all samples, the toilet handle located in the same area was chosen for PMA samples from the toilet area.

Samples were taken and processed independently, including field blanks and laboratory extraction controls. All samples were transported to laboratory on ice and were immediately frozen at -80°C until processing. PMA samples were frozen after treatment.

In addition, all sampling areas were sampled with CASO agar plates (Heipha Diagnostika, Eppelheim, Germany). These were given to the Institut für Krankenhaushygiene und Mikrobiologie for cultivation. 139 direct contact tests (DCT) were performed. Samples were taken on predefined sites and incubated at room temperature for 4 days. Then colony forming units (CFU) were counted.

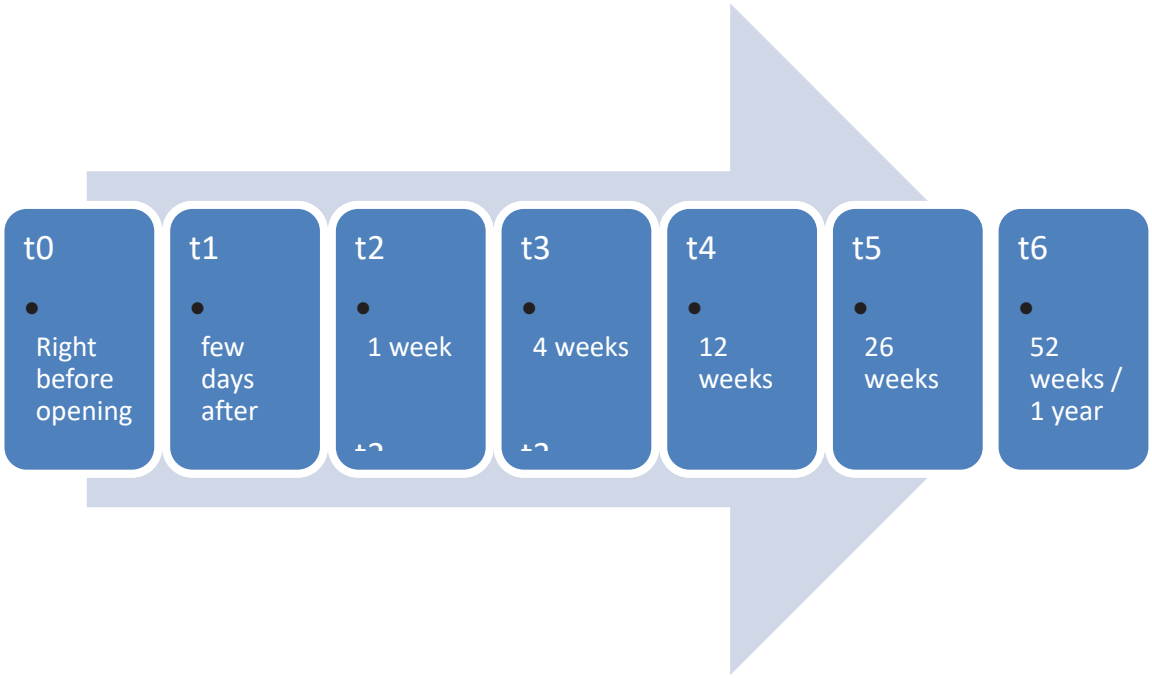

Figure 6 Timeline of sampling

2.2 Propidium monoazide treatment

This treatment was used to distinguish between DNA from dead cells or cell fragments and viable, intact cells. Propidium monoazide (PMA) is a high-affinity photoreactive DNA-binding dye that favourably binds to dsDNA with high affinity. The dye is faintly fluorescent by itself but becomes extra fluorescent after binding to DNA. Visible light induces a photoreaction that will cause a covalent bond of PMA and dsDNA, resulting in permanent DNA modification. Because the dye is cell membrane-impermeable, it only binds to exposed DNA from cell fragments or dead cells, while it leaves DNA from intact cells untouched. This allows to obtain only amplified DNA of living or intact cells.[120,121]

Samples from the lightbars, sinks, toilet flush buttons and remote controls were taken in doubles and additionally treated with a 50µM solution of PMA.

2.3 Floor samples

For the 16S rRNA gene investigation, floor samples were taken with sterile gloves (Kimtech Pure G3 Sterling Nitril-Gloves, Kimberly-Clark Professional, Koblenz, Germany) from under patients' beds using gamma sterilised bonded polyester cleanroom wipes (TX3211; ITW Texwipe, Kernesville, NC, USA). Before sampling, wipes were baked at 170 °C for 24 h (to destroy possible remnants of DNA), then pre-moistened with 15 ml sterile water (microfiltered at 0.2 µm; LiChrosolv, Merck Millipore), and finally autoclaved in sterile and pyrogen-free PP tubes (Sarstedt, Germany). For each new wipe sample, a fresh pair of sterile gloves was used.

During sampling, the wipe was placed flat on the floor and rubbed over the entire surface of a square meter of floor using a firm, steady pressure. This area of floor was wiped three times overall, rotating the direction of motion first 90° and then 135°. For the field negative control samples, a sterile sampling wipe was removed from its sterile and pyrogen-free PP tube, the wipe was opened and placed back into the tube. In laboratory, extraction blank samples were used to control the sterility of reagents and equipment. Controls were processed and analysed in the same way as samples.

In the laboratory floor samples were filtered and concentrated before DNA extraction by using Amicon™ Ultra-15 filters as follows:

The thawed floor samples on wipes were placed in DNA free Erlenmeyer bottles (baked in 250°C for 24 hours) filled with 80 ml of PCR grade water (microfiltered at 0.2 µm; LiChrosolv, Merck Millipore). They were shaken strongly for one minute by hand and then placed in an ultrasonic bath with a maximal power of 240 W and a frequency of 40 kHz for two minutes. The liquid was then poured on UV sterilised Amicon™ Ultra-15 filters, which were UV-treated for one hour, and centrifuged at 4000 rpm at 4°C for 5 min to concentrate the samples. This step was repeated until the whole sample was filtered and concentrated to about 200µl.

2.4 DNA extraction

The DNA from the collected samples was extracted in a using the "DNeasy® PowerSoil® Kit" by QIAGEN. The instructions of the Quick-Start Protocol were followed except for a few variations included in the description below.

For the DNA extraction 60 µl of Solution C1 were added to the provided PowerBead Tubes. The swab head or liquid sample was placed in the PowerBead Tube and gently vortexed to mix. The PowerBead Tubes were then placed in a MagNA Lyser machine and shaken twice for 30s at 3400 rpm with an incubation of one minute on ice in-between. The tubes were centrifuged at 10,000 x g for 30 s. The supernatant was transferred to a clean 2 ml collection tube. 250µl of Solution C2 were added and tubes were vortexed for 5 s and then incubated at 4°C for 5 min. After that the tubes were centrifuged for 1 min at 10,000 x g. Avoiding the pellet, supernatant was transferred to a clean 2 ml collection tube. 200 µl of Solution C3 were added and tubes were vortexed briefly. Again, the tubes were incubated at 4°C for 5 min. Then the tubes were centrifuged for 1 min at 10,000 x g. Avoiding the pellet, supernatant was transferred to a clean 2 ml collection tube. 1200 µl of Solution C4 were added and tubes were vortexed for 5 s. 675 µl were loaded onto a MB Spin Column and centrifuged at 10,000 x g for 1 min. The flow through was discarded. The last two steps were repeated until all of each sample has been processed. 500 µl of Solution C5 were

added to the column and centrifuged for 30 s at 10,000 x g. Flow through was discarded. Columns were centrifuged again for 1 min at 10,000 x g. The MB Spin Column was placed into a clean 2 ml collection tube. 100 µl of Solution C6 were added to the centre of the white filter membrane. The tubes were then centrifuged at room temperature for 30 s at 10,000 x g. The MB Spin Column was discarded, and the gained extraction was frozen at -80°C.

2.5 Amplification by PCR

For the use of next generation sequencing (NGS; Illumina MiSeq), the variable region V4 of 16S rRNA gene was amplified with "universal" PCR primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using TaKaRa Ex Taq polymerase (Clontech, Japan). The protocol detailed here mainly targets prokaryotes (Bacteria and Archaea). Five microliters of total DNA extract were used for a 25 µl PCR reaction containing 10x Buffer (Roche, Mannheim, Germany), 5 U/µL Enzyme (Roche, Mannheim, Germany), 2.5 mM dNTPs (Roche, Mannheim, Germany), 10µM primers and PCR-grade water (Roche, Mannheim, Germany). Cycling conditions involved an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation 94 °C 45 s, annealing 50 °C 60 s, extension 72 °C 90 s, and a final extension at 72 °C 10 min.[122]

Amplification was verified by using a 3% agarose gel. The target band size was verified at 295bp.

2.6 Sequencing

The gained DNA fragments were sequenced at ZMF Core Facility Molecular Biology in Graz, Austria, using the present Illumina MiSeq platform.

The library preparation was performed by following the manufacturer's instructions on a SequalPrep Normalization Plate (Life Technologies, Vienna, Austria). 15 µl of the

normalized PCR product were used as the template for indexing PCR in a 50 µl single reaction performed as described for targeted PCR to introduce barcode sequences to the samples according to Kozich et al. (2013).[123] Cycling conditions were identical to the targeted PCR with only eight cycles for amplification. Further, 5 µl of each sample were pooled, 50 µl of the unpurified library were loaded to a 1% agarose gel (Sigma–Aldrich, St. Louis, MO, USA), and then purified from the gel with a Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. To quantify the pool, PicoGreen dsDNA reagent (Life Technologies, Vienna, Austria) was used as recommended in manufacturer's instructions. Then it was visualized for size validation on an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) by using a high sensitivity DNA assay following the manufacturer's instructions. The sequencing library pool was than diluted to 4 nM until running on a MiSeqII desktop sequencer (Illumina, Eindhoven, Netherlands). Version 3 600 cycles chemistry (Illumina, Eindhoven, Netherlands) was used as stated by manufacturer's instructions to run the 6 pM library with 20% PhiX (Illumina, Eindhoven, Netherlands). The gained FASTQ files were used for further data analysis described below.

During the processing of the samples, it became clear that 43 of the total 279 samples had less than 1000 reads in sequencing, which is the recommended limit for diversity analysis. One of the reasons for this could be, that the sampled area was to "clean" or poor in microbial life due to e.g. cleaning, and there was simply not much to find at all. This is a known problem of low biomass samples, because of bacterial DNA contamination in extraction and sequencing reagents.[124] This is why extraction kit controls were added to the samples.

A general problem with DNA extraction or further processing could be excluded, since these samples were from different timepoints and were sampled and processed on different days. Most of these samples were PMA samples from the toilet handle, water tap or bed remote. The read limit was therefore set to 500 reads, to include as many samples as possible without negatively influencing the results of diversity analysis.

2.7 Bioinformatic analysis, statistics and visualization

Data processing was performed in Galaxy, which is an open source web-based platform for data processing and analysis using the QIIME2 software.[125] This platform was offered by the Centre for Medical Research (ZMF), Medical University of Graz. Low quality reads were filtered out. Quality trimming, classifying and denoising was performed using "DADA2" software package in QIIME2. DADA (Divisive Amplicon Denoising Algorithm) is a divisive partitioning algorithm, which helps model and correct amplicon errors.[126] Taxonomic classification was done with SILVA rRNA gene database.[127]

To further analyse and visualise the results, Calypso (Version 8.84) was used. Calypso is an online platform for mining, visualisation and comparison of microbial community composition data (cgenome.net/calypso).[128]

Due to the dissimilar number of sequences among samples, the data was normalized before analysis. Following settings were used: Samples with insufficient read count were filtered. Samples with less than 500 sequence reads were removed. Taxa that have less than 0.01 percent relative abundance across all samples were removed. Top 3,000 taxa were included.

Taxa associated with different biological conditions were identified using the linear discriminant analysis (LDA) effect size method (LEfSe). LEfSe determines the taxa most likely to explain differences between classes by coupling standard tests for statistical significance with additional tests encoding biological consistency and effect relevance.[129]

For a part of visualisation Prism software by Graphpad Software, Inc. was used.

The grade of diversity was calculated by using the Shannon index. This commonly used mathematical model for calculating diversity was developed by the American mathematician C.E. Shannon within the framework of information theory. This index measures the diversity within a set of species.[130]

Analysis of variance (ANOVA) is a group of data-analytical statistical methods that allow many different applications. They have in common, that they calculate variances and test variables in order to obtain information about the data. The variance of one or more target variables is explained by the influence of one or more influencing variables. The simplest form of variance analysis is therefore an alternative to the t-test, which is suitable for

comparisons between more than two groups.[131] Here it was used to compare abundancies of different taxa in regard to several different explaining variables.

3 RESULTS

3.1 The hospital microbiome is dominated by human-associated taxa

In total 279 samples including 21 controls were sequenced and analysed. After quality trimming the minimal read count was zero in several samples, the maximal count was 30.224 reads in sample Allg-57 which was sampled in timepoint 1 (t1) from the light bar above the bed in room 05-D-015. The median overall was 7.353 reads and the mean was 8.877,405 reads, with a standard deviation of 7.416,055 reads.

There were 9.595 ribosomal sequence variants (RSVs) found overall. In total 26 different phyla were found. The five most abundant phyla are *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria* and *Bacteroidetes* (Figure 8). Together they represent 86.9% of all taxa found. A small part of taxa was not classifiable on this level.

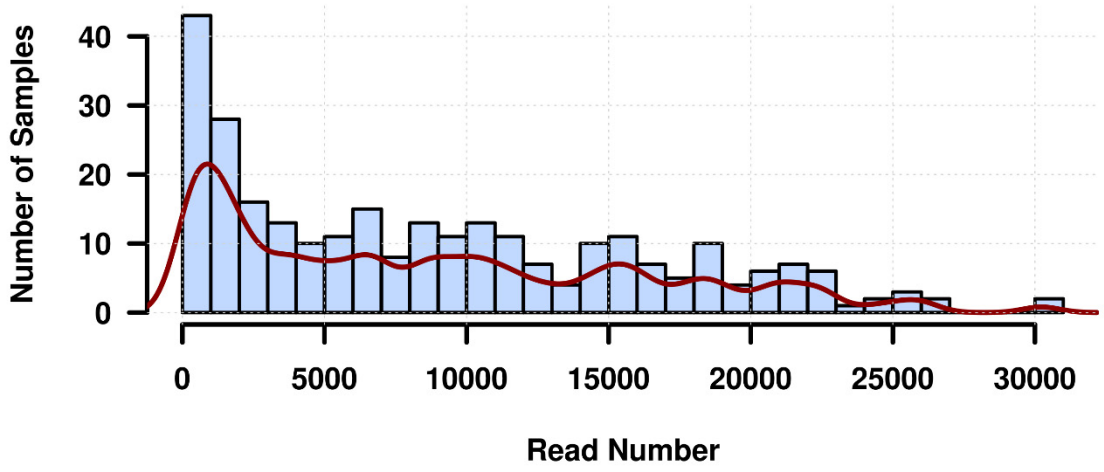

Figure 7 Distribution of reads per sample

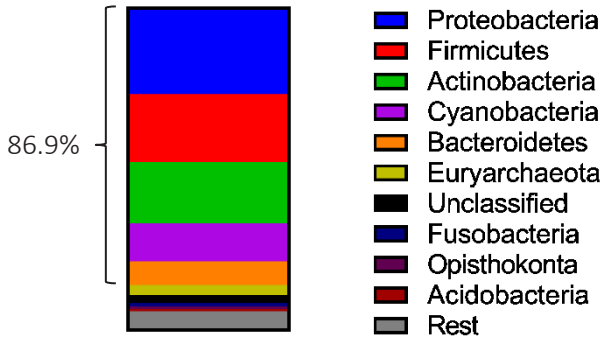

Figure 8 Phyla found in respective to their abundance

14 of 21 controls were contaminated. Non-PMA controls were dominated by unclassified species as well as *Ralstonia* and *Corynebacterium*. PMA-controls had high abundancies of *Ralstonia* and *Methanobrevibacter.* Two Kit controls were contaminated with *Ralstonia* and *Lactobacillus* as well as many unclassified species.

The composition of the overall top ten microbial community on genus level, calculated from all samples, is shown in Figure 9. In many samples a great part of organisms was not classifiable on this level, which is also reflected in the image. The following five genera were most abundant overall: *Corynebacterium*, *Staphylococcus*, S*treptococc*us, *Acinetobacter* and *Ralstionia*. *Corynebacterium* species are Gram-positive and aerobic bacteria which are mostly innocuous and commonly found on human skin.[132] Also commonly found on human skin but facultative anaerobic are *Staphylococcus* species. Although most members of this genus are harmless residents of the skin, one member – *Staphylococcus aureus* - is especially famous for its ability to cause disease in humans and its common antibiotic resistance.[133] Another genus of gram-positive bacteria is *Streptococcus,* which is commonly found in the salivary microbiome. Species of this group are responsible for diverse infections like streptococcal pharyngitis, meningitis, endocarditis, necrotising fasciitis and many more.[134,135] *Acinetobacter* species are typically found in soil, water or sewage, but also on human skin or in the respiratory tract. Some species (e.g. *A. lwoffii, A. baumannii*) can cause hospital acquired infections.[136] The genus *Ralstonia* was previously included in the genus *Pseudomonas* and consists of gram negative bacteria, which include opportunistic pathogens.[137] It can be found in water, soil and plants,

and also in the human oral cavity and respiratory tract. *Ralstonia pickettii* is also known to be a contaminant of high-purity water.[138]

A List of the 50 most abundant genera can be found in the appendix on page 76.

To distinguish living organisms from the total found genomes, PMA was used as described above. In Figure 10 the abundancies of the 19 most abundant genera are shown in comparison between PMA treated and untreated samples. Except for two genera, all show significantly lower abundancies in PMA treated samples. Notably *Methanobrevibacter* and *Methylobacterium* show significantly higher abundancies in PMA treated samples, this probably due to contamination of PMA solution.

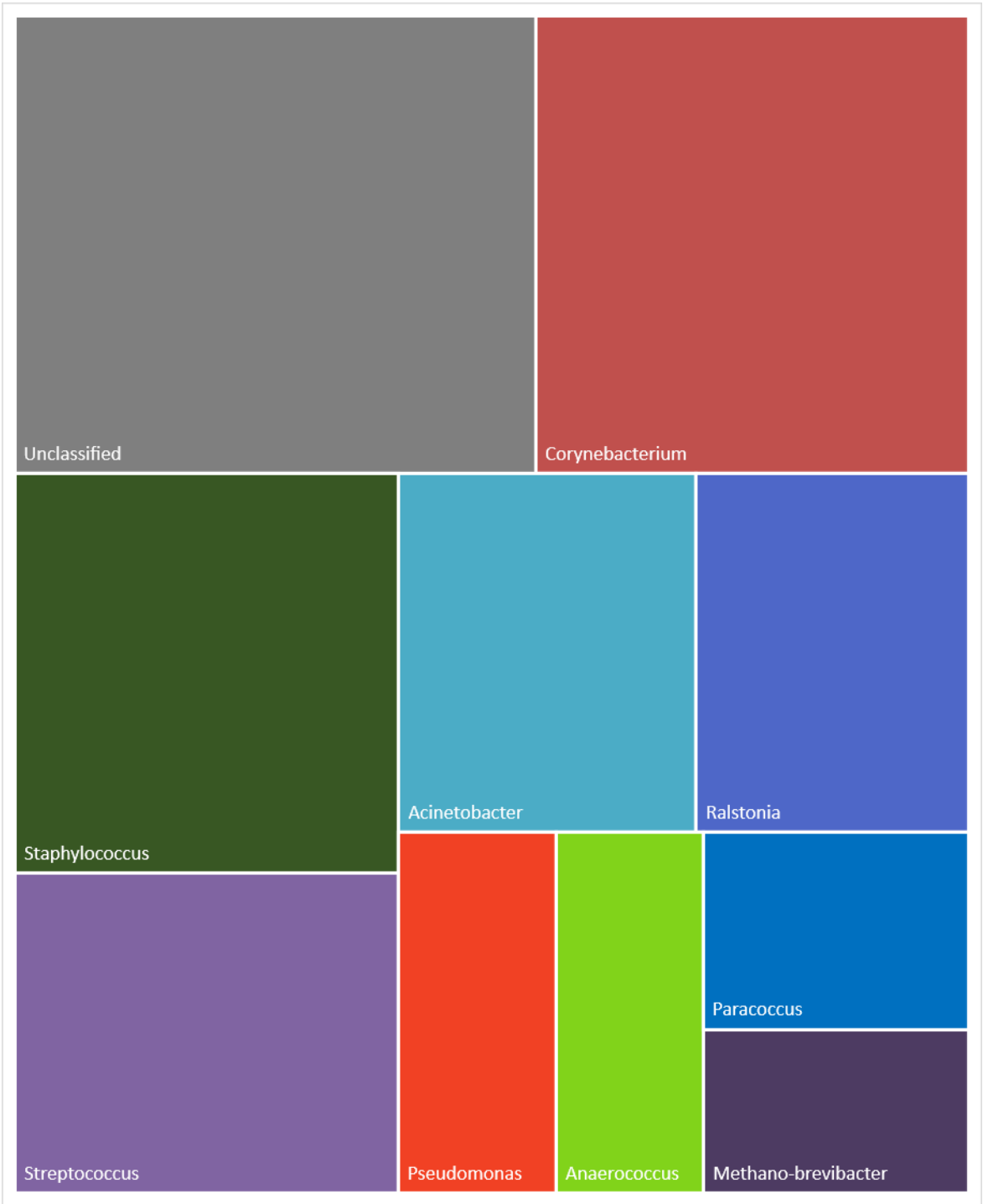

Figure 9 Relative representation of the overall 10 most abundant genera.

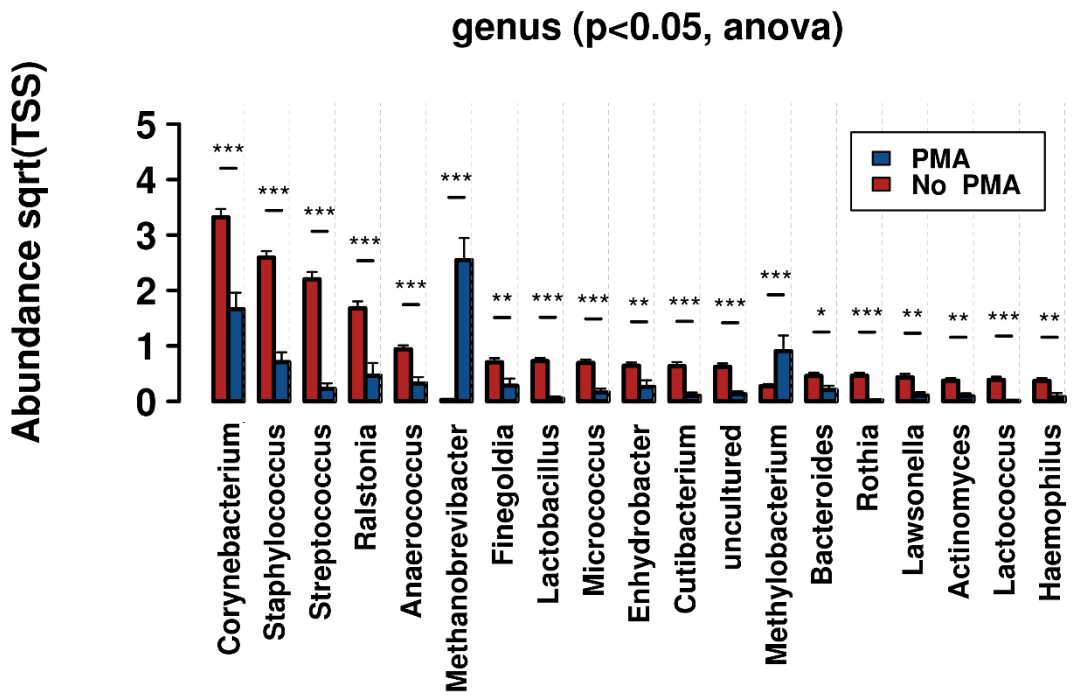

Figure 10 Abundance of the top 19 genera compared in samples that were treated with PMA (blue) versus untreated samples (red). * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001

3.2 Locations harbour different microbiomes

One of the main interests of this project was the composition of microbial communities in different locations inside patient rooms. Figure 11 shows the diversity index for each location on RSV level. Overall floor and door handle samples have the significantly highest diversities compared to other locations, although light bar, medical workplace and nightstand samples partially also have high diversities. Toilet and water tap samples harbour different communities, although these samples both have significantly low richness. Both have *Pseudomonas* as a member of the top ten most abundant genera. Pillow and night stand samples belong to the highly divers samples. These samples are dominated by *Corynebacterium*, *Staphylococcus* and *Streptococcus*, as well as *Ralstonia* and *Acinetobacter*.

Figure 12 shows the composition of the found microbiomes in all chosen locations. A great part of the species found is always unclassified. The 10 most abundant genera make up approx. between 40-60% of the total species found in each location. The bed remotes seem to be dominated by *Corynebacterium* along with *Staphylococcus* and *Streptococcus* which is similar to door handles, although *Acinetobacter* seem to be more abundant here. Floor samples seem to have a different composition. Although *Staphylococcus* and *Acinetobacter* alongside *Corynebacterium* and *Streptococcus* have the highest abundancies in floor samples, which is similar to other locations, several "uncommon" genera are found under the top ten most abundant, such as *Stenotrophomonas* and *Micrococcus*.

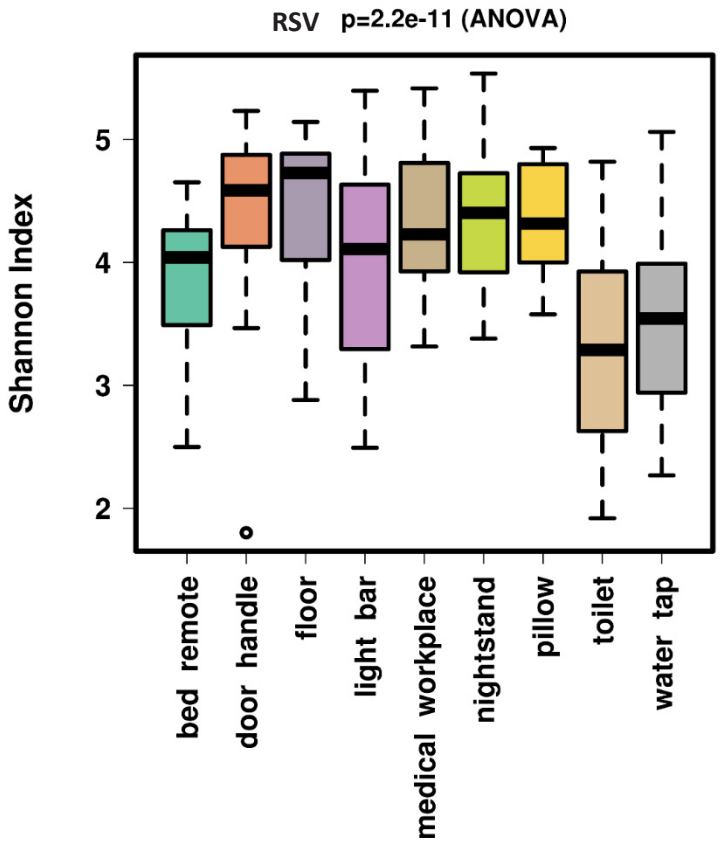

Figure 11 Microbial diversity in all chosen locations on RSV level

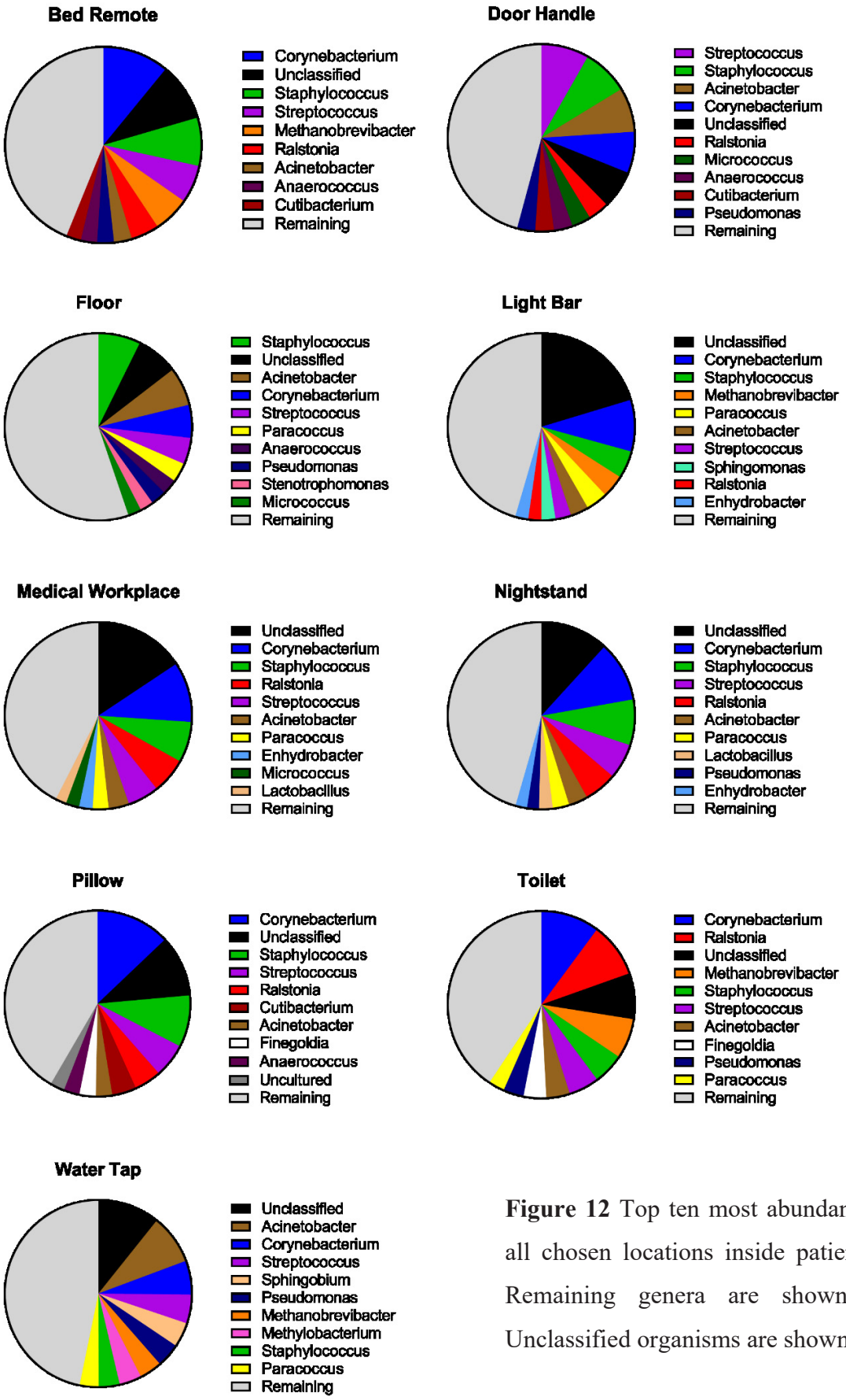

Figure 12 Top ten most abundant genera in all chosen locations inside patients' rooms. Remaining genera are shown in grey. Unclassified organisms are shown in black.

The LEfSe analysis, shown in Figure 13, revealed taxa on genus level which are statistically associated with a certain location. Several taxa including *Rheinheimera, Lactococcus, Gaiella, Atopobium, Rubellimicrobium, Skermanella* and *Dyadobacter* are associated to floor samples. Other locations had less taxa associated: *Stenotrophomonas* and *Blastomonas* are linked to the water tap area, while *Shpingomonas* and *Megashaera* are associated to light bar samples. *Lawsonella*, *Rothia* and *Cloacibacterium* are associated to door handle samples. Only one taxon was found for each of the following locations: toilet, pillow and nightstand.

Further, the chosen locations were assigned to three categories: Frequently touched locations, rarely touched locations and floor samples. Frequently touched locations include

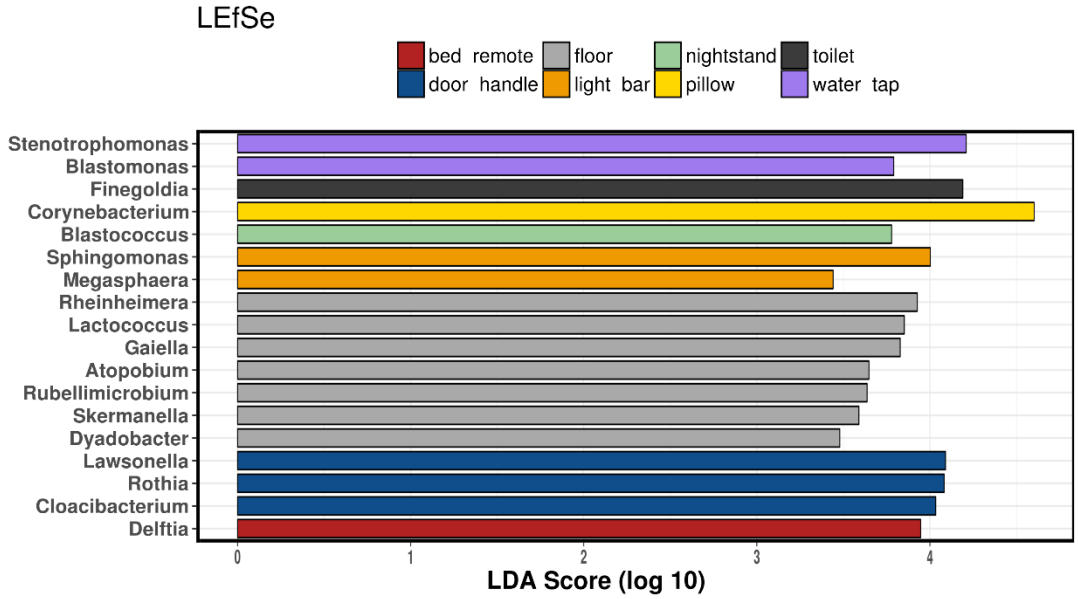

Figure 13 LEfSe analysis of several locations, calculated from the 150 most abundant genera.

toilet, door handle, bed remote, nightstand and pillow. Rarely touched locations include light bar, medical workplace and water tap.

In Figure 14 a network analysis of these three categories is shown. The top 100 most abundant RSVs are clustered by their co-occurrence. There are numerous taxa which are co-occurring in frequently touched areas (green nodes). Four taxa with high abundancy co-occure in both frequently touched areas and floor samples (blue). Several taxa from rarely touched locations also form a cluster (red). Also, a cluster of unassigned taxa formed, which shares no co-occurrences with other categories of samples (black, upper left corner). The

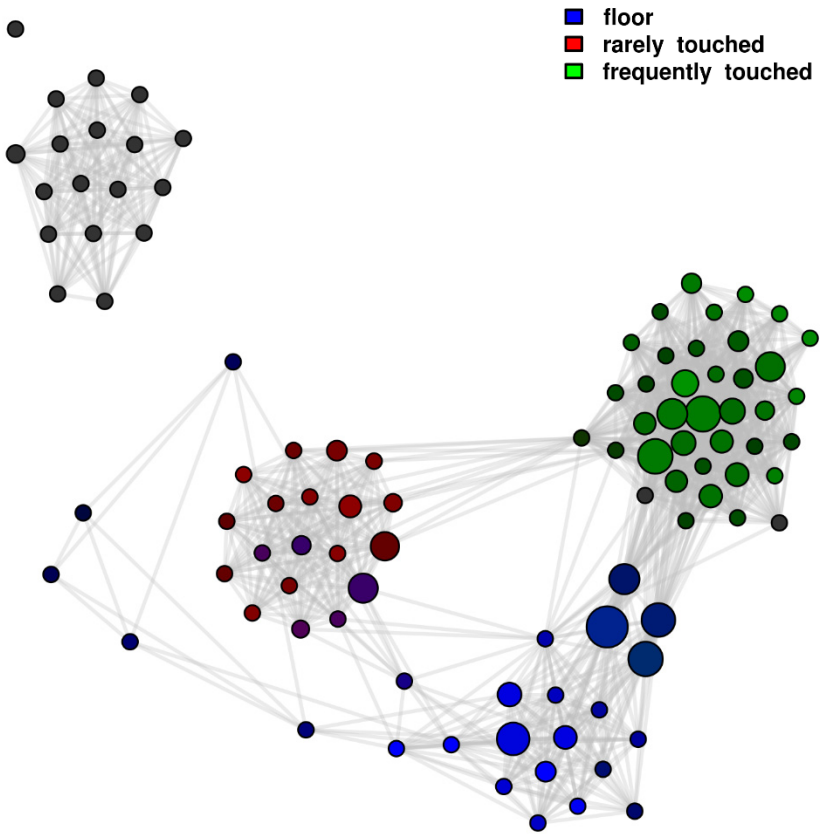

Figure 14 Network analysis of categorised samples. Identification of co-occurring bacteria on RSV level. Taxa are represented as nodes, taxa abundance as node size, and edges represent positive associations. Includes Top 100 most abundant RSVs.

diversity of these three categories was analysed in Figure 15. Floor samples were significantly more diverse than rarely touched and frequently touched areas.

While the diversity in different locations was significantly unalike, the changes in diversity over time, presented in Figure 16 were not significant. The calculated median individual diversities of each time point are not vastly different. However, within one time point the diversity of samples can vary a lot, as is visible for t3.

While the was found no significant difference in general abundance between winter (September-February) and summer (March-August) season, some individual genera were found to be significantly different between seasons (see Figure 17). *Staphylococcus* and *Pseudomonas* species were more abundant in winter samples. Furthermore, numerous unclassified species were found to be more common in summer season.

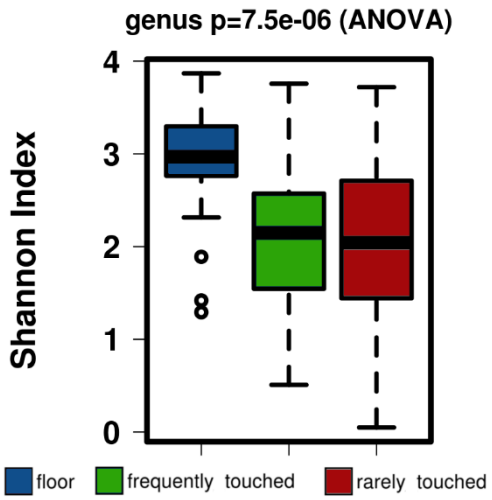

Figure 15 Calculated diversity of categorised samples

3.3 Microbial communities change over time

Figure 16 shows the general diversity of samples in every time point. While there were no significant differences between time points overall, seasonal changes in abundance of *Staphylococcus* and *Pseudomonas* as well as many unclassified species were found (see Figure 17).

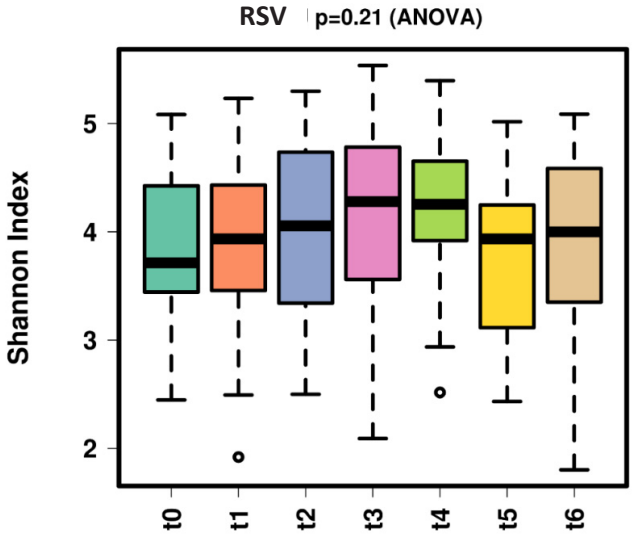

Figure 16 Microbial diversity over the period of one year on RSV level. t0=before opening, t1=day of first occupation, t2=one week after t3=four weeks after, t4= 12 weeks after, t5=26 weeks after, t6= 52 weeks after

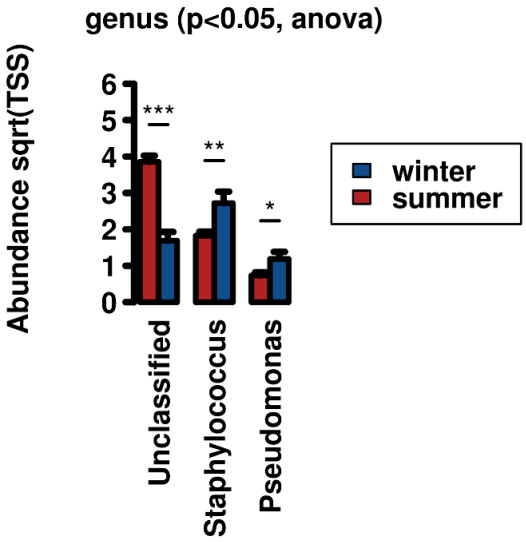

Figure 17 Abundance of genera in comparison between winter (September-February) and summer (March-August) season.

3.3.1 Abundance of selected taxa

Several species change in abundance over time, a few interesting dynamics are shown in Figure 18. A significant difference in abundance of *Pseudomonas aeruginosa* was noticed over time (see Figure 18). *Pseudomonas aeruginosa* first appeared in t1 (day of first occupation) and reappeared in few samples on the following time points. From t5 on there is a significant rise in abundance and number of samples it was detected in.

Other interesting changes are visible also for *Legionella spec., Shigella spec., Lactobacillus delbrueckii* and *Clostridium scindens.* Notably, *Legionella* and *Shigella spec.* are often pathogenic to humans and may cause serious illness, while *Lactobacillus delbrueckii* is not pathogenic (even has been used in the food industry for a long time) and *Clostridium scindens* is a faecal bacterium known to be associated with resistance to *C. difficile* infection.[139]

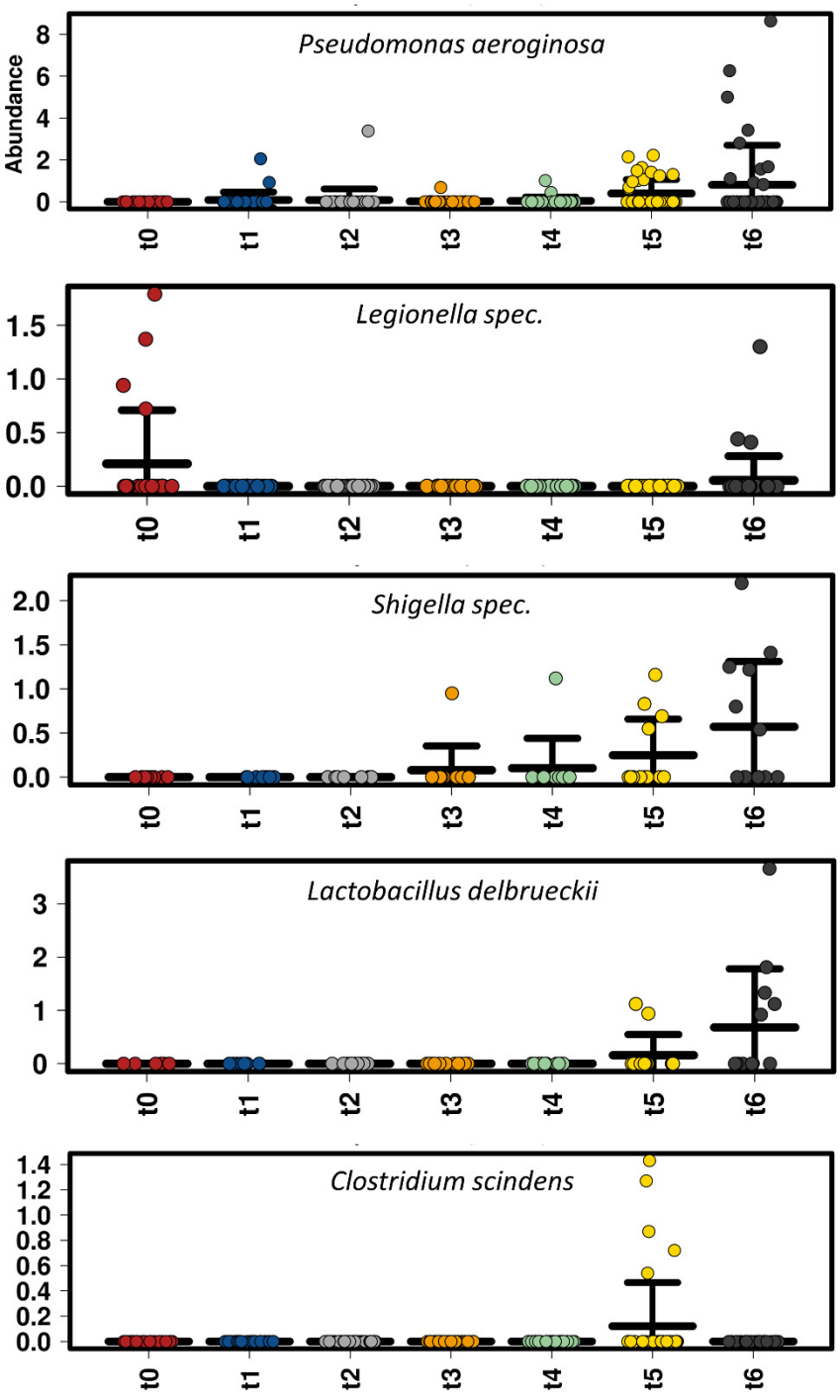

Figure 18 Overall abundance of *Pseudomonas aeruginosa* and other selected taxa over time. Y axis shows abundance. t0=before opening, t1=day of first occupation, t2=one week after t3=four weeks after, t4= 12 weeks after, t5=26 weeks after, t6= 52 weeks after

All three rooms had samples carrying *Pseudomonas aeruginosa* as is visible in Figure 19. Highest abundance was found in room 05-D-038 on t6 (one year after opening). While it was not detected in room 05-D-039 on t6, it was also found in room 05-D-015.

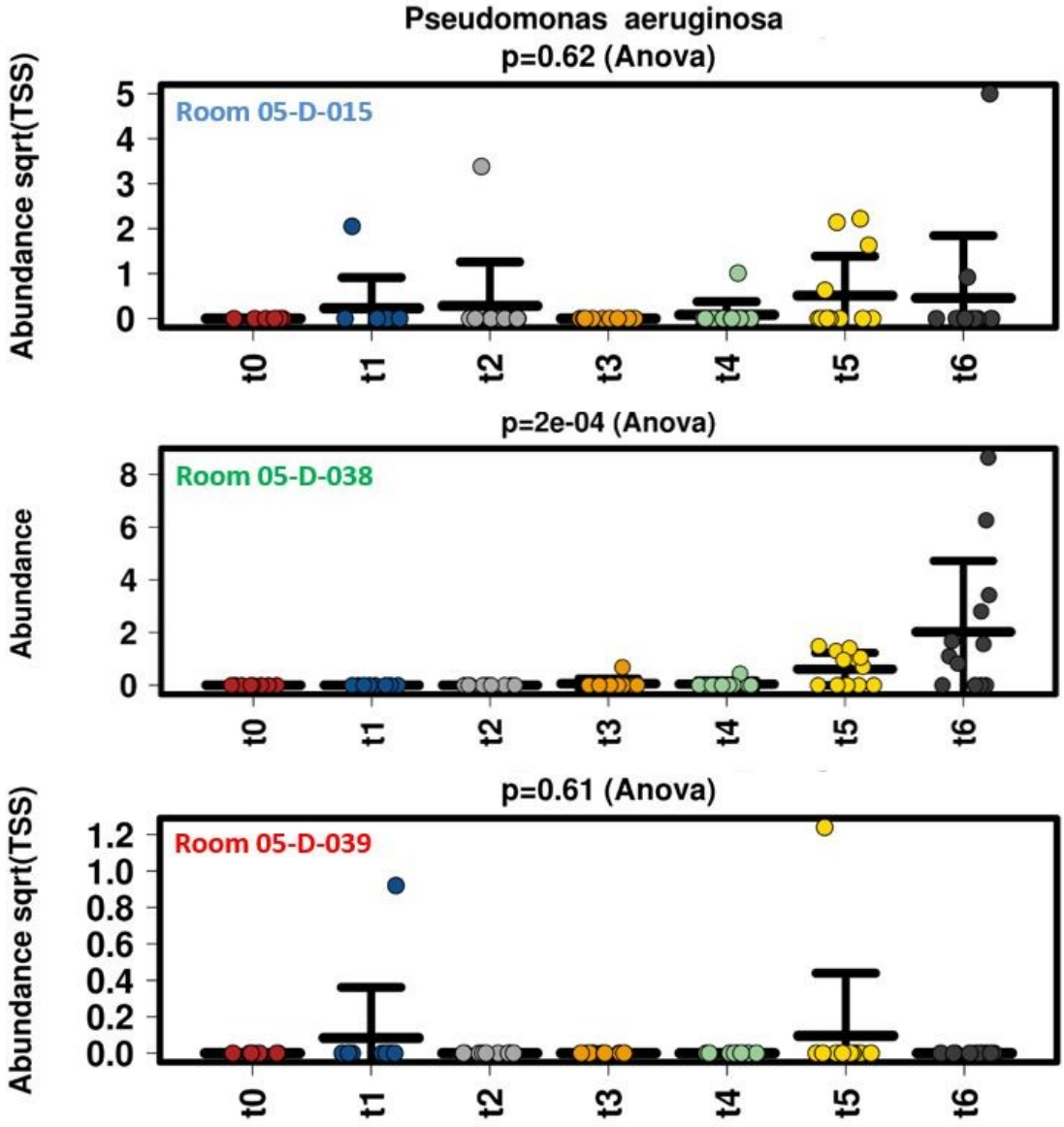

Figure 19 Difference in abundance of *Pseudomonas aeruginosa* shown separately for each room over time. t0=before opening, t1=day of first occupation, t2=one week after t3=four weeks after, t4= 12 weeks after, t5=26 weeks after, t6= 52 weeks after

Room 05-D-038 is used as an isolation room for patients carrying multi resistant bacteria when necessary. Interestingly at sampling of t5 and t6 isolated patients were occupying this room. On t5 the patient was isolated because of a multidrug resistant Gram-negative bacterium resistant to 4 groups of antibiotics (4MRGN) and on t6 a different patient carried a 3MRGN. It is not known whether the bacteria in question were *Pseudomonas aeruginosa,* but this pathogen is known for its common multidrug resistance.

Further inspection of the data revealed the distribution of *Pseudomonas aeruginosa* inside room 05-D-038 (compare Figure 20). Highest abundance was found in individual samples on patients pillow and from the water tap, followed by nightstand and bed remote, but *Pseudomonas aeruginosa* was detectable in almost every location except the toilet.

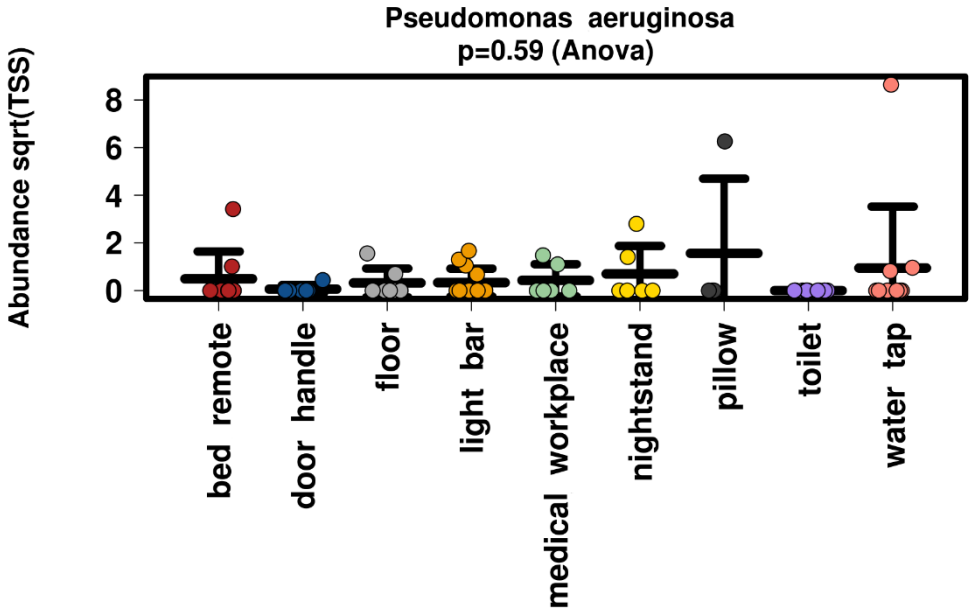

Figure 20 Distribution of *Pseudomonas aeruginosa* in room 05-D-038

3.4 Direct Contact Tests - Cultivation

A total of 139 direct contact tests (DCT) were included. 21 of these were above the safe level of 300 CFU/100cm² (KBE = "Koloniebildende Einheiten" / colony forming units) as is visible in Figure 21. In one sample, taken from a medical workplace, the CFU was not countable, because a thick layer formed rather than individual colonies. In this sample coagulase negative (c.n.) Staphylococci, aerobic spore-formers and *Klebsiella pneumoniae* could be identified. The remaining samples which exceeded the safe level mainly originated from floor and bed remote samples taken on several different days.

In general, three main categories of microorganisms were found in the DCT (see Figure 23). Most samples harboured c.n. staphylococci or a combination of c.n. staphylococci and aerobic sporeformers. In 21 samples molds were identified. 10 DCT carried all three

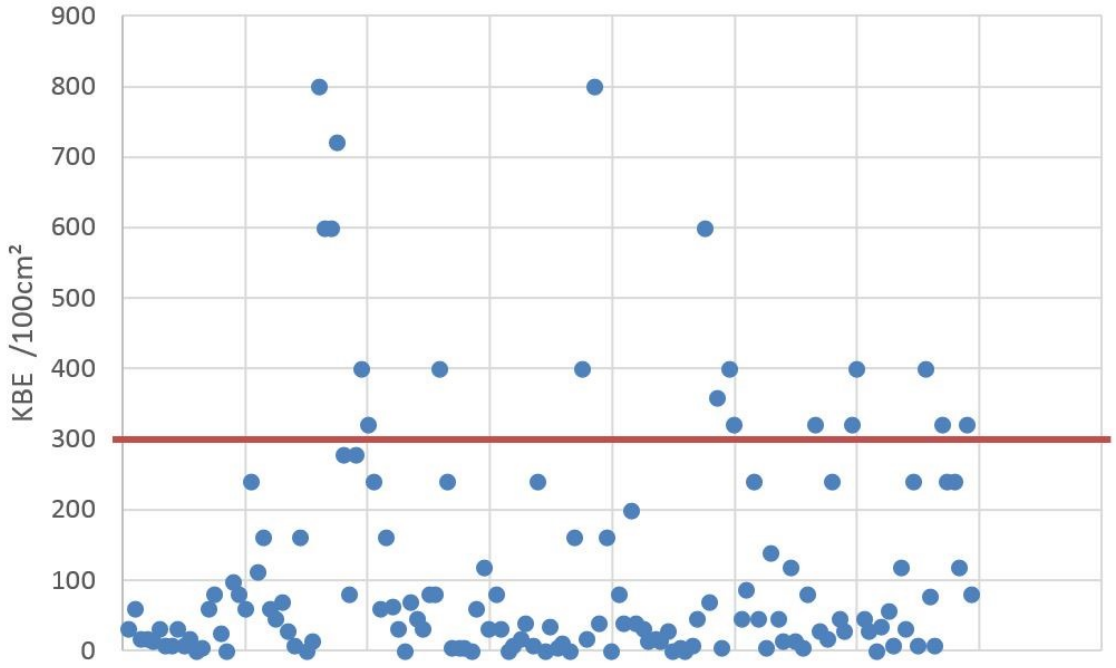

Figure 21 Quantities of CFU /100cm² (KBE = "Koloniebildende Einheiten" / colony forming units) shown for all included DCT. Red bar indicates safe level at 300 KBE /cm²

categories of microorganisms, nevertheless these DCT did not necessarily show high CFU/100cm² numbers. 11 DCT showed no growth at all. These DCT were mainly taken in bathrooms and on medical workplaces.

Apart from the three categories some DCT were more closely analysed, when found necessary. In these 10 DCT the following specific microorganisms were identified: *Cronobacter sakazakii*, *Acinetobacter lwoffii, Klebsiella pneumonie, Pseudomonas aeruginosa, Pseudomonas luteola, Acinetobacter calcoacet. /baumanii comp.* and *Staphylococcus aureus.*

Overall average CFU /100cm² levels categorised by location are shown in Figure 22. Highest average CFU /100cm² levels were found in floor samples, lowest in water tap samples.

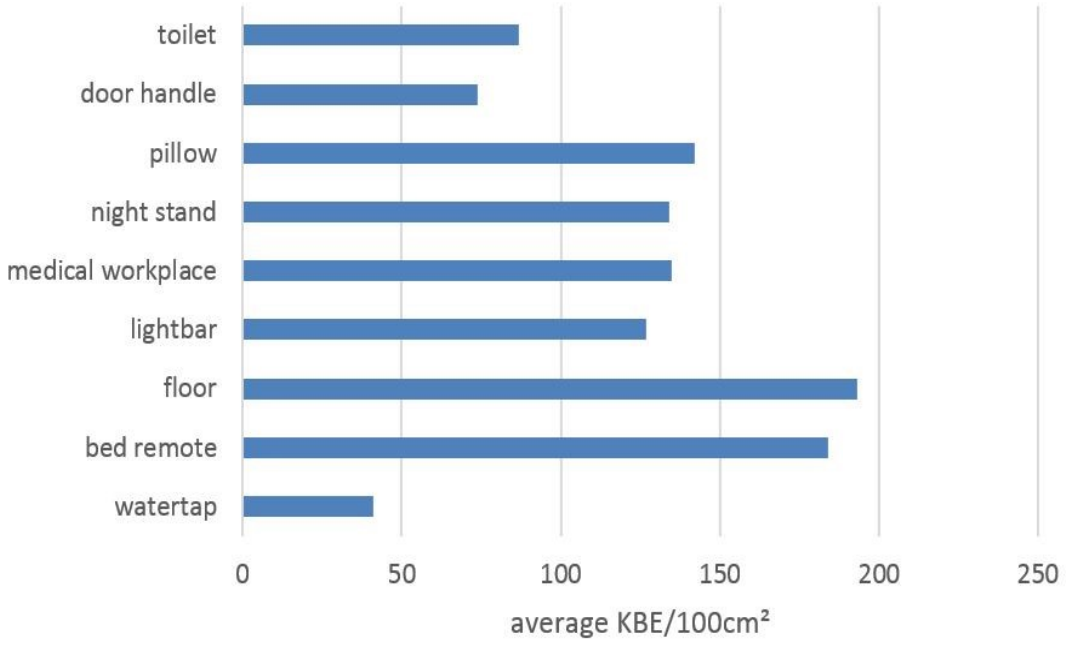

Figure 22 Overall average CFU /100cm² levels of DCT categorised by location

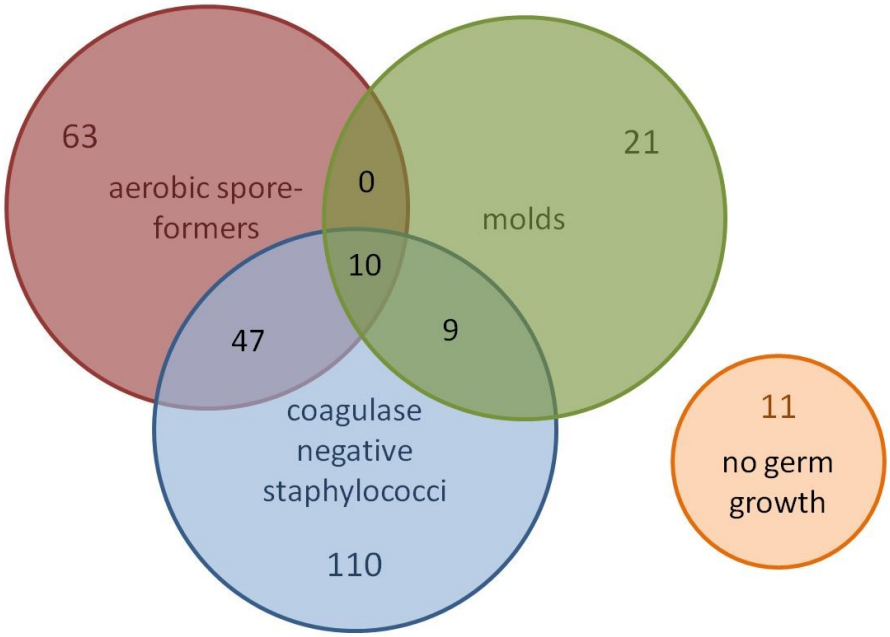

Figure 23 Venn-Diagram showing the distribution of identified groups of microorganisms and DCT with no germ growth.

4 DISCUSSION

The amount of gained data from this (comparatively small) study was very great. The reading and interpretation of this data was accordingly complex and time-consuming. It delivered many results of which minor results were considered to not be relevant enough. Therefore, not all results found in the data are shown in this thesis. The demonstrated results will be further discussed in this chapter.

It is not very surprising that the examined microbiome is generally dominated by human-associated taxa, because other studies already proved that the main sources of the indoor microbiome are the human skin and gut microbiome. A comparable study from a Brazilian hospital found similar abundances of the most common taxa.[140] The hospital microbiome is partially similar to microbiomes of public buildings or homes,[11] but also shows some decisive differences. Several (nosocomial) pathogens were identified. It is also visible that these were probably spread from room to room, although strict isolation measures were taken (see Figure 19). Though the pathogenic species are outnumbered by harmless taxa, in this environment even rarely abundant species might lead to fatal nosocomial infections.

It was also confirmed that the sampling sites carry different communities. The selective pressure here is depending on whether they are frequently touched, regularly cleaned, getting wet and many other factors, that could not always be determined specifically. Among these sites the floor is influenced by many factors and it is therefore not surprising that it has one of the richest microbiotas. The changing of seasons did not have a large impact on the microbiota present. Only few taxa had significantly different abundancies in summer and winter season. So, the effect of changing climatic outdoor conditions on the indoor hospital microbiome is not high, which could be explained by the controlled indoor conditions of the hospital, like steady temperature and humidity.

Interestingly some taxa (like *Corynebacterium*, *Streptococcus*, *Staphylococcus*) are permanent residents of the hospital surfaces, since they were found in almost every sample, while other taxa seem to come and go and be spread from room to room (like *Pseudomonas*

aeruginosa). The microbiota of the hospital is dynamic and constantly changes its composition.

Due to contamination of extraction kit reagents and other used solutions, the results need to be looked at with caution.[141] While some taxa can be easily identified as contaminants (e.g. *Ralstonia*), others, such as *Corynebacterium* and *Staphylococcus*, are more difficult to exclude since they are strongly human associated. *Methylobacterium* has also been identified as a contaminant of DNA extraction kits[141] and was found in two controls. *Methanobraevibacter* was found to exclusively contaminate PMA controls and samples, probably because of contaminated PMA solution.

Many comparable microbiome studies depend on the amplification of regions of the 16S rRNA gene and its sequencing, because it has proven to be an effective method to investigate the microbial communities without the bias or effort of cultivation. However, this technique is known to be limited by short read lengths gained, sequencing errors, variances coming from the different regions chosen, and complications in assessing OTUs or RSVs. Furthermore, there are difficulties in defining bacterial species as well as the restricted resolution of the 16S rRNA gene among closely related species.[142] There are several bioinformatic strategies to reduce influences of these shortcomings, which were also used in this study.[143] But the identification on species level is still limited by the databases used. As is reflected in the results of this study, many found organisms are simply not classified yet.

The data from the DCT cultivation revealed that part of the sampled locations carried microbial life above the safe level. This might be another indication to overthink the current cleaning routine. Also, in comparison to the 16S rRNA gene analysis, it revealed the composition of the microbiota in much smaller depth, since in most samples only three different categories of microorganisms were classified. Only when the examiner saw it fit, further analysis was used to determine exact species, so there might also be a subjectivation bias. Whether more depth in analysis is needed for routine controls of hospital hygiene is needed cannot be answered here, but it might be worth considering to use 16S rRNA gene analysis in certain settings.

The demonstrated results provide new insight in characteristics of the hospital microbiome but also show that more research in this field is needed. The main method of this study

- 16S rRNA gene sequencing - might serve as a strong tool with high resolution for microbiological identification and characterization of clinically relevant bacterial taxa in hospital surroundings and in patients. It might also be particularly useful for the identification and control of nosocomial pathogens and the fight against antibiotic resistance by mapping the AMR genes.

Moreover, we need to rethink the routine cleaning and disinfection methods used in this building and hospitals in general. The results demonstrate that most taxa found are harmless or even beneficial to human health and should not be eliminated by cleaning. Newer studies tried using specified bacteriophages to control nosocomial infection rates and were highly successful.[50,52] This might offer a way to leave harmless or beneficial bacteria in their environment while specifically targeting and reducing harmful species.

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6 APPENDICES

6.1 List of Samples and Controls

Sample name	*Timepoint/Sampling date*	*Room number*	*Location*	*Reads*
Allg-1	t0 (6.6.2017)	05-D-038	Light bar	6565
Allg-2	t0 (6.6.2017)	05-D-038	Light bar PMA	2442
Allg-3	t0 (6.6.2017)	05-D-038	Medical workplace	17090
Allg-5	t0 (6.6.2017)	05-D-038	Water tap PMA	5046
Allg-6	t0 (6.6.2017)	05-D-038	Toilet (button)	11611
Allg-7	t0 (6.6.2017)	05-D-038	Toilet (handle) PMA	891
Allg-8	t0 (6.6.2017)	05-D-038	Floor (under the bed)	16117
Allg-9	t0 (6.6.2017)	05-D-038	Door handle	18079
Allg-10	t0 (6.6.2017)	05-D-039	Light bar	18734
Allg-11	t0 (6.6.2017)	05-D-039	Light bar PMA	3447
Allg-12	t0 (6.6.2017)	05-D-039	Medical workplace	4907
Allg-13	t0 (6.6.2017)	05-D-039	Water tap	9348
Allg-14	t0 (6.6.2017)	05-D-039	Water tap PMA	4604
Allg-15	t0 (6.6.2017)	05-D-039	Toilet (button)	1853
Allg-16	t0 (6.6.2017)	05-D-039	Toilet (handle) PMA	190
Allg-17	t0 (6.6.2017)	05-D-039	Floor (under the bed)	21863
Allg-19	t0 (6.6.2017)	05-D-015	Light bar	11215
Allg-20	t0 (6.6.2017)	05-D-015	Light bar PMA	21672
Allg-21	t0 (6.6.2017)	05-D-015	Medical workplace	15578
Allg-22	t0 (6.6.2017)	05-D-015	Water tap	14943
Allg-23	t0 (6.6.2017)	05-D-015	Water tap PMA	2914
Allg-24	t0 (6.6.2017)	05-D-015	Toilet (button)	14117
Allg-25	t0 (6.6.2017)	05-D-015	Toilet (handle) PMA	804
Allg-26	t0 (6.6.2017)	05-D-015	Floor (under the bed)	14463
Allg-27	t0 (6.6.2017)	05-D-015	Door handle	170
Allg-28	t1 (8.6.2017)	05-D-038	Floor (under the bed)	2211
Allg-29	t1 (8.6.2017)	05-D-038	Water tap	50

Allg-30	t1 (8.6.2017)	05-D-038	Water tap PMA	1590
Allg-31	t1 (8.6.2017)	05-D-038	Light bar	869
Allg-32	t1 (8.6.2017)	05-D-038	Light bar PMA	1207
Allg-34	t1 (8.6.2017)	05-D-038	Toilet (button)	157
Allg-35	t1 (8.6.2017)	05-D-038	Toilet (handle) PMA	535
Allg-36	t1 (8.6.2017)	05-D-038	Door handle	2775
Allg-38	t1 (8.6.2017)	05-D-038	Bed remote	3627
Allg-39	t1 (8.6.2017)	05-D-038	Bed remote PMA	100
Allg-40	t1 (8.6.2017)	05-D-038	Night stand	3345
Allg-41	t1 (9.6.2017)	05-D-039	Floor (under the bed)	17332
Allg-42	t1 (9.6.2017)	05-D-039	Water tap	21063
Allg-43	t1 (9.6.2017)	05-D-039	Water tap PMA	1574
Allg-44	t1 (9.6.2017)	05-D-039	Light bar	11980
Allg-45	t1 (9.6.2017)	05-D-039	Light bar PMA	1375
Allg-46	t1 (9.6.2017)	05-D-039	Medical workplace	21221
Allg-47	t1 (9.6.2017)	05-D-039	Toilet (button)	6262
Allg-48	t1 (9.6.2017)	05-D-039	Toilet (handle) PMA	184
Allg-49	t1 (9.6.2017)	05-D-039	Door handle	9554
Allg-50	t1 (9.6.2017)	05-D-039	Pillow	15417
Allg-51	t1 (9.6.2017)	05-D-039	Bed remote	11384
Allg-52	t1 (9.6.2017)	05-D-039	Bed remote PMA	473
Allg-53	t1 (9.6.2017)	05-D-039	Night stand	20767
Allg-54	t1 (12.6.2017)	05-D-015	Floor (under the bed)	24344
Allg-55	t1 (12.6.2017)	05-D-015	Water tap	5922
Allg-56	t1 (12.6.2017)	05-D-015	Water tap PMA	146
Allg-57	t1 (12.6.2017)	05-D-015	Light bar	30224
Allg-58	t1 (12.6.2017)	05-D-015	Light bar PMA	4625
Allg-59	t1 (12.6.2017)	05-D-015	Medical workplace	30221
Allg-61	t1 (12.6.2017)	05-D-015	Toilet (handle) PMA	1162
Allg-62	t1 (12.6.2017)	05-D-015	Door handle	23309
Allg-63	t1 (12.6.2017)	05-D-015	Pillow	13862
Allg-64	t1 (12.6.2017)	05-D-015	Bed remote	13888
Allg-65	t1 (12.6.2017)	05-D-015	Bed remote PMA	0
Allg-67	t2 (14.6.2017)	05-D-038	Floor (under the bed)	14989
Allg-68	t2 (14.6.2017)	05-D-038	Water tap	5567
Allg-69	t2 (14.6.2017)	05-D-038	Water tap PMA	748
Allg-70	t2 (14.6.2017)	05-D-038	Light bar	17519
Allg-71	t2 (14.6.2017)	05-D-038	Light bar PMA	10255
Allg-72	t2 (14.6.2017)	05-D-038	Medical workplace	7928
Allg-73	t2 (14.6.2017)	05-D-038	Toilet (button)	10383
Allg-74	t2 (14.6.2017)	05-D-038	Toilet (handle) PMA	5624
Allg-75	t2 (14.6.2017)	05-D-038	Door handle	19161

Allg-77	t2 (14.6.2017)	05-D-038	Bed remote	5475
Allg-78	t2 (14.6.2017)	05-D-038	Bed remote PMA	234
Allg-79	t2 (14.6.2017)	05-D-038	Night stand	2323
Allg-80	t2 (16.6.2017)	05-D-039	Floor (under the bed)	22533
Allg-81	t2 (16.6.2017)	05-D-039	Water tap	9950
Allg-82	t2 (16.6.2017)	05-D-039	Water tap PMA	4853
Allg-83	t2 (16.6.2017)	05-D-039	Light bar	14174
Allg-84	t2 (16.6.2017)	05-D-039	Light bar PMA	8865
Allg-85	t2 (16.6.2017)	05-D-039	Medical workplace	20470
Allg-86	t2 (16.6.2017)	05-D-039	Toilet (button)	202
Allg-87	t2 (16.6.2017)	05-D-039	Toilet (handle) PMA	227
Allg-88	t2 (16.6.2017)	05-D-039	Door handle	11271
Allg-89	t2 (16.6.2017)	05-D-039	Pillow	9159
Allg-90	t2 (16.6.2017)	05-D-039	Bed remote	6197
Allg-91	t2 (16.6.2017)	05-D-039	Bed remote PMA	1369
Allg-92	t2 (16.6.2017)	05-D-039	Night stand	24539
Allg-93	t2 (19.6.2017)	05-D-015	Floor (under the bed)	17458
Allg-94	t2 (19.6.2017)	05-D-015	Water tap	9334
Allg-95	t2 (19.6.2017)	05-D-015	Water tap PMA	7171
Allg-96	t2 (19.6.2017)	05-D-015	Light bar	20090
Allg-97	t2 (19.6.2017)	05-D-015	Light bar PMA	14993
Allg-98	t2 (19.6.2017)	05-D-015	Medical workplace	2190
Allg-99	t2 (19.6.2017)	05-D-015	Toilet (button)	1242
Allg-100	t2 (19.6.2017)	05-D-015	Toilet (handle) PMA	304
Allg-101	t2 (19.6.2017)	05-D-015	Door handle	16605
Allg-102	t2 (19.6.2017)	05-D-015	Pillow	6337
Allg-103	t2 (19.6.2017)	05-D-015	Bed remote	7009
Allg-104	t2 (19.6.2017)	05-D-015	Bed remote PMA	542
Allg-105	t2 (19.6.2017)	05-D-015	Night stand	25343
Allg-106	t3 (6.7.2017)	05-D-038	Floor (under the bed)	18118
Allg-107	t3 (6.7.2017)	05-D-038	Water tap	936
Allg-108	t3 (6.7.2017)	05-D-038	Water tap PMA	822
Allg-109	t3 (6.7.2017)	05-D-038	Light bar	25345
Allg-110	t3 (6.7.2017)	05-D-038	Light bar PMA	1305
Allg-111	t3 (6.7.2017)	05-D-038	Medical workplace	12893
Allg-112	t3 (6.7.2017)	05-D-038	Toilet (button)	1716
Allg-113	t3 (6.7.2017)	05-D-038	Toilet (handle) PMA	586
Allg-114	t3 (6.7.2017)	05-D-038	Door handle	8610
Allg-115	t3 (6.7.2017)	05-D-038	Pillow	15959
Allg-116	t3 (6.7.2017)	05-D-038	Bed remote	3457
Allg-117	t3 (6.7.2017)	05-D-038	Bed remote PMA	222
Allg-118	t3 (6.7.2017)	05-D-038	Night stand	25884

Allg-119	t3 (6.7.2017)	05-D-039	Floor (under the bed)	5370
Allg-120	t3 (6.7.2017)	05-D-039	Water tap	7582
Allg-121	t3 (6.7.2017)	05-D-039	Water tap PMA	5066
Allg-122	t3 (6.7.2017)	05-D-039	Light bar	19330
Allg-123	t3 (6.7.2017)	05-D-039	Light bar PMA	8131
Allg-124	t3 (6.7.2017)	05-D-039	Medical workplace	26206
Allg-125	t3 (6.7.2017)	05-D-039	Toilet (button)	6565
Allg-126	t3 (6.7.2017)	05-D-039	Toilet (handle) PMA	836
Allg-127	t3 (6.7.2017)	05-D-039	Door handle	9232
Allg-128	t3 (6.7.2017)	05-D-039	Pillow	7192
Allg-129	t3 (6.7.2017)	05-D-039	Bed remote	6445
Allg-130	t3 (6.7.2017)	05-D-039	Bed remote PMA	1850
Allg-131	t3 (6.7.2017)	05-D-039	Night stand	8521
Allg-132	t3 (6.7.2017)	05-D-015	Floor (under the bed)	16535
Allg-133	t3 (6.7.2017)	05-D-015	Water tap	11258
Allg-134	t3 (6.7.2017)	05-D-015	Water tap PMA	12931
Allg-135	t3 (6.7.2017)	05-D-015	Light bar	21735
Allg-136	t3 (6.7.2017)	05-D-015	Light bar PMA	274
Allg-137	t3 (6.7.2017)	05-D-015	Medical workplace	16215
Allg-138	t3 (6.7.2017)	05-D-015	Toilet (button)	4072
Allg-139	t3 (6.7.2017)	05-D-015	Toilet (handle) PMA	6606
Allg-140	t3 (6.7.2017)	05-D-015	Door handle	10105
Allg-141	t3 (6.7.2017)	05-D-015	Pillow	22206
Allg-142	t3 (6.7.2017)	05-D-015	Bed remote	6518
Allg-143	t3 (6.7.2017)	05-D-015	Bed remote PMA	434
Allg-144	t3 (6.7.2017)	05-D-015	Night stand	6082
Allg-145	t4 (31.8.2017)	05-D-038	Floor (under the bed)	14601
Allg-146	t4 (31.8.2017)	05-D-038	Water tap	10827
Allg-147	t4 (31.8.2017)	05-D-038	Water tap PMA	339
Allg-148	t4 (31.8.2017)	05-D-038	Light bar	14236
Allg-149	t4 (31.8.2017)	05-D-038	Light bar PMA	8097
Allg-150	t4 (31.8.2017)	05-D-038	Medical workplace	4250
Allg-151	t4 (31.8.2017)	05-D-038	Toilet (button)	1618
Allg-152	t4 (31.8.2017)	05-D-038	Toilet (handle) PMA	494
Allg-153	t4 (31.8.2017)	05-D-038	Door handle	20895
Allg-154	t4 (31.8.2017)	05-D-038	Pillow	11427
Allg-155	t4 (31.8.2017)	05-D-038	Bed remote	12901
Allg-156	t4 (31.8.2017)	05-D-038	Bed remote PMA	2885
Allg-157	t4 (31.8.2017)	05-D-038	Night stand	10445
Allg-158	t4 (31.8.2017)	05-D-039	Floor (under the bed)	18662
Allg-159	t4 (31.8.2017)	05-D-039	Water tap	8355
Allg-160	t4 (31.8.2017)	05-D-039	Water tap PMA	380

Allg-161	t4 (31.8.2017)	05-D-039	Light bar	20639
Allg-162	t4 (31.8.2017)	05-D-039	Light bar PMA	8831
Allg-163	t4 (31.8.2017)	05-D-039	Medical workplace	6725
Allg-164	t4 (31.8.2017)	05-D-039	Toilet (button)	14021
Allg-165	t4 (31.8.2017)	05-D-039	Toilet (handle) PMA	471
Allg-166	t4 (31.8.2017)	05-D-039	Door handle	9117
Allg-167	t4 (31.8.2017)	05-D-039	Pillow	26029
Allg-168	t4 (31.8.2017)	05-D-039	Bed remote	7455
Allg-169	t4 (31.8.2017)	05-D-039	Bed remote PMA	1258
Allg-170	t4 (31.8.2017)	05-D-039	Night stand	4095
Allg-171	t4 (31.8.2017)	05-D-015	Floor (under the bed)	21034
Allg-172	t4 (31.8.2017)	05-D-015	Water tap	22411
Allg-173	t4 (31.8.2017)	05-D-015	Water tap PMA	1066
Allg-174	t4 (31.8.2017)	05-D-015	Light bar	21578
Allg-175	t4 (31.8.2017)	05-D-015	Light bar PMA	8393
Allg-176	t4 (31.8.2017)	05-D-015	Medical workplace	5119
Allg-177	t4 (31.8.2017)	05-D-015	Toilet (button)	6876
Allg-178	t4 (31.8.2017)	05-D-015	Toilet (handle) PMA	2817
Allg-179	t4 (31.8.2017)	05-D-015	Door handle	3650
Allg-180	t4 (31.8.2017)	05-D-015	Pillow	22551
Allg-181	t4 (31.8.2017)	05-D-015	Bed remote	8481
Allg-182	t4 (31.8.2017)	05-D-015	Bed remote PMA	329
Allg-183	t4 (31.8.2017)	05-D-015	Night stand	18402
Allg-184	t5 (7.12.2017)	05-D-038	Floor (under the bed)	18139
Allg-185	t5 (7.12.2017)	05-D-038	Water tap	12615
Allg-186	t5 (7.12.2017)	05-D-038	Water tap PMA	15423
Allg-187	t5 (7.12.2017)	05-D-038	Light bar	9655
Allg-188	t5 (7.12.2017)	05-D-038	Light bar PMA	2137
Allg-189	t5 (7.12.2017)	05-D-038	Medical workplace	7353
Allg-190	t5 (7.12.2017)	05-D-038	Toilet (button)	4147
Allg-191	t5 (7.12.2017)	05-D-038	Toilet (handle) PMA	976
Allg-192	t5 (7.12.2017)	05-D-038	Door handle	2517
Allg-193	t5 (7.12.2017)	05-D-038	Pillow	7542
Allg-194	t5 (7.12.2017)	05-D-038	Bed remote	13067
Allg-195	t5 (7.12.2017)	05-D-038	Bed remote PMA	911
Allg-196	t5 (7.12.2017)	05-D-038	Night stand	10188
Allg-197	t5 (7.12.2017)	05-D-039	Floor (under the bed)	15100
Allg-198	t5 (7.12.2017)	05-D-039	Water tap	11698
Allg-199	t5 (7.12.2017)	05-D-039	Water tap PMA	8839
Allg-200	t5 (7.12.2017)	05-D-039	Light bar	6560
Allg-201	t5 (7.12.2017)	05-D-039	Light bar PMA	1787
Allg-202	t5 (7.12.2017)	05-D-039	Medical workplace	22695

Allg-203	t5 (7.12.2017)	05-D-039	Toilet (button)	1732
Allg-204	t5 (7.12.2017)	05-D-039	Toilet (handle) PMA	1748
Allg-205	t5 (7.12.2017)	05-D-039	Door handle	12194
Allg-206	t5 (7.12.2017)	05-D-039	Pillow	3867
Allg-207	t5 (7.12.2017)	05-D-039	Bed remote	8703
Allg-208	t5 (7.12.2017)	05-D-039	Bed remote PMA	1161
Allg-209	t5 (7.12.2017)	05-D-039	Night stand	9793
Allg-210	t5 (7.12.2017)	05-D-015	Floor (under the bed)	2174
Allg-211	t5 (7.12.2017)	05-D-015	Water tap	1128
Allg-212	t5 (7.12.2017)	05-D-015	Water tap PMA	10782
Allg-213	t5 (7.12.2017)	05-D-015	Light bar	1135
Allg-214	t5 (7.12.2017)	05-D-015	Light bar PMA	631
Allg-215	t5 (7.12.2017)	05-D-015	Medical workplace	6030
Allg-216	t5 (7.12.2017)	05-D-015	Toilet (button)	1462
Allg-217	t5 (7.12.2017)	05-D-015	Toilet (handle) PMA	5004
Allg-218	t5 (7.12.2017)	05-D-015	Door handle	6626
Allg-219	t5 (7.12.2017)	05-D-015	Pillow	4481
Allg-220	t5 (7.12.2017)	05-D-015	Bed remote	4872
Allg-221	t5 (7.12.2017)	05-D-015	Bed remote PMA	2785
Allg-222	t5 (7.12.2017)	05-D-015	Night stand	3350
Allg-225	08.06.2017	ALTE 501	Bed remote	17719
Allg-226	08.06.2017	ALTE 501	Bed remote PMA	16222
Allg-227	08.06.2017	ALTE 501	Floor (under the bed)	18110
Allg-228	08.06.2017	ALTE 501	Door handle	18898
Allg-229	08.06.2017	ALTE 501	Night stand	22440
Allg-230	14.06.2017	ALTE 501	Light bar	18434
Allg-231	14.06.2017	ALTE 501	Light bar PMA	1899
Allg-232	14.06.2017	ALTE 501	Floor (under the bed)	10888
Allg-233	14.06.2017	ALTE 501	Door handle	20785
Allg-238	t6 (8.6.2018)	05-D-038	Floor (under the bed)	5850
Allg-239	t6 (8.6.2018)	05-D-038	Water tap	10363
Allg-240	t6 (8.6.2018)	05-D-038	Water tap PMA	16478
Allg-241	t6 (8.6.2018)	05-D-038	Light bar	15590
Allg-242	t6 (8.6.2018)	05-D-038	Light bar PMA	3690
Allg-243	t6 (8.6.2018)	05-D-038	Medical workplace	11027
Allg-244	t6 (8.6.2018)	05-D-038	Toilet (button)	9654
Allg-245	t6 (8.6.2018)	05-D-038	Toilet (handle) PMA	1446
Allg-246	t6 (8.6.2018)	05-D-038	Door handle	19889
Allg-247	t6 (8.6.2018)	05-D-038	Pillow	11804
Allg-248	t6 (8.6.2018)	05-D-038	Bed remote	10421
Allg-249	t6 (8.6.2018)	05-D-038	Bed remote PMA	16154
Allg-250	t6 (8.6.2018)	05-D-038	Night stand	5448

Allg-251	t6 (8.6.2018)	05-D-039	Floor (under the bed)	11899
Allg-252	t6 (8.6.2018)	05-D-039	Water tap	6980
Allg-253	t6 (8.6.2018)	05-D-039	Water tap PMA	8678
Allg-254	t6 (8.6.2018)	05-D-039	Light bar	10144
Allg-255	t6 (8.6.2018)	05-D-039	Light bar PMA	19384
Allg-256	t6 (8.6.2018)	05-D-039	Medical workplace	9486
Allg-257	t6 (8.6.2018)	05-D-039	Toilet (button)	15662
Allg-258	t6 (8.6.2018)	05-D-039	Toilet (handle) PMA	1298
Allg-259	t6 (8.6.2018)	05-D-039	Door handle	12215
Allg-260	t6 (8.6.2018)	05-D-039	Pillow	8449
Allg-261	t6 (8.6.2018)	05-D-039	Bed remote	10351
Allg-262	t6 (8.6.2018)	05-D-039	Bed remote PMA	3931
Allg-263	t6 (8.6.2018)	05-D-039	Night stand	2113
Allg-264	t6 (8.6.2018)	05-D-015	Floor (under the bed)	15389
Allg-265	t6 (8.6.2018)	05-D-015	Water tap	18673
Allg-266	t6 (8.6.2018)	05-D-015	Water tap PMA	13532
Allg-267	t6 (8.6.2018)	05-D-015	Light bar	10638
Allg-268	t6 (8.6.2018)	05-D-015	Light bar PMA	1616
Allg-269	t6 (8.6.2018)	05-D-015	Medical workplace	14787
Allg-270	t6 (8.6.2018)	05-D-015	Toilet (button)	15091
Allg-271	t6 (8.6.2018)	05-D-015	Toilet (handle) PMA	915
Allg-272	t6 (8.6.2018)	05-D-015	Door handle	799
Allg-273	t6 (8.6.2018)	05-D-015	Pillow	12726
Allg-274	t6 (8.6.2018)	05-D-015	Bed remote	15153
Allg-275	t6 (8.6.2018)	05-D-015	Bed remote PMA	2347
Allg-276	t6 (8.6.2018)	05-D-015	Night stand	15802
PMA-t1	t1 (8.6.2017)	NA	PMA control	899
PMA-t2	t2 (14.06.2017)	NA	PMA control	1285
PMA-t3	t3 (06.07.17)	NA	PMA control	1411
PMA-t4	t4 (31.8.2017)	NA	PMA control	2228
PMA-t5	t5 (7.12.2017)	NA	PMA control	1203
PMA-t6	t6 (8.6.2018)	NA	PMA control	2090
PMA12-6-17	t1 (12.06.2017)	NA	PMA control	340
KC-5-4	05.04.2018	NA	Kit control	533
KC-8-5	08.05.2018	NA	Kit control	3067
KC-9-5	09.05.2019	NA	Kit control	133
KC-15-5	15.05.2018	NA	Kit control	600
KC-27-4	27.04.2018	NA	Kit control	0
Allg-234	9.6.2017	NA	Wipe field control	466
Allg-235	12.6.2017	NA	Wipe field control	3933
Allg-236	16.6.2017	NA	Wipe field control	3817
Allg-237	19.6.2017	NA	Wipe field control	3556

6.2 50 most abundant genera

1	Unclassified
2	Corynebacterium
3	Staphylococcus
4	Streptococcus
5	Acinetobacter
6	Ralstonia
7	Pseudomonas
8	Anaerococcus
9	Paracoccus
10	Methanobrevibacter
11	Finegoldia
12	Lactobacillus
13	Micrococcus
14	Cutibacterium
15	Enhydrobacter
16	uncultured
17	Sphingomonas
18	Bacteroides
19	Rothia
20	Lawsonella
21	Methylobacterium
22	Actinomyces
23	Lactococcus
24	Haemophilus
25	Peptoniphilus
26	Stenotrophomonas
27	Massilia
28	Enterococcus
29	Kocuria
30	Cloacibacterium
31	Neisseria
32	Gemella
33	Porphyromonas
34	Corynebacterium
35	uncultured_bacterium
36	Sphingobium
37	Prevotella
38	Ferritrophicum
39	Hymenobacter

40	Basidiomycota
41	Prevotella
42	Unclassified.uncultured_bacterium
43	Brevibacterium
44	Veillonella
45	Brevundimonas
46	Fusobacterium
47	AllorhizobiumNeorhizobiumPararhizobiumRhizobium
48	Picea_glauca_white_spruce
49	Chryseobacterium
50	Pedobacter