Diplomarbeit

Antibiotic Resistances In Human Associated Indoor Microbiomes

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unter der Anleitung von

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Graz am 03.05.2021

Eidesstattliche Erklärung

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Graz, am 03.05.2021 *Benedikt Pilshofer eh*

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Glossary und Abbreviations

AME	Aminoglycoside modifying enzyme
AMR	Antimicrobial Resistance
BLDB	Beta lactamase database
CB	Confined building
ECDC	European Center for Disease Control
CDC	Center for Disease Control
CFU	Colony-forming unit
HAI	Healthcare-Acquainted Infection
HGT	Horizontal gene transfer
ICU	Intensive Care Unit
ISO	International Organization for Standardization
ISS	International Space Station
MCP	Microbe carrying particle
MDR	Multi drug resistant
MGE	Mobile genetic element
MRSA	Methicillin resistant *Staphylococcus aureus*
PBP	Penicillin binding protein
UB	Unconfined building
VRE	Vancomycin resistant Enterococcus
WHO	World Health Organization

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Zusammenfassung

Innenräume sind von einer großen Vielfalt und Anzahl an Mikroorganismen besiedelt. Da sich Menschen durchschnittlich 90% ihrer Zeit in Innenräumen aufhalten, sind sie in ständigem Kontakt mit diesen Mikroorganismen. Innenräume können jedoch sehr unterschiedlich von voneinander sein (etwa aufgrund ihrer Funktion oder ihrer Architektur), wodurch sich auch ihre Mikrobiome sehr voneinander unterscheiden können. Faktoren wie Abgeschlossenheit, Belüftung, Reinigung der Innenräume oder der Kontakt mit anderen Mikrobiomen können die Beschaffenheit und Eigenschaften von Innenraummikrobiomen erheblich beeinflussen. Darüber hinaus beinhalten Innenraummikrobiome auch zahlreiche pathogene Mikroorganismen, die eine Gefahr für die Gesundheit von den Personen, die mit ihnen in Kontakt kommen, darstellen können. Diese Gefahr wird dadurch erhöht, dass viele Pathogene Resistenzmechanismen entwickeln und dadurch weniger Behandlungsoptionen zur Verfügung stehen, wenn diese Infektionen verursachen.
Das Ziel dieser Literaturrecherche ist es einen Überblick des aktuellen Wissens über die Beschaffenheit und Charakteristika verschiedener Innenraummikrobiome zu geben und durch welche Faktoren sie beeinflusst werden. Außerdem soll in dieser Arbeit ein spezielles Augenmerk auf das Problem der antimikrobiellen Resistenzen in diesen Mikrobiomen gelegt werden, welche Ursachen dahinterstecken und welche Ideen und Strategien die Wissenschaft hat um diese Herausforderung zu bewältigen.

Abstract

Indoor environments are inhabited by a great variety and abundance of different microorganisms. As people spend about 90% of their time indoors, they almost constantly interact with these microorganisms. However, as indoor environments can differ greatly from each other (e.g. in function and architecture), their indoor microbiomes can as well quite substantially differ from each other. Factors such as confinement, ventilation and cleaning of indoor environments or influences from other microbiomes can severely impact the composition and characteristics of indoor microbiomes. Furthermore, indoor microbiomes also include numerous pathogenic microorganisms that can pose a threat to the health of people interacting with these pathogens. The microbial threat is enhanced by resistance mechanisms that microorganisms can develop, which lead to fewer treatment options for infections caused by them.

The aim of this literature review is to give an overview of the current knowledge of indoor microbiomes, of their composition and characteristics and what factors influence them. Furthermore, it will especially focus on the problem of antimicrobial resistances in indoor microbiomes, what causes them and what the latest ideas and strategies to tackle this problem are.

1 Introduction

On average, people living in industrialized countries spend approximately 90% of their time in indoor environments (1), where they are surrounded by a great number of microorganisms such as bacteria, fungi, archaea, algae and many more (2). The knowledge about these commensals has increased tremendously over the last years. Due to new scientific methods introduced in the last decades such as high- throughput 16S rRNA gene sequencing, scientist have been able to identify a huge number of new microorganisms living in indoor microbiomes. Furthermore, these new methods have not only allowed to identify which microbes live in these environments but also made it possible to study their genome and enabled the discovery of detailed genetic information such as the number and diversity of resistance genes. This has given rise to many new insights in how indoor microbiomes function and what factors lead to changes in their composition or features.
Indoor environments comprise a diverse group of places and can thus differ greatly from each other. Whether it is due to the amount of people visiting or inhabiting these places, the geographic region, the confinement of the buildings or the applied cleaning regiments, many factors influence the indoor environment (3).

The goal of this literature review is to provide an overview of the current knowledge and research of the field of indoor microbiomes. It will answer the question of which microorganisms typically live in certain indoor environments and how environmental factors influence these microbiomes. How do the indoor microbiomes of private or public buildings differ from the ones in hospitals? And which microorganisms live in much more confined environments, such as cleanrooms or the International Space Station (ISS)?
This literature review will also discuss the effects of actions such as cleaning or disinfecting on indoor microbiomes and what effects these altered microbiomes potentially have on people in return. As a closely linked topic to microbiome research a special focus will be on the problem of antimicrobial resistances, which the World Health Organization (WHO) rates as one of the major health hazards for

the coming decade (4). Thus, the current scientific knowledge of the resistome of the different indoor microbiomes will be reviewed. The review will also discuss how the factors influencing indoor microbiomes can also affect their resistomes as well as what the effects of an altered resistome can be.

Finally, this review will also give an outlook on important future research fields and how the knowledge obtained so far can be implemented to decrease the proportion of harmful and resistant microorganisms in indoor environments. This includes the question whether the current practices to eradicate pathogens in indoor environments, especially in health care facilities, are the best approaches or if it is time to perhaps rethink some of the current principles.

2 Methods

The primary research method used for this literature review was literature research in textbooks, on platforms particularly PubMed, Science Direct and Google Scholar, as well as guidelines and reports from institutions such as the World Health Organization, the Center for Disease Control and the European Center for Disease Control. The initial literature research was performed on PubMed with the MeSH terms “microbiota” and “built environments”, as well as “microbiota” and “drug resistance, microbial”. The obtained articles where then searched for relevant titles and abstracts which were then fully read. Based on these articles and a list of relevant articles provided by my supervisor a broad overview over the topic was established. The articles were then forward and backwards reference searched for additional articles related to the topic to develop a deeper knowledge of the topic. Further research was conducted on the terms “resistome”, “healthcare-associated infections”, “antimicrobial resistances”, “biofilms” and “disinfectants”.

Overall, 178 sources, including articles, textbooks, reviews, guidelines and websites were considered relevant for the final version of the literature research (references).

3 Results

3.1 The Microbiome

Almost everywhere on earth, surrounding us and even living on and in us are microorganisms. Normally these small organisms are invisible to our eyes but the play a vital role in our lives.

The term ‘microbiome’ has risen to huge popularity in the past 30 years when new research methods revealed the sheer number and abundance of organisms in, on, and around us. Today the word microbiome is not only very popular in science and research but also all over the media and our everyday life. The increasing popularity can be seen when looking at the rise of articles and books containing the word microbiome that have been published in recent years (Fig. 1; Fig. 2).

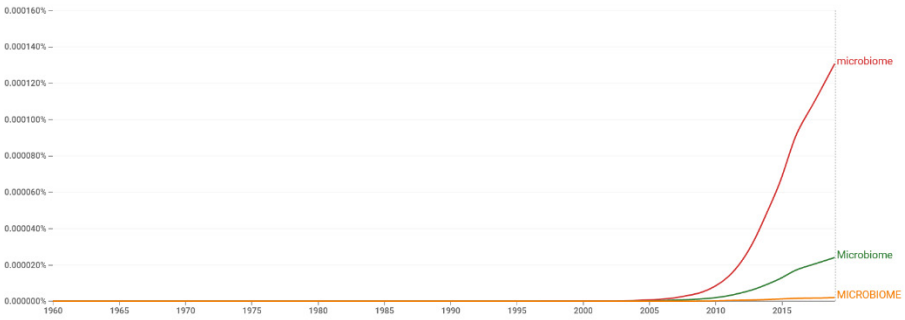

Figure 1: Rise in books containing the word microbiome (Google Books)

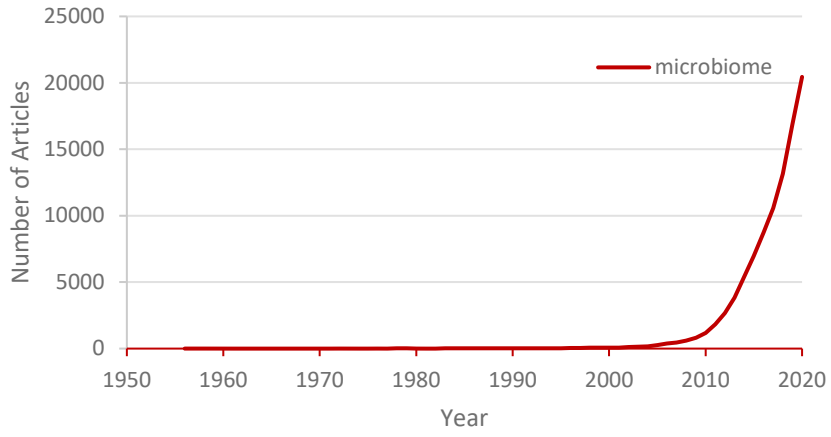

Figure 2: PubMed search results containing the word microbiome over the last 70 years (6)

But what exactly does the word microbiome mean and what is its definition? One of the first definitions of the term microbiome comes from the year 1988 and is still quite accurate today. It defines a microbiome as:
"[…] a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity" (7).

However, over the last decades there have been many new proposals and adaptions for the definition of the term microbiome from different scientific point of views.
The probably most popular one being a definition by Lederberg and colleagues from 2001, which looks at the microbiome from an ecological perspective describing it as a *"ecological community of commensal, symbiotic, and pathogenic microorganisms within a body space or other environment"* (8).
Other definitions have looked at the term microbiome from different angles such as from a genomic perspective or a host-dependent perspective. The genomic definition describes the microbiome as the collective genomic inventory of microorganisms in a specific environment. This genomic approach in contrast then uses the term microbiota for the microorganisms present in an environment (9). These different definitions of the words microbiome and microbiota have caused some confusion in the scientific community.

In 2020 the MicrobiomeSupport project tackled this problem and proposed a novel consensus definition. The new definition is based on the 1988 definition by Whipps but extends the old definition and also differentiates between the terms microbiome and microbiota (10).
The new definition lines out that the microbiome "*forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health"* (10).

The microbiota is defined as all the living organisms present in a specific environment. The difference to the microbiome being that the microbiota does not

include the “theatre of activity” which are all “*microbial structures, metabolites, mobile genetic elements (e.g., transposons, phages, and viruses), and relic DNA embedded in the environmental conditions of the habitat”* (10).

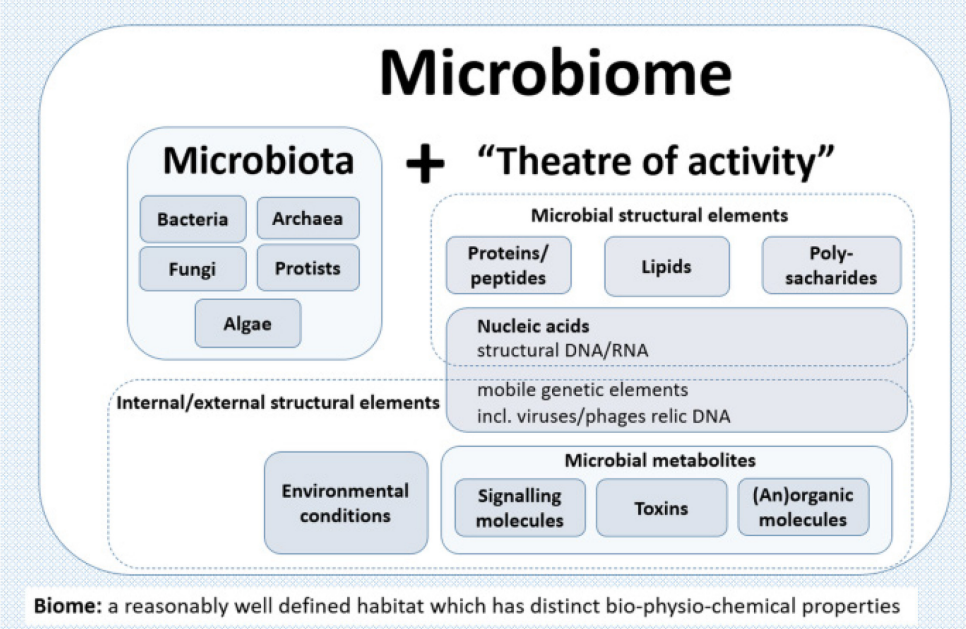

Figure 3: The microbiome consist of the microbiota, which are the living microorganisms, and their “theatre of activity” as well as internal and external structural elements (Microbiome by Berg et al. 2020 (9), CC by 4.0, https://creativecommons.org/licenses/by/4.0/)

3.1.1 The Indoor Microbiome

An average person living in an industrialized country spends up to 90% of their time per day in an indoor environment (1). Only 7-8% of our time is spent outside. So, most of our time we stay in built environments like our homes or our workplaces.
Therefore we spend a lot of our time surrounded by an indoor microbiome, meaning bacteria, fungi, archaea etc. and their theatre of activity. These microorganisms are very well adapted and sometimes even specific to the indoor environment and can differ a lot from the outdoor microbiome (11,12). However, there are a lot of different kinds of indoor environments, ranging from our homes or public buildings, which are connected to the outside quite well, to rather isolated environments like an intensive care unit (ICU) in an hospital or in the most extreme case the International Space Station (ISS) with no contact to any outdoor environment at all. These different indoor environments also offer quite different conditions for microorganisms and thus the indoor microbiomes can also be very dissimilar in these distinct places.

3.1.1.1 Basic Mechanisms of transmission & influences

There are many different factors that influence the indoor microbiome: humans living in the houses, the type of ventilation, temperature, humidity, geographic location, mold, pets, plants, plumbing systems and many more (3). However the factor that seems to be the most important one are the humans inhabiting the buildings (2).
The human microbiome is transferred to its surroundings through several different processes.
Every person harbors their unique human microbiome. There are microorganisms living on our skin, in our oral and nasal cavities, our respiratory system, our gut or urogenital system (13).
And since we are in constant contact with our surrounding environment, we also spread our human microbiome. Research suggests that we emit up to 10^6 biological particles per hour. The main mechanism of how we spread these

particles are by direct contact and by dispersing bioaerosols when talking, coughing, and breathing (14).

Depending on what kind of contact is dominant with a certain surface the microbiome of the surface will differ e.g. toilet surfaces have a gut associated microbiome whereas high-touch surfaces in restrooms show microorganisms similar to the skin microbiome (15).
Decisive for the microbiome on a surface is not the surface itself and the material it is made of but is the location of the surface and its usage patterns. It does not matter whether the floor is a carpet floor or a tile floor, but it matters whether a carpet is on the floor or on the wall (16).

It has also been shown that every human carries a kind of 'microbial cloud' around them and thus emits bioaerosols in the surrounding air. Said bioaerosols consist of microorganisms from our breath, skin, hair and clothes. This cloud is individual for every human being and might even one day be used to identify individuals for forensic purposes (14).

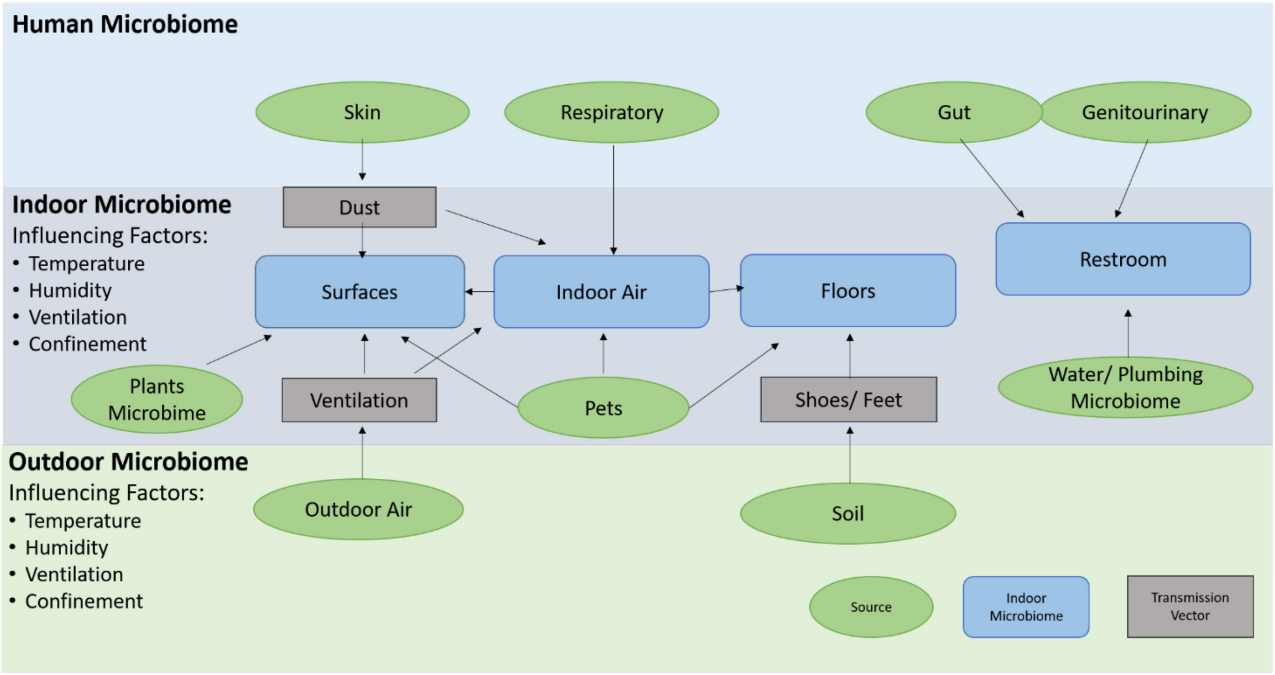

Figure 4: The indoor microbiome, its sources and transmission vectors

However, also other factors such as the transfer of microorganisms associated with the outdoor environment into a house greatly influence the indoor microbiome. This transfer happens for example via dirt on our shoes, via pets and via air (17) (Fig. 4).
The indoor microbiome is substantially influenced by the outdoor environment i.e. changes in the microbial profile can be observed according to different seasons of the year (18) and the composition of the microbiome also depends on the exact geographic location, the climate and the surrounding environment (11,19).

Furthermore, also factors like architectural building design or the habits of the occupants can lead to a change in the composition of the microbiome. It has been shown that the air in rooms that are mechanically ventilated is more distinct from outdoor air than the air in rooms that are window ventilated (20).
Besides ventilation, also the connectedness of a room with other rooms and the amount of movement of people through a certain room, the usage patterns, humidity, temperature, interiors and many more affect its indoor microbiome (21,22).

Moreover, we do not only live with the indoor microbiome, we also try to manipulate and attempt to control it, especially in places we want to keep clean and sanitary such as hospitals or onboard the ISS. We do so by cleaning and disinfecting our surroundings and attempt to reduce harmful microorganisms and to reduce the potential negative effects such as infections the organisms living in our surrounding microbiomes may have on us. Over the last decades we have focused mainly on the pathogenic potential of our surrounding microbiomes and little on the microbiome as a cohabitant. However, this approach to try to create a sterile environment with as few microorganisms as possible might be a little shortsighted as this seems to lead to the emerging of new problems like resistances to antimicrobial agents such as antibiotics or disinfectants.

3.2 Healthcare-associated infections

According to the World Health Organization (WHO) about one in ten people acquires a healthcare-associated infection (HAI) while receiving care (23). Thirty percent of them would be preventable with the right interventions and measures. HAIs do not only lead to prolonged stays in hospitals, higher costs for health care systems but also to a higher mortality and to an increase of antimicrobial resistances. Especially patients staying in an ICU are at high risk for HAIs. The European Center for Disease Control and Prevention (ECDC) estimates that in the year 2017 almost 12,000 patients (roughly 8% of all ICU patients), which were staying at an ICU for more than two days acquired at least one HAI (24). In total it is estimated that at least 2.5 million people get infected with an HAI each year in the European Union (25).

According to a study from 2014 in the US the most common types of HAI are pneumonia, surgical site infections and gastrointestinal infections, followed by urinary tract and bloodstream infections (26).

A few microbial groups cause about 80% of these HAIs. The most important pathogens are *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli, Enterococcus* spp*. Pseudomonas aeruginosa, Candida* spp*., Klebsiella* species and *Enterobacter* spp. (27,28). Furthermore, up to 20% of the bacteria causing HAI are multi- drug resistant pathogens. The most important among them are MRSA (Methicillin resistant *Staphylococcus aureus*), vancomycin- resistant enterococci and carbapenem or cephalosporine resistant species (29).

And these multi drug resistant pathogens lead to higher mortality, longer hospital stays and higher costs for the health care systems because infections caused by them cannot be treated as well as diseases caused by non-resistant pathogens (30).

3.2.1 Transmission of infectious microorganisms

In attempt to lower the rate of infections in the community and especially in health care facilities one of the major goals is to stop the transmission of infectious organisms. On one hand we try to decrease the transmission from one individual to another, on the other hand we try to stop the contamination of our surroundings or to decontaminate them before someone else can come in contact with them. There are many ways infections can be transmitted with two major ways. The first is transmission by direct contact from one person to another while the second is transmission by indirect contact. The routes of transmissions (Fig. 5) listed below are the most important ways of infection transmission and thus also where the most effective measures can be taken to prevent the spreading of diseases (31).

Direct contact

1. Droplets: Describes the transmission of sneezed, breathed or coughed out pathogens by an infected person which then enter the respiratory system of another person
2. Fecal-oral: Pathogens present in feces of an infected person enter another person’s body through their mouth
3. Blood: Pathogens present in the blood of an infected person directly enter the blood stream of another person
4. Wound: A defect in a person´s skin or mucous membrane leads to the invasion of surrounding pathogens
5. Sexual: Describes the transmission of pathogens through fluids and blood during sexual contact
6. Vertical: Diaplacental transfer from mother to child

Indirect contact:

1. Airborne: Pathogens that occur in the air and are carried by droplet nuclei or dust and are transmitted this way
2. Objects: The transmission of the germs occurs through contact with contaminated surfaces or objects
3. Vehicle: Transmission via food, water or inanimate objects that are contaminated with pathogens.

4. Vectors: The pathogens are transmitted by mosquitos, ticks or fleas which function as a host for the pathogens.

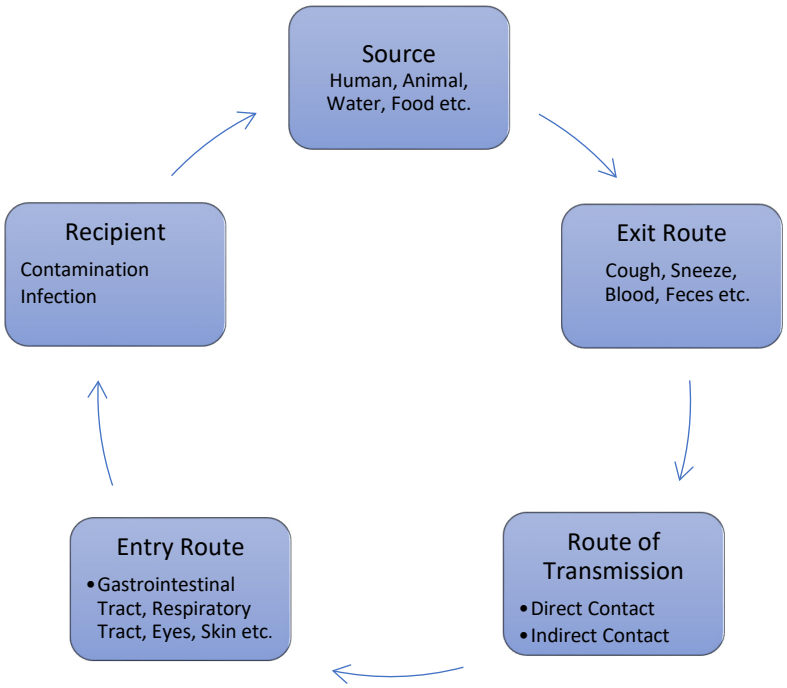

Figure 5: Route of transmission of infectious particles

3.2.2 Prevention of HAIs

There are a couple of basic methods that lead to a significant decrease of transmission especially in the context of healthcare associated infections. The CDC lists the following precautions as among the most important ones to prevent the spread of infections in hospitals: Hand hygiene, protective personal equipment, respiratory hygiene principles, isolation precautions, proper cleaning and disinfecting (32).
Hygiene in general leads to a significant reduction of illnesses of more than 20% (33).
There are different levels of risk for transmission of infectious microorganisms or microbial toxins for different sites. Whereas floors and walls generally are low risk sites, food contact surfaces, hand contact surfaces and even more hands themselves are sites with a very high risk of transmission of infectious particles (34).
To reduce the risk for the transmission of infectious microorganisms we clean, disinfect, or sterilize certain surfaces and wash or disinfect our hands to reduce the number of pathogens and opportunistic pathogens that can cause those infections. Pathogens are any microorganisms that cause diseases to their hosts, they can be divided into two categories: obligate and opportunistic pathogens (35). Obligate pathogens are microorganisms that always cause infections. Opportunistic pathogens are microorganisms that often live as commensals with their hosts and just cause infections if the host's resistance is weakened or compromised. In places like hospitals or ICUs where many people are weakened and have preexisting conditions, they can however become a big problem. Examples of opportunistic pathogens are common HAI-causing microorganisms such as *Candida albicans, Staphylococcus aureus* or *Pseudomonas aeruginosa* (35).

Cleaning is the first step of creating a sanitary pathogen-free environment. It is defined as the removal of all visible dirt, foreign material and organic matter. It also leads to a significant reduction of the number of microorganism present on a surface. Only if cleaning is done sufficiently, the next step, disinfection, can be successfully achieved (36).

Disinfection describes the process of destroying most of the microorganisms present on a surface. However, disinfection is not sporicidal. Sterilization is defined as a process which eliminates all forms of microbial life. This also includes the elimination of endospores, thus after sterilization no microorganisms at all should be present at the treated surfaces. Sterilization is needed for tools that reach sterile places for example surgical instruments (36).

In the next subchapter, the precautions of hand hygiene and environmental control will be reviewed in more detail as these are the ones that have direct impact on the microbiomes on our hands and in our direct environment, respectively.

3.2.2.1.1 Mechanisms of disinfectants & sterilization

Disinfection either works by using physical methods such as UV-light and washer-disinfectors or chemical substances like chlorine, alcohol, quaternary ammonium, hydrogen peroxide or glutaraldehyde (Fig. 6) (36).
There are different ways of how disinfectants work and how they eradicate microorganisms. Chemical disinfectants can be separated into two classes of disinfectants, namely oxidizing disinfectants and coagulating or non-oxidizing agents (37).
Oxidizing agents have a few different points of attack. On one hand, they produce radicals which directly break the bacterial DNA and RNA. Or the radicals damage the purines and pyrimidines and thus make replication or translation of the DNA or RNA impossible. On the other hand, oxidizing agents work by destroying proteins. They oxidize amino acids, which leads to a destruction of the bonds connecting them to other amino acids and thus the protein is damaged. A third way is by oxidizing lipids, which leads to a destruction of the double bonds and thus collapse of the cell wall and membranes (37).
Oxidizing agents include substances such as hydrogen peroxide (H_2O_2), sodium hypochlorite (NaClO), povidone and peracetic acid (CH_3CO_3H).
Non-oxidizing agents have different mechanisms. One mechanism is cross linking the bases in DNA and RNA leading to an impossibility separating the DNA and

RNA strands and making replication and transcription impossible. This mechanism is especially used by alkylating agents. Another mechanism is cross linking proteins and amino acids especially those with a -NH_2 group leading to a destruction of the protein structure as seen in aldehyde disinfectants (37).
Non- oxidizing agents include substances like alcohol, chlorhexidine, quaternary ammonium or glutaraldehyde.
Alcohol is a very common disinfectant especially as a skin disinfectant for hands. Alcohol works by binding to microbial proteins with its hydroxyl group and then denaturizing and coagulating these proteins resulting in the death of the cell. (36)Alcohol needs to be in concentrations from 60- 80%, above 80% alcohol is unable to reach the cytoplasm and can also be harmful to skin.
Quaternary ammonium compounds like chlorhexidine use their positive charge to bind to the negative charged cell wall and membrane. There, they lead to a malfunction of the membrane potential and the cell's pH value resulting eventually in cell death (37).
Another important and commonly used disinfectant is glutaraldehyde. Glutaraldehyde is a very potent disinfectant able to kill not only bacteria but also endospores if applied long enough. When glutaraldehyde is in an alkaline surrounding it becomes very acidic, thus kills bacteria through destruction of their proteins (36).

Sterilization means not only eliminating most of the microorganisms on a surface but destroying all microorganisms including endospores (36).
There are many different methods for sterilization which can be separated in two groups, physical or chemical sterilization. The most popular methods are steam sterilization and flash sterilization (physical) or ethylene oxide sterilization and hydrogen peroxide gas plasma (chemical) (36).
Steam sterilization is most often performed via autoclaves. These are containers that increase the pressure inside them when boiling water thus leading to an increase of the boiling point of water. The water then does not boil at 100°C; it reaches its boiling point at around 120°C which is high enough to not only kill bacteria but also endospores which are more resistant to heat than the bacterial vegetative cell (37).
Other important sterilization techniques especially for materials that cannot endure

such high temperatures include substances like ethylene oxide or hydrogen peroxide plasma. Ethylene oxide gas works by alkylating the bacterial RNA and DNA (38).

Depending on the risk of infection transmission exposed by certain instruments or surfaces different combinations of disinfection or sterilization have to be performed.

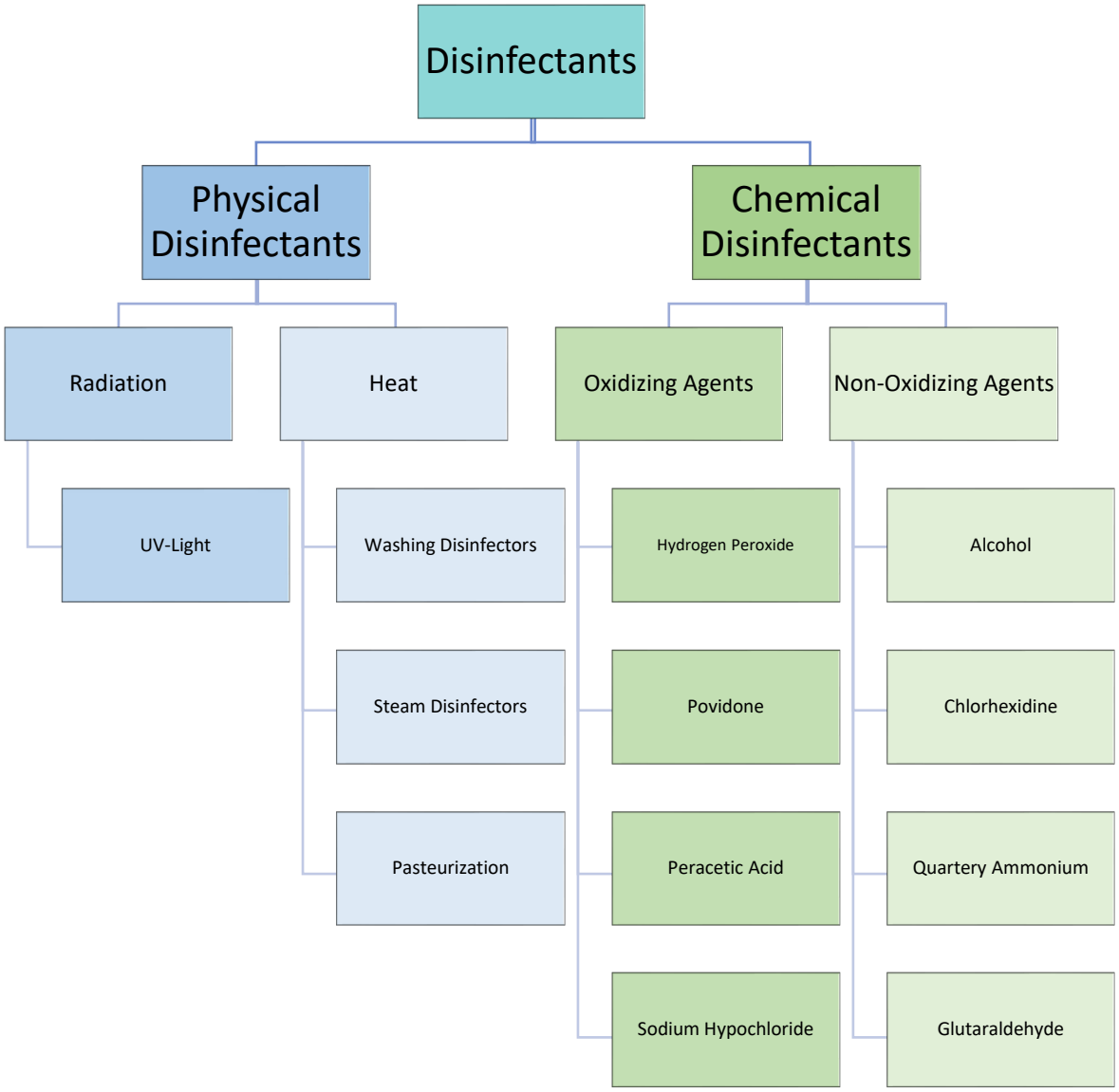

Figure 6: Overview on the most common disinfectants methods

3.2.2.2 Environmental Control

Environmental control encompasses methods like cleaning, disinfecting surfaces or filtering air and water with the goal to create a sanitary pathogen-free environment.

The principle behind cleaning and disinfecting surfaces in hospitals is that especially surfaces that are often touched by staff or patient hands (“high-touch surfaces) are contaminated with various microorganisms and pathogens and should therefore be regularly cleaned and disinfected to prevent the transmission of germs. For even low levels of microbial concentrations can lead to a transmission of infectious diseases (39).

It has been shown in several studies that disinfection of surrounding surfaces lowers certain infection rates. However, the exact extent of this measure is not yet enough researched (40,41).

The CDC has formulated guidelines on cleaning and disinfecting surfaces in healthcare facilities (36).

They recommend cleaning surfaces such as tabletops or floors on a regular basis (e.g. once or twice per day) and when visibly dirty. Disinfection of these surfaces is also recommended regularly (daily or every two days) and when visibly dirty. For non-critical surfaces and patient care items (e.g. blood pressure cuffs) they advise to use low-level disinfectants (disinfectants that destroy bacteria and viruses but not bacterial spores). Semi-critical patient care items such as endoscopes, respiratory equipment, etc. should be disinfected with high level disinfectants (disinfectants that destroy all microorganisms including spores). Low-level disinfectants include quaternary ammonium detergents, ethyl or isopropyl alcohol (concentration of 70-90%) and sodium hypochlorite with >100 ppm free chlorine. Hydrogen peroxide (7.5%) and glutaraldehyde formulas (>2%) and sodium hypochlorite (>650ppm) are considered high-level disinfectants (36).

3.2.2.3 Hand Hygiene

Healthcare workers hands are the number one vehicle for transmission of health care associated infections, being responsible for up to 40% of nosocomial infection (42,43).
Therefore a good compliance to hand washing and even more importantly hand disinfection is a major factor in preventing the transmission of pathogens via medical staff and other people (44).

The importance of hand cleanliness has been known for more than one hundred years now. In 1847, a time when microbes were not even discovered yet, Ignaz Semmelweis recognized that women giving childbirth treated by medical students who were coming straight from the autopsy room had a higher rate of puerperal fever than women giving childbirth treated by midwives. But after hand washing with chlorine this rate dropped to about the level of those of the midwives (45).
Today we know that our hands can be reservoir to many different pathogens like *Staphylococcus aureus*, *Enterococcus* or *Clostridium difficile* (46). We shed about 10^6 cells of skin from our hands daily which contaminate the surrounding objects and surfaces (14).
And these shed cells can lead to the transmission of the microorganisms residing on them and thus spread diseases.
However, hand washing and disinfection is one of the most effective and cheapest ways to stop transmission of microbes and to prevent Infections. Studies show that hand washing and disinfection significantly reduce the number of pathogens and also the number of healthcare associated infections. A 2008 meta-analysis of studies on hand hygiene came to the conclusion that appropriate hand hygiene leads to a 31% reduction of gastrointestinal illnesses and 21% of respiratory illnesses. Good hand hygiene education and usage of antibacterial soaps even lead to case reductions of 50 % (47).
The recommended and preferred substance for hand disinfection is alcoholic hand rub which is superior to hand washing with soap (42).

Due to the importance of hand hygiene the WHO has launched programs like *“Save lives: Clean your hands”* to reduce the rate of hospital acquired infections worldwide. They propose the five moments for hand hygiene which are: “*Before*

touching a patient, before performing a clean/aseptic procedures, after body fluid exposure risk, after touching a patient and after touching patient surroundings" (48). (Fig. 7)

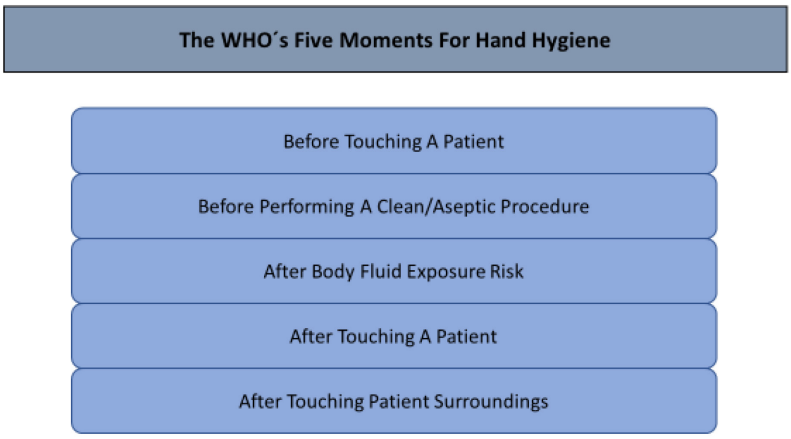

Figure 7: The WHO's 5 moments for hand hygiene according to their SAVE LIVES: Clean Your Hands campaign (48)

3.3 Antimicrobial Resistances

According to the WHO "*antimicrobial resistance is one of the biggest threats to global health, food security and development today*" (30).
The most prominent of antimicrobial resistances (AMR) are the resistance to antibiotics.
In the US approximately 2.8 million people get an infection caused by antibiotic resistant microorganisms per year and over 35,000 die (49). In Europe studies estimate that almost 700,000 infections caused by antibiotic resistant bacteria occurred in 2015, of which 63.5% were associated with health care. And more than 33,000 people are estimated to have died due to infections with antibiotic resistant bacteria in that time span (50).
Antibiotics are one of the most prescribed drugs in modern medicine. About one in three patients receives antimicrobial agents when treated in an acute care facility in Europe, with antibiotics accounting for more than 90% of these antimicrobials (51).

The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Antibiotics revolutionized medicine, and made it possible to treat bacterial wound infections, urinary tract infections, infections of the pulmonary tract, or any other type of bacterial infection more effectively than ever before.
Antibiotics tremendously changed the therapy and outcome of infectious diseases. In the 'Golden Era' of antibiotics which started in the 1940s, numerous new antibiotics like streptomycin, erythromycin and tetracyclines were introduced to the market. However shortly after each discovery and the use of antibiotics, also organisms resistant to those antibiotics were detected. The first resistances to penicillin were discovered in 1940 even before penicillin was used as a therapeutic (52).
Even for antibiotics that were especially designed for the treatment of drug-resistant bacterial infections (like vancomycin which was introduced in 1958 to treat methicillin resistant staphylococci and streptococci) resistant bacteria were discovered already a few years later (53).
In the early days of antibiotics this did not seem to be concerning because so

many new antibiotics kept coming to the market, that whenever there was a resistance, physicians could just prescribe a newer, maybe even more powerful antibiotic (54).
Unfortunately, the development of new antibiotics slowed down dramatically. And there is not much hope for a rise in discovery and development of new antibiotics as many big pharmaceutical companies choose not to invest in the field of antibiotics anymore because of increasing economic and regulatory obstacles (55).

Moreover, resistances against antibiotics have become much more common. Nowadays many bacteria are not only resistant to only one antibiotic but are multi resistant organisms. One example is MRSA which is resistant to an entire class of antibiotics namely ß-lactams and is responsible for many healthcare associated infections (54).

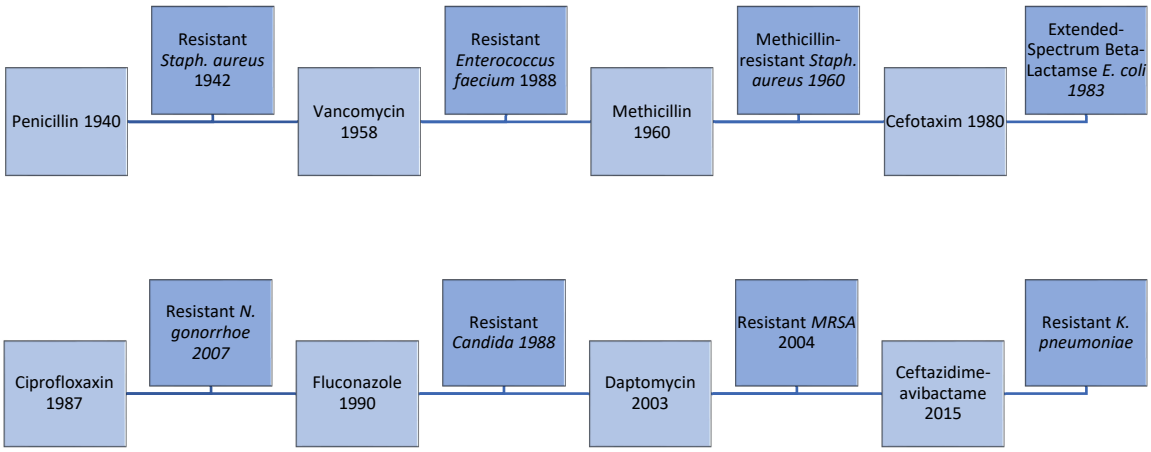

Figure 8: Evolution of antimicrobials and their resistances (56)

3.3.1 Resistance Mechanisms

Acquiring resistances is a normal evolutionary mechanism that microorganisms undergo when facing selective pressure: The ‘fittest’ bacterium, the one that is able to withstand the toxic influences, will survive. Resistance mechanisms to antibiotics have been found in 30,000 year-old permafrost and are believed to be millions of years old (57,58). Most of the antibiotics used today were detected in soil bacteria called Actinomycetes which produce antibiotic substances. Common antibiotics such as streptomycin, tetracycline or vancomycin derived from these bacteria. These bacteria themselves had to develop resistance mechanisms to their own substances in order to survive and are discussed to be the origin of antibiotic resistances (59). There is data that indicates that the old evolutionary mechanisms providing resistances were originally encoded on chromosomal genes but were over time transferred onto mobile genetic elements such as plasmids, thus enabling the spread of resistances between bacteria (60). Furthermore it is believed that most resistance features of pathogens were gained through horizontal gene transfer (HGT) from environmental microorganisms (61). However, even though antibiotic resistances are very old mechanisms that have been around for ages and not just emerged since the use of antibiotics, research suggest that the usage of antibiotics in agriculture and medicine has led to an increased prevalence of resistance genes as studies of the resistome of soil microorganisms have shown (62). This indicates that the excessive use of antibiotics leads to a selective pressure in the environment and as HGT constantly happens between bacteria the prevalence of resistance features increases and the environmental resistome transfers to pathogenic bacteria. Acquiring a mechanism to gain resistance may be good for the survival of bacterium itself but leads to a lot of problems in treating infections caused by these resistant bacteria.
There are several different resistance mechanisms, the most important ones will be described in more detail and are displayed in Figure 9.

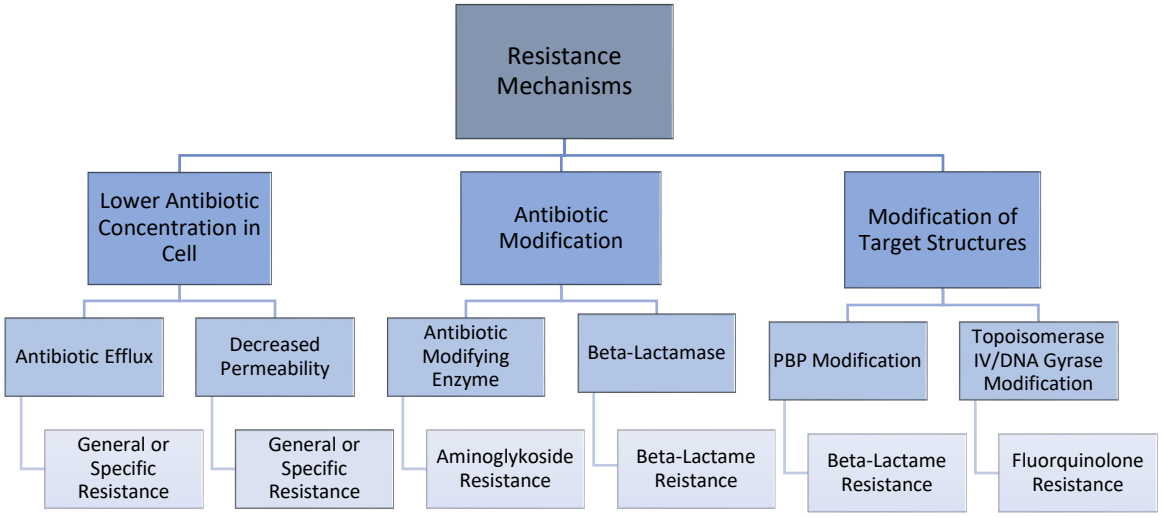

Figure 9: Antibiotic resistance mechanisms

3.3.1.1 Antibiotic Efflux

Using efflux pumps to transport antimicrobials out of the cytoplasm of a cell is a common mechanism to gain resistance to antibiotics. These pumps actively transport the antibiotic out of the cell using energy and are either ATP dependent (ABC proteins) or depend on an ion gradient (MFS, RND, MATE, SMR proteins) (63).

The genetic information for these pumps either is on intrinsic genes for example *norA* in *S. aureus* or *acrAB/tolC* in *E. coli* but can also be acquired. The pumps can also be either specific to a certain substrate that they can transport, or they are polyspecific which allows them to pump different kind of drugs across the cell wall (63).

Acquiring plasmids with information on resistance mechanism such as tetracycline efflux pumps is another way. This happens by acquiring certain genes e.g., *tet*-genes, coding for efflux pumps (64).

Another similar mechanism that also aims for obtaining a lower concentration of antibiotics in the cell is decreasing the permeability of the cell membrane as seen

in some bacteria, especially Gram-negative ones which have an additional outer membrane that decreases the permeability of their cell wall. Additionally, fewer porins or modifications of porins in the bacterial membrane can lead to a lower ability for antimicrobials that use these channels to cross the membrane and thus lead to antimicrobial resistances. This is seen in many different bacterial species for example *E. coli,* where mutations of the *ompF* gene which encodes for certain porins, leads to a lower susceptibility to certain beta-lactams (65).

3.3.1.2 Antibiotic Modification

Another common resistance mechanism is the modification of drugs to neutralize them. The most prevalent mechanism for resisting aminoglycosides is synthesizing aminoglycoside modifying enzymes (AME). AME catalyze the modification of the 2-deoxystreptamine nucleus of aminoglycosides or the sugar moieties of the aminoglycoside structure, thus rendering them ineffective (66). Another mechanism of antibiotic modification used by bacteria especially Gram-negatives is synthesizing β- lactamase which destroys the β-lactam ring of certain antibiotics the beta-lactams (54). The mechanism is explained in more detail further below.
There are many kinds of AMEs known today and many are able to be transferred to other bacteria by horizontal gene transfer making these resistance mechanisms quite common among bacteria.

3.3.1.3 Modification of antibiotic target structures

A further important mechanism to gain resistance to antibiotics is by modifying the biological structures that the antimicrobials target. These types of resistances are gained by synthesizing different proteins or enzymes that fulfill the same function but cannot be targeted themselves by antimicrobials or by protecting the targeted structure from the antimicrobials (63).
On a genetic level these resistances often are caused by point mutations in genes encoding for subunits of enzymes. These mutations lead to a slightly different enzyme which then cannot be targeted by antibiotics. An example of this

mechanism are bacteria that have a mutation on the *parC or gyrA* gene which leads to a slightly altered version of the enzyme topoisomerase IV or DNA gyrase, respectively. Thus fluoroquinolones that normally inhibit these enzymes cannot target them anymore (63,67).

Another example is the expression of Penicillin-binding-protein 2a (PBP 2a) which has a lower affinity to β-lactams than normal PBPs, thus making the organism resistant to those drugs (54).

3.3.2 Transfer of Resistance Genes

There are naturally two forms of genetic information that code for resistances in bacteria. Either intrinsic resistance genes or acquired resistance genes.
The intrinsic resistome describes naturally occurring genes coding for mechanisms that make bacteria resistant to certain antibiotics. It can be defined as "*the ensemble of chromosomal genes that are involved in intrinsic resistance and whose presence in strains of a bacterial species is independent of previous antibiotic exposure and is not due horizontal gene transfer*" (68).
Intrinsic resistances are often species specific and provide resistance to certain classes of antibiotics. These are mostly very old evolutionary mechanisms, encoded on chromosomal genes, and are not the result of the use of antibiotics as a therapeutic drug (69).
However, new intrinsic mechanisms can be acquired by spontaneous mutations in chromosomal genes.

The other form of resistance is acquired resistance. The mobile resistome is encoded on mobile antibiotic resistance genes which are able to be transferred from one bacteria to another and is thus called horizontal gene transfer (63).
The major mechanism of HGT of resistance genes is through mobile genetic elements (MGEs). These include transfer via plasmids, transposons, integrons, phages or integrative conjugative elements (70).
MGE transfer mechanisms can further be separated into transport within a cell like transposons and gene cassettes or transport from one cell to another as seen in plasmids or conjugative transposons. There are three ways of transport of mobile genetic elements from one cell to another (Fig. 10): transformation, transduction and conjugation (71).
Transformation describes the mechanism of free DNA being taken up by bacterial cells and then getting integrated in the cells own genome. However, this mechanism works only for DNA from the same or closely related species.
Transfer of bacterial DNA via bacteriophages, viruses that are specific to bacteria, is called transduction. This occurs when bacteriophage particles are unintentionally packed with bacterial DNA and upon infecting other bacteria the foreign DNA gets transduced into the new cell (71).

Conjugation is considered to be the most relevant mechanism for transfer of resistance genes. This mechanism uses conjugative plasmids or transposons to transport DNA from one cell to another (70).

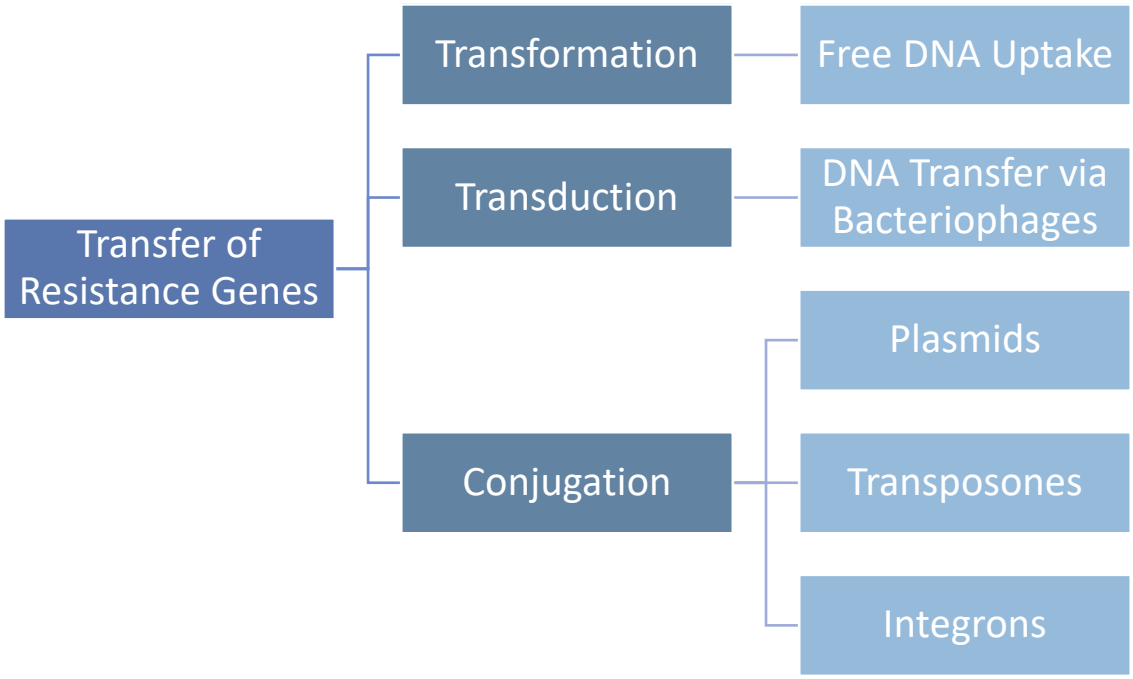

Figure 10: Mechanisms of horizontal gene transfer

3.3.2.1 Plasmids

Plasmids are double stranded, generally circular DNA molecules of different sizes that exist separately from the core DNA of a bacterial cell. They carry genes not coding for any essential functions of the regular bacterial activity but code for functions that can come in handy in certain situation for example genes carrying information about antibiotic resistances or resistance to toxic metals (70). Conjugative plasmids also carry genes which can mediate the coupling of cells and the following transfer of plasmids. These genes are called mpf *genes* (mating pair formation) and code for DNA transfer replication (DTR) proteins, e.g., relaxase, transferase and a complex called Type IV secretion system (T4SS). This complex is responsible for the transport of the DNA and the synthesis of pili. The transfer of plasmids requires direct cell-to-cell contact which is achieved through extracellular filaments called pili, which connects donor and recipient cell (Fig. 11) (72). The plasmid DNA contains a special sequence, the *oriT* gene (origin of

transfer). The relaxosome, a complex of relaxase and auxiliary proteins, recognizes the *oriT* gene and starts to cleave it at this site using the enzyme relaxase. After the cleavage reaction the plasmid's single strand DNA is transported to the T4SS and then transported through the channel into the other cytoplasm. There it is formed to a circular shape again and replicated to a double stranded DNA plasmid (73).

None-conjugative plasmids do not carry *mpf* genes and can therefore not mediate their own transfer. However, they can also be transferred with help from conjugative plasmids that initiate the transfer.

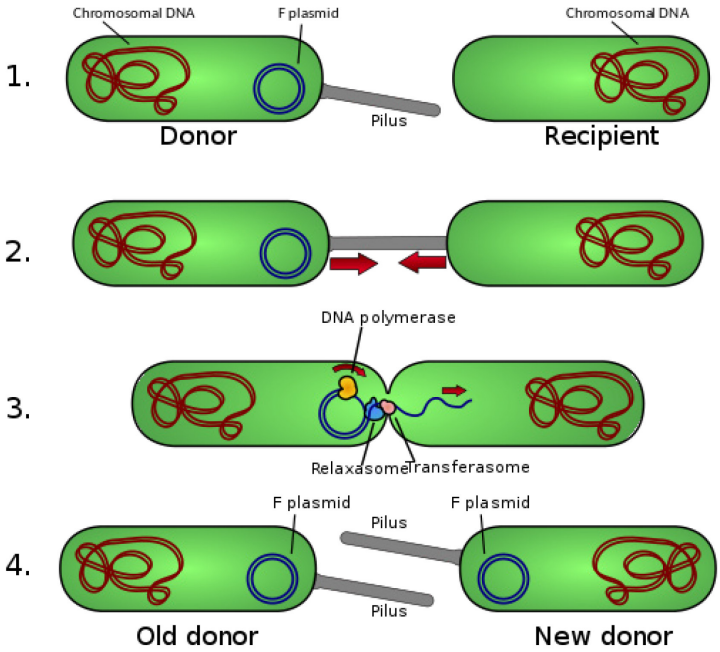

Figure 11: Bacterial conjugation (Conjugation by *Adenosine, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via* Wikimedia Commons, 2018 (74)

Example for resistance mechanisms that can be transferred via plasmids are *mls* resistances (macrolides, lincosamides, streptogramines), or vancomycin resistances in enterococci. Also plasmids encoding aminoglycoside resistance in staphylococci or streptomycin and lincomycin resistance in *Acinetobacter baumanii* and many more have been detected (75).

3.3.2.2 Transposons

Transposons are small mobile genetic elements that are able to move around the genome freely. They can jump almost freely between different DNA molecule locations, between different plasmids or between plasmids and bacterial chromosomes (70).

The main parts of transposons are the insertion sequences. The insertion sequences consist of a gene (sometimes two) coding for the transposase which is needed for transposition and is flanked by terminal inverted repeats. Inverted repeats are nucleotide sequences that are mirroring each other in terms of nucleotide order. The insertion sequences can follow either a conservative mechanism of transposition, meaning they are cut out of their current location and inserted into another DNA site (cut-paste mechanism) or a replicative mechanism, either copy- paste mechanism or copy-out-paste-in mechanism, leaving a copy of the transposon in the old location and at a new site (Fig. 12). If two same or similar kinds of insertion sequences flank a strain of DNA e.g., a resistance gene, they can move that gene with them from one site to another. These are named composite transposons. Transposons are responsible for many antibiotic resistances such as kanamycin, neomycin or tetracycline resistance in many Gram-negative bacteria and are encoded on the transposons *Tn5 and Tn10*, respectively (70).

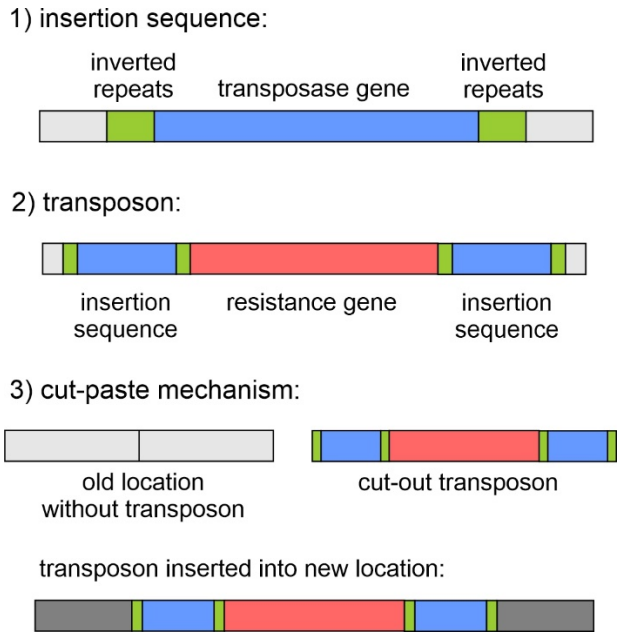

Figure 12: Transposon & cut- paste mechanism

3.3.2.3 Integrons and Gene Cassettes

Bacterial integrons are rather small DNA sites that are able to capture DNA sequences or gene cassettes from other location and integrate them. Gene cassettes are small mobile genetic elements that usually consist only of one gene. They do not carry a promoter site on them and are therefore unable to express the gene they carry on them. Thus, they need to be located on an integron, which has these promotor regions to express what they encode (70).
The *int* gene of integrons encodes for a specific enzyme, the integrase, which can detect the gene cassette at its *attC* site and then can insert it into the DNA of the integron at the insertion site *attI*. After the insertion of the gene cassette the integrons promoter (Pc) enables the gene encoded, e.g., a resistance gene, on the gene cassette to be expressed. Even cassette arrays encoding multiple resistance mechanisms can be expressed this way if multiple gene cassettes coding for resistance mechanisms are inserted into one integron. Gene cassettes are responsible for many different antibiotic resistances such as resistance to β-lactams or resistance to aminoglycosides (70,75).

3.3.2.4 Genomic Islands

"*A genomic island is a distinct region of a bacterial chromosome that has been acquired via horizontal transfer,…"* (75).
Genomic islands can consist of numerous different genes and can be further divided into subclasses based on the genes they carry. A GI with lots of resistance genes are named resistance island.
An example for a resistance island is the Staphylococcal Cassette Chromosome (SCCmec) which encodes resistance to β- lactams of all kinds.

3.3.3 Clinically Important Antibiotics and their resistance mechanisms

3.3.3.1 B-Lactam Antibiotics

Beta-lactam antibiotics are a group of antibiotics which share a common molecular structure, a so-called β-lactam ring. There are four subgroups: Penicillin, cephalosporin, monobactams and carbapenems.
They are the most used and produced group of antibiotics and make up about 65% of the world market for antibiotics (76).
Most of the β-lactams work by inhibiting the biosynthesis of the cell wall of the bacteria. They bind to penicillin-binding proteins (PBPs) and inhibit the synthesis of peptidoglycan, which is part of the cell wall. However, many bacteria evolved resistances to this mechanism. Either by producing an enzyme called β-lactamase which leads to the destruction of the β- lactam ring or by reducing the access or the binding affinity to the PBPs (54).
Due to the outer membrane of Gram-negative bacteria which acts as an additional diffusion barrier, it is generally harder for β-lactam antibiotics to reach the PBPs of Gram-negative bacteria compared to gram-positive bacteria (54).

3.3.3.1.1 Beta-Lactam Resistances of Gram-positives

The most important form of resistance of Gram-positives to β-lactams is the expression of a special variation of PBP. The variation PBP 2a for example has a lower affinity for β- lactams. This leads to a decreased binding of almost all β-lactams to the PBP 2a which can continue to produce peptidoglycan and therefore ensure the survival of the cell (54).
Bacteria resistant to all β- lactams are so called β- lactam or methicillin resistant bacteria, for example MRSA.
PBP 2a is encoded on the *mecA* gene which is located on the SCC*mec* (Staphylococcal Cassette Chromosome) island alongside its regulatory genes *mecR1* and *mecI*. This SCCmec is presumed to be a mobile chromosomal

element and therefore be able to be transferred between different *Staphylococcus* strains. Thus it is believed to contribute to the spread of methicillin resistance (77).
Another way for gaining resistance is synthesizing a β-lactamase as found in some staphylococci, destroying the β-lactam ring of the antibiotic. However, this lactamase is often a very specific penicillinase and therefore only effective against penicillin.
Enterococcus faecium uses PBP5 to gain resistance to β-lactams, which same as PBP 2a has a low affinity to β-lactams. PBP5 is intrinsic to *E. faecium* but transferable between different strains (78).
Another way of acquiring resistance is through expressing mosaic genes which is seen in species like *Streptococcus pneumoniae* or *Neisseria gonorrhoe*, which expresses a mosaic *penA* gene meaning multiple genetic changes in the gene. This *penA* then leads to an altered PBP2 variation (79,80).
Moreover, *N. gonorrheae* has been observed expressing efflux pumps, which actively pump the antibiotics out of the cell. Mutations of certain genes, in this case the *mtrR* gene, can lead to an increase of the pumps and therefore to an increased efflux resulting in a higher resistance to antibiotics. Furthermore, a mutation in the *penB* gene can lead to a lower permeability of the outer membrane for antibiotics (79).

3.3.3.1.2 Beta-Lactam Resistances of Gram-Negatives

Gram-negative bacteria, besides being generally more resistant due to their outer membrane, acquire resistance mostly through expression of β-lactamase. The Beta-Lactamase DataBase (BLDB) currently counts an overall number of more than 7000 different enzymes (81).
Beta-lactamases generally work by hydrolyzing the β-lactam ring of the antibiotics. The Ambler classification describes four different classes of lactamases (A,B,C,D) (82).
Class A describes penicillinases and these were first reported in plasmids of Gram- positives, but are also described in Gram-negatives. They were the first β-lactamases that occurred after the first antibiotics were introduced. They are mostly encoded on the TEM-1, SHV-1 and CTX-M genes which are plasmid-

borne and can be found all around the earth and in many different species of bacteria (83).
Class B β- lactamases are also called carbapenemases and are metallo β-lactamases whereas the others are serin lactamases. They are able to hydrolise the metal containing ring of carbapenemes.
Class C describes cephalosporinases and are present in most Gram- negatives. They were originally encoded on chromosomes but also are plasmid borne.
Class D enzymes are Oxicillinases and their lactamases are generally encoded on OXA genes, which are either located on plasmids or in chromosomes (54).

3.3.3.2 Glycopeptides

Glycopeptides work by binding to the D-alanyl-D-alanine termini of the peptidoglycan precursors and by this inhibiting the transpeptidase and transglycolase which are essential for synthesis of the cell wall by cross linking the peptidoglycans (54).
Especially enterococci have developed mechanisms for resistance to glycopeptides like Vancomycin or Teicoplanin. VREs (Vancomycin resistant enterococci) do so by producing an alternate peptidoglycan precursor ending with D-alanyl-D-lactate instead and therefore having a much lower affinity to the antibiotics. Responsible genes are among others *vanS, vanR* and *vanHAX* (84).
And although the possibility of transfer of these genes to other species of bacteria like staphylococcus aureus has been shown in experiments this does not seem to have happened on a large scale in nature.

3.3.3.3 Fluoroquinolone Resistance

The mechanism of fluoroquinolones is inhibiting the DNA synthesis of the bacteria by interacting with the DNA gyrase and the topoisomerase IV. This mechanism leads to an inhibition of the supercoiling of bacterial DNA which then inhibits the replication of the bacterial DNA which first results in a cell not being able to replicate and ultimately in the death of the cell (85).
Amino acid changes in certain subunit genes like *gyrA* or *parC* lead to a mutation

of the subunits which prevents the quinolones from binding to the gyrase and topoisomerase. Other mechanisms are an increased amount of efflux pump which actively pump the antibiotic out of the cell, generally the pumps are not specific to fluoroquinolones but are so called multi drug resistant pumps (85).

3.4 Resistances to disinfectants

Bacteria naturally bear or have developed not only resistances to antibiotics but also resistances to disinfecting agents. Especially spores are resistant to many disinfecting agents.

The basic principle of most of the resistance mechanism is to lower the concentration of disinfectants in the cytoplasm. This can be achieved through several different ways such as changes in the cell walls or membrane with decreased permeability or higher cell efflux (86).

Gram-negative bacteria often are more resistant than Gram-positives due to structural advantages such as their outer membrane which acts as an additional diffusion barrier or efflux pumps in the outer membrane that pump antiseptics out of the periplasm and thus decrease the amount of antiseptics reaching the cytoplasm (87). Bacterial species like *P. aeruginosa* or *Proteus* spp. can show high levels of resistance to disinfecting agents (88).

Endospores are special cell forms that can only be developed by some Gram-positive bacteria like certain *Bacillus* or *Clostridium* species. They have no or a very reduced metabolism and a very thick peptidoglycan cortex and a protein coat that protects them from almost all environmental hazards and makes them resistant to many antiseptics. These forms are usually developed when the bacteria encounter a low nutrient supply in their environment (88).

Mycobacteria are another example for bacteria with a high intrinsic resistance due to their complex structure of their wall which reduces the permeability for antiseptics. Substances like chlorhexidine or quaternary ammonium compounds are rather ineffective against mycobacteria and even in very high concentration do not reach a bactericidal level but stay bacteriostatic. Responsible for the resistance is the high amount of lipids in the cell wall resulting in a very hydrophobic cell wall which prevents hydrophilic substances from entering the cell (88).

However, besides this natural resilience of certain microorganisms to disinfectants, they can also acquire new resistance mechanisms to disinfectants by horizontal gene transfer. This is achieved by the same mechanisms as seen in antibiotic resistances. There are plasmid-mediated acquired resistances to disinfecting

agents like mercury, silver or quaternary ammonium compounds (70).

A different mechanism that helps bacteria to withstand disinfecting agents is by producing a biofilm that protects them not only from disinfectants but also from other agents like antibiotics.

3.5 Biofilms

„Biofilms are multimicrobial communities enclosed in self-synthetized polymeric matrices, attached to biotic or abiotic surfaces” (89).
Today we know that up to 80% of the bacteria live in biofilms which makes it the most common form of bacterial state in nature. They do so because biofilms offer a lot of advantages for bacterial cells. However not all accumulations of cells attached to a surface fit the criteria of a biofilm. Biofilms are not only cells sticking together, they are complex and dynamic structures which also change the behavior of the bacterial cells in it e.g. the genes they express, their growth rate and their intercellular interaction (90).
Biofilms are preferably formed in so called high shear environments meaning environments where high shear forces are present as seen e.g. in rapidly flowing milieus (91). And although biofilms were mainly believed to be associated with wet and rough surfaces recent research has shown that they also occur on smooth and dry surfaces (92).
These multimicrobial communities not only include different species of bacteria. Other microorganisms such as fungi, algae, archaea and viruses have also been found in biofilms (92).

3.5.1 General Structure

There is no uniform, standard biofilm, every biofilm is unique depending on the bacterial species forming it and also the environment in which it occurs (93). However certain features are seen in every biofilm and define biofilms.
One main feature of biofilms is the extracellular matrix, often composed of polysaccharide biopolymer. This matrix surrounds the cells, sticks them together and protects them from harmful agents. It is produced by the bacteria in the biofilm. There are many different polysaccharides that can be found in biofilms e.g., cellulose, an important component of many matrices which is secreted by bacteria such as *Gluconacetobacter xylinus* or *Escherichia coli*. Other species like *Pseudomonas aeruginosa* can produce different polysaccharides like alginate, PSL polysaccharid (polysaccharide synthesis locus), which is high in mannose, or PEL (pellicle) polysaccharide containing a lot of glucose. One further especially

clinically important extracellular polymer is polysaccharide intercellular adhesion, short PIA, which is produced by species like *Staphylococcus aureus* and *Staphylococcus epidermis* (94).
The exact composition of the biofilm depends on the bacterial species present in a biofilm and what kind of extracellular matrix these species produce. However, bacteria of the same species can also produce different kinds of polymers, for example when different genes encoding for different polysaccharides are expressed.
Moreover, the extracellular matrix does not only consist of polysaccharides, additional components found in it are, among others, proteins and DNA. Proteins such as the biofilm- associated proteins (Bap) or enterococcal surface protein (Esp) are often essential for certain biofilms to be formed. If genes encoding these proteins are knocked out some bacteria lose the ability to form biofilms. Other biofilms rely on extracellular DNA, eDNA, to form solid and sturdy biofilms (93,95).

3.5.2 Development

There are several steps biofilm formation. They can be described as following different states: planktonic, attachment, microcolony formation, macrocolony and dispersal state (96). However, the real development is probably not stepwise but occurs rather as a continuous process (Fig. 13).
At first planktonic bacterial cells form cell- surface contacts and adhere to a surface and become sessile cells. These cells then start to grow and divide and other free- living bacteria form cell-to-cell contacts with the attached bacteria, leading to small groups of cells, so-called microcolonies that are attached to a surface. The microcolonies then grow and mature forming macrocolonies. When cells have adhered onto a surface, they start producing extracellular polymer, forming a mature and developed biofilm. Finally, single cells or small cell groups detach from the biofilm returning to the planktonic state again and thus reentering

the cycle of biofilm formation at the beginning (96).

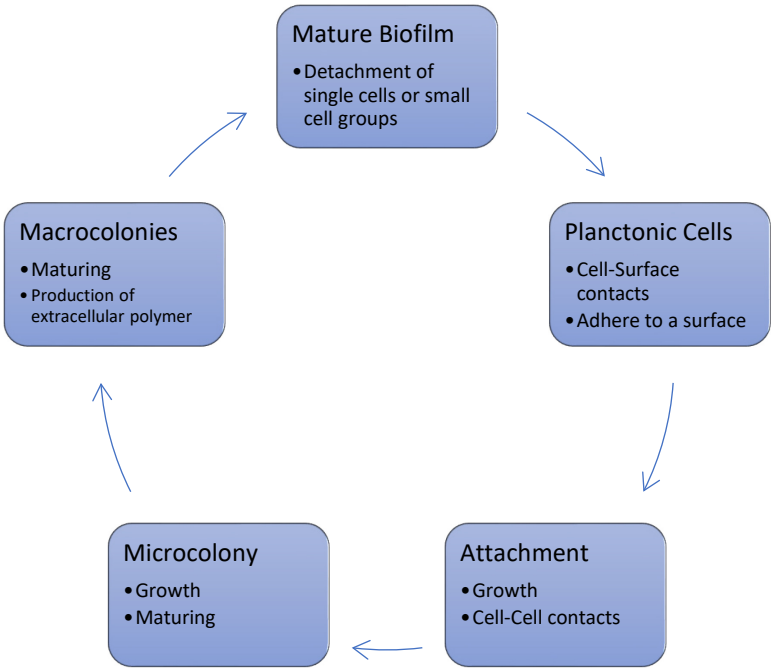

Figure 13: Biofilm formation

3.5.3 Resistances

The formation of a biofilm brings many advantages to the bacterial cells living in it. Among them is an increased resistance to antimicrobial substances which leads to many problems regarding infections caused by biofilm bacteria. The minimal bactericidal concentration for biofilms can be as much as 1,000 times higher than the minimal bactericidal concentration for planktonic phenotype (91).
Biofilm bacteria are also less susceptible to UV- light, pH-gradients, toxicity and other harmful environmental stressors (96).
There are different mechanisms that provide biofilms with an increased level of resistance. The extracellular matrix acts as a diffusion barrier for antimicrobial substances thus decreasing the transport rate of the substances. Together with a changed cell density this leads to a decreased penetration of the cell wall (91).
Another mechanism that can increase the antimicrobial resistance is the slower growth rate of bacteria in biofilms, leading to a slower metabolism and a lower uptake of substances (97).
Moreover acquired resistances also play an important role in biofilms. The rate for

horizontal gene transfer is higher in biofilms than it is in non-biofilm microbial cultures. Reasons for this are the high density of cells in biofilms with sometimes many different microorganisms in them as well as an increased level of mutations in the biofilm organisms. Furthermore, also the protection of the cells in the biofilm from environmental forces, e.g., shear forces that can separate conjugated bacteria during gene transfer, may lead to an increased horizontal gene transfer. All these factors lead to a higher level of acquired resistance and thus to higher resistance to antimicrobial substances itself (92).

3.5.4 Health Issues

All those factors explain why biofilms play such an important role in our health care system and why biofilms are clinically relevant. Biofilms attached to medical devices such as catheters or biofilms forming on prosthetic elements such as artificial heart valves cause major problems for the health care system. Biofilms also play an important role in infections like bacterial endocarditis, cystic fibrosis pneumonia and periodontitis. Furthermore, the increased level of resistance of biofilms against disinfecting agents, cleaning detergents and other antimicrobial substances also contributes to the health hazard presented by biofilms (92).
The prevalence of biofilms in patients' surroundings in hospitals seems to also have an impact in increasing hospital associated infections (98). This study also comes to the conclusion that biofilm bacteria occur on more than 90% of surfaces found in an ICU among them many pathogenic and multi drug resistant strains. Furthermore, biofilms may be responsible for approximately 60% of nosocomial infections (99).
Other problematic environments concerning biofilms are surfaces in water pipes or in food industry, which may also play a role in the spread of infections (89).

3.5.5 Biofilm control

The insusceptibility of biofilms to all kinds of antimicrobial substances obviously raises a lot of questions of how to best prevent biofilm development and how to

eradicate established biofilms. Strategies to prevent and remove biofilm formation underline the importance of regular cleaning of the surfaces to prevent the attachment and the importance of mechanical force to remove biofilm from the surfaces (100).

New materials for surfaces, such as liquid glass, special polymer coatings or copper and silver surfaces might also proof beneficial for a successful prevention of attachment but are not yet fully studied and developed (92).
Other novel approaches suggest the additional usage of enzymatic digestion e.g., usage of alginate lyase and DNase to break down the extracellular polysaccharides and reach the cells or the usage of substances that inhibit signaling molecules e.g., quorum sensing inhibitors, and thus disrupt the biofilm development (101,102).

3.6 Allergies

A rather new health concern in contrary to infectious diseases is the rise of allergic diseases over the last century. Diseases like allergic rhinitis, asthma, eczema and food allergy have risen to levels never seen before. However, these diseases did not rise simultaneously but one after another. Hay fever started in the mid-1800s and peaked in the 1940s, increases in pediatric asthma were first seen in the 1950s and reached its plateau in the 1990s and a rise in food allergies like peanuts has been observed since the 1990s (103).

The causes for this rise have been heavily debated in the scientific community. Different hypotheses have been proposed to explain this: From shifts in our hygiene standards, to the change in our behavior e.g. the time we spend outdoor per day or the change in the food we consume compared to earlier centuries.

The 'hygiene hypothesis' proposed by Dr. Strachan in 1989 stated that the rise in allergic diseases could be caused by reduced exposure to infectious microorganisms and thus fewer infections in childhood (104).

This theory of too stringent hygiene regimes has been accompanying the public since then and has created dangerous theories that childhood diseases like measles might be essential for the development of the immune system. However studies have shown that childhood infections do not prevent allergic diseases (105).

A newer theory suggests that not infectious microorganisms but mainly non-pathogenic microorganisms i.e., the microbes living on skin and in human guts or just around us, are necessary for a normal development of our immune system. These 'old friend' microorganisms seem to be important for a normal and not overshooting activity of our immune system. Contrary to the 'hygiene hypothesis' which stated that the contact with pathogens leads to a normal development of our immune system, newer studies however show that especially the diversity of different harmless microorganisms encountered in the early childhood helps to develop a normal regulation of our immune system (106).

Contact with these 'old friends' seems to be especially important during pregnancy, delivery and the early infantry. Several studies show that influences like natural delivery, breastfeeding, living on farms or on the countryside, having siblings, contact with animals, spending time outdoors and other factors that

broaden the diversity of microorganisms people are exposed to, benefit the immune system in terms of a regular function (106).

What seems to be especially important is the development of a healthy and diverse gut microbiome. The diversity of the gut microbiome depends among other factors on a healthy and balanced diet (107) and is disturbed by an excessive use of antibiotics. Studies have shown that the use of antibiotics especially during pregnancy and early infantry can have long lasting effects on the gut microbiome and thus lead to a higher risk of developing an allergic disease (108,109).

What does not seem to affect the likelihood of allergic disorders in contrary to the ‘hygiene hypothesis’ is the level of personal hygiene and the level of cleanliness in our homes. Modern research suggests that the shift to different kinds of microorganism we encounter today compared to the species of microbes we shared our environment with one century ago, may be a more important factor than the number of microbes on and around us (106).

Moreover the approach to increase our encounters with these ‘old friend’ microbiomes must not be confused with lowering certain hygiene standards such as hand hygiene or cleaning high-touch surfaces, which are essential for preventing the transmission of infectious diseases. The goal should be to diversify the contact with harmless microorganisms to develop a well-regulated immune system, without increasing the contact with infectious pathogens that cause diseases but do not lead to a better regulated immune system.

3.7 Indoor Microbiomes

The next chapter focuses on specific indoor microbiomes of built environments and will particularly address the following questions: What are the distinctions between different indoor microbiomes? What are the main factors influencing the microbiomes? And what do the resistance profiles of the specific microbiomes look like?
The habitats in built environments differ greatly from those that occur naturally in the outdoor world, which leads to changes in the microbiomes inhabiting them. Moreover, the built environment habitats can also be quite dissimilar from each other. Built environments can be in contact with outdoor environments or quite confined; they can be inhabited by many people or by none; they can be situated in different geographic locations from rural villages to urban cities or even in space.

As there are many different factors influencing the composition of indoor microbiomes it is impossible to say what an average indoor microbiome looks like. However, there are several phyla of bacteria e.g., Firmicutes, Proteobacteria and Actinobacteria, which are very common in most indoor microbiomes and make up a big proportion of the microorganisms inhabiting the indoor microbiomes (110).

3.7.1 Private and Public Houses Microbiome

Since we spend a lot of time indoors in our homes or in public houses such as offices, schools etc. it is important to know about the microorganisms that we share our homes with and what influences them.
Research suggests that the microbiomes on the different surfaces in a normal family houses largely depend on the people living in those homes. People transmit their own unique microbiome to their surroundings as shown in the ‘home microbiome project’ (111). The project showed that the people living in a house are the main factor for the composition of the house´s microbiome and after only one day when moving into a new home its microbiome matches the microbiome of the old home. And when the inhabitants of the new houses left their homes for a longer period of time, their distinct human associated bacteria decreased until they

returned (111). Only approximately one third of all the taxa found in the study were detectable in all the different homes, but these taxa accounted for the vast majority (~95%) of all sequences found. This means a small proportion of species of microorganisms makes up a big percentage of the number of microorganisms. The study also showed that our own microbial profile depends on who we share our home with, as it is more similar between people living in the same home. Moreover, the similarity of the microbial profiles is greater among different sites in the same house than among the same sites (e.g. the bathroom floors) in different houses. This again indicates that the most important factor for the microbiome in a private house is the people living in it (17).
It is also debated how much the number of people living in a house, the occupancy and the sex of these people contribute to differences in microbiomes (12).

When looking at the general composition of the microbiome of private or public houses, the number of bacteria seems to be up to 100 times higher than the number of eukaryotes (112). Among these bacteria it is generally seen that the most common bacterial phyla and bacteria classes in family homes are Proteobacteria (Alpha-, Beta- and Gammaproteobacteria), Firmicutes (Bacilli and Clostridia), Actinobacteria, and Bacteroidetes (Bacteroidia, Flavobacteria and Sphingobacteria) (111,112). Firmicutes were often the most abundant phyla in private houses with a proportion from 30% to up to more than 50% in these studies. Proteobacteria were also quite common with an abundance of 20-35%. The third big group was Actinobacteria. These three phyla were dominant in private houses as well as in public buildings. Together with Bacteroidetes they account for approximately 90% of the bacteria found in these built environments. The different numbers in these studies indicate that generally these phyla and classes make up the majority of the indoor bacteria, the exact numbers however vary between the indoor microbiome depend on multiple influencing factors (113). When looking at the level of bacterial classes, Alpha-, Beta- and Gammaproteobacteria are the most abundant ones, as well as Bacilli and Clostridia (both Firmicutes), Actinobacteria (Actinobacteria) an Bacteroidia (Bacteroidetes) (111).
Generally speaking, the indoor microbiome of private homes and public houses consists of more Gram-positive bacteria than Gram-negatives and genera like

Janibacter and *Arthrobacter* were found to be more specific for in public buildings e.g., buildings used for schooling, whereas genera like *Enhydrobacter, Kocuria* and *Panotea* were more specific for private homes (112).

A different study observed the effect of urbanization on the indoor microbiome by comparing very rural buildings like jungle huts to semi urban buildings and further to western style urban buildings. It found that with increased urbanization the amount of skin associated taxa increased, even though on average the number of people per house decreased with increasing urbanization. This is most likely a result of an increased isolation from the outside in more urbanized buildings, limiting the influence of outdoor factors (114).

3.7.1.1 Indoor Air Microbiome and where it comes from

The main sources for airborne microorganisms in houses are humans, ventilation systems, plumbing systems, heating, outdoor air, mold, pets, plants and settled dust that is whirled up again (3).
The indoor air microbiome of unrestricted buildings generally seems to resemble the outdoor air microbiome and changes in the outdoor microbiome also lead to changes in the indoor microbiome. However the levels of human microbiome associated bacteria show a far greater abundance in the indoor air microbiome than in the outdoor air microbiome (11).
Studies showed that the impact of people on the air microbiome rises with the number of people in a room and decreases with increased ventilation. Depending on these factors an indoor microbiome can look either similar to an outdoor microbiome when few people are in the building and when it is well ventilated. But it can also differ significantly from the outdoor environment when more isolated from the outside especially regarding the levels of human microbiome associated bacteria (20,115).
The mode of ventilation also has a big impact on the indoor microbiome. Depending on the amount of ventilation and the type of ventilation whether it is window ventilated or mechanically ventilated and the air is filtered, certain bacterial species have been shown to be almost twice as abundant in a mechanically

ventilated or window ventilated room compared to the other one (21). This leads to indoor microbiomes being quite distinct from one another and either resemble outdoor air or are quite different from it.

When comparing the diversity of indoor and outdoor microbiomes it has been shown that the indoor microbiome of normal house dust is more diverse than outdoor dust. A possible explanation for this is that indoors, in addition to outdoor microorganisms which are transmitted into the house, also indoor specific microorganisms inhabit the indoor environment (12).
Research suggests that indoor air contains ten times more bacteria than outdoor air, on the contrary, fungal levels are up to 50 times higher in outdoor air (116). Most fungi that are found in these indoor environments come from the outside and only a few sources of fungi (mold, plants, human associated fungi, etc.) appear to reside inside a house. Therefore, the similarity between the indoor and outdoor fungal communities is quite high and exceeds the similarity between the indoor and outdoor bacterial communities (116).
When comparing indoor and outdoor bacteria, especially bacterial species associated with human skin or gut such as *Staphylococcus, Streptococcus, Lactobacillus, Corynebacterium* or *Faecalibacterium,* these bacteria are far more abundant indoors than outdoors (12).
Humans have been shown to emit as much as 37×10^7 bacterial genome copies per hour into the surrounding air, approximately 18% of them being human skin associated bacteria (117). Other microorganisms in the indoor air seem to be microorganisms that are resuspended from settled dust or microorganisms from the outside that are transmitted into the buildings.
It has been shown that humans and their clothes can act as a transport vector for certain microorganisms from one place to another (118).
Although we know that people have a big impact on the indoor microbiome it remains unclear to what extent people influence the microbiome and which so far unknown factors additionally contribute to the composition of these microbiomes.

Another factor that has been shown to significantly influence the composition of the indoor microbiome is pets. Increased numbers of *Porphyromonas, Moraxella,* and *Prevotella* species associated with pets have been found in houses with cats

or dogs. On basis of the microbiome one can also quite accurately predict whether there are pets in the house and what kind of pets there are (12).
Other sources of microorganisms are plants, which do not only influence the indoor microbiome by being carried into a building from the outside on shoes or by air but also indoor plants like pot plants seem to have effects on their surroundings. Plant associated microorganisms lead not only to an increased biodiversity in homes but plants themselves can filter certain contaminants out of the air and lead to a change in their microbiome (119,120).
Furthermore, microorganisms which inhabit humid microenvironments in homes such as toilets or showers can be aerosolized. Bacterial species such as *Legionella* can be aerosolized when showering and gut associated bacteria like *E.coli* have been shown to be detectable as bioaerosols after flushing the toilet (3). Also, other sources like mold, improperly maintained heating ventilation and air conditioning (HVAC) systems, areas of water suspension and dust resuspension play a role for the exact composition of the indoor air microbiome (3).
In conclusion, the air microbiome of buildings is a mixture of many different microorganisms from many different sources. Although further research has to be done on to what extent exactly the different sources contribute to the composition of this microbiome the two major factors seem to be microorganisms from outdoor air and human associated microorganisms.

3.7.1.2 Resistome of private and public building microbiome

For understanding and to increase the knowledge of our surrounding microbiome we obviously not only have to study which microorganisms make up our environment but also what features our surrounding microorganisms have. And one of these major features is the resistome of said organisms. This question is very important to understand the microbiome and perhaps also to be able to control it and shift it to a way that is beneficial for the inhabitants. However, since the whole field of indoor microbiomes is quite new, information about the resistome of indoor microbiomes in private and public buildings is even scarcer.
A study comparing microorganisms of unrestricted buildings with confined buildings (hospitals, cleanrooms) showed that bacteria in public and private houses have a lower diversity of resistances than bacteria in confined buildings (CB). Moreover microorganisms in unrestricted buildings showed mostly a

resistance to drug classes such as aminoglycosides, diaminopyrimidines and macrolides (112).

3.7.2 Hospital Microbiome

Hospitals have many preconditions that differ vastly from other built environments like public or private houses. First, a hospital is home to many sick people with infections who naturally emit a lot of infectious particles through e.g., coughing, sneezing, vomiting, bleeding. Secondly, hospitals also house people with very weak immune systems who are in danger of getting an infection even from opportunistic pathogens which normally would not pose a threat to immunocompetent people.
Thus, to prevent pathogens from spreading and sickening patients, establishing quite harsh cleaning protocols, including the application of disinfectants to eradicate these infectious microorganisms and create an environment with as few microbes as possible, is recommended by organizations like the CDC (36,121). These measures induce a lot of stress and selective pressure on the microorganisms. Together with other factors like restricted access to certain areas and often only mechanical ventilation this leads to a decrease in the diversity of the indoor microbiome of hospitals. One study found that in hospitals the diversity of microorganism is 50% less than in normal public or private houses (112). However, there are again many factors that influence the hospital microbiome and affect its diversity and composition, thus it is hard to deduce generally valid assumptions.

A study by Lax *et al.* in 2017 observed a newly opened hospital for one year and tracked the shift of the hospital microbiome during this period. It was shown that after the opening the abundance of human skin associated bacterial genera such as *Corynebacterum, Staphylocoocus* and *Streptococcus* increased on floors and contact surfaces while observing a decrease of the *Actinetobacter* and *Pseudomonas* species which were the dominant genera before the opening (122). It was also observed that the microbial profile on staff-associated surfaces resembled the microbial communities of the staff and the Shannon diversity index increased on these surfaces while staying at the same level on floors. On contrary the microbial community of a patient's rooms became similar to the microbiome of the patient occupying the room especially after the patient had spent a night in the room. However, also a change in the patient's skin microbiome could be observed

as bacteria associated with the room's initial microbial profile were also more abundant on the patient's skin after entering a room (122).
Moreover, it has been shown that differently ventilated rooms in hospitals show very distinct microbiomes. Window ventilated rooms were more similar to outdoor air than mechanically ventilated rooms. Further, these rooms also showed a greater diversity in their microbiome (21). Also, the composition of the microbiome shifted to Betaproteobacteria and several human-microbiome associated microorganisms were found to be more abundant in mechanically ventilated rooms. The study also found that the abundance of pathogens depends on the air flow rate and not on the type of ventilation (21).

3.7.2.1 Intensive Care Unit Microbiome

Intensive care units (ICUs) are special departments in hospitals where patients with life-threatening diseases are treated. These departments undergo even harsher cleaning protocols and the access to ICUs is generally very limited for outsiders and visitors to protect the severely weakened patients from acquiring new infections (123).
But nonetheless health care associated infections acquired in ICUs are a global concern to health agencies as the CDC estimates that about 8% of patients get an HAI when staying in an ICU for more than two days (24).
When looking at the microbiome of an ICU we see that as a result of more confinement and the frequent and aggressive cleaning the microbiome diversity was lower in the ICU than in other indoor environments. Nonetheless surfaces are still quite full of microorganisms with up to one hundred different genera living on a surface. In general ICUs show a greater abundance of microbes associated with the human microbiome than other indoor microbiomes. The main bacterial phyla similar to other human associated environments still seem to be Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria (124,125).
However a shift towards more Gram-negative bacteria has been observed and the proportion of bacteria to eukaryota also is lower in ICUs with only 55% bacteria compared to the 99% in private houses (112).
On the level of bacterial genera especially *Bradyrhizobium*, *Burkholderia*, *Propionibacterium*, *Staphylococcus, Streptococcus, Pseudomonas*, *Acinetobacter*

and others were detected on the floor, workplace or medical device surfaces and are characteristic genera for an ICU microbiome (124).
The microbial composition differed from each other in these locations especially the workplace and medical devices were harboring many human skin associated microbes, most likely as a result of frequent touching whereas the floor microbiome showed a higher association with outdoor microorganisms.

Even though the majority of microbes found in ICUs is harmless to humans also many potentially or obligatory pathogens occur.
There are several bacteria which regularly occur in an ICU's microbiome and can cause HAIs. The most prominent among them are species like *Clostridium difficile*, *Pseudomonas aerugiosa*, *Acinetobacter baumanni*, *Staphylococcus aureus* or *Enterococcus* spp (126).

3.7.2.2 HAIs transmission and biofilms in ICUs and hospitals

Although many HAIs are transmitted directly from patient to patient, a substantial amount is also transmitted indirectly. Many pathogens can become a part of the microbiome of a patient's room or surrounding and infect other patients through indirect contact with these surfaces. Health care workers can transmit microorganisms from one location to another via their hands after touching patients or their surrounding environment (127).
Microbes, including multi drug resistant (MDR) species, can be found on many different surfaces in an ICU including medical equipment like stethoscopes, mobile phones, monitors, ultrasound devices etc. or on several inanimate hospital surfaces especially high-touch surfaces close to a patient (126).
These surfaces obtain their microbiome and thus also the pathogens either by direct contact, shedding or emitting of microorganism from a patient or via the hands of health care workers who after touching a patient or their environment, touch other surfaces or devices and thus contaminate them (128).

Bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Mycobacterium tuberculosis* and many others can survive for several months on dry surfaces if these surfaces are not cleaned and disinfected. Furthermore, also

fungi such as *Candida albicans* and viruses like *HAV* or *Rotavirus* can survive on these surfaces for a few months. As a result of this durability surfaces can be a source of transmission and infection for a long period of time if not cleaned properly (129).

An important mechanism to withstand environmental stressors and cleaning when residing on a dry inanimate surface is the formation of biofilms. Whereas biofilms are more commonly associated with catheters or other (especially invasive) medical devices, they are also a major factor for bacterial survival in the indoor environment (91).
Biofilms, as explained before, grant the microorganisms living in it more resistance to desiccation, drugs and cleaning detergents and enable them to survive in harsher environments.
One study found that 90% of surfaces in an ICU harbored microorganism-containing biofilms, including pathogenic species such as MDR *S. aureus* (98). It also showed that biofilms closer to the patient were richer in different microbial species, most of them skin associated, than biofilms further away from the patient. Biofilms can also be found on dry surfaces even after regular cleaning.
The fact that biofilms and thus pathogenic bacteria can be found on almost every surface in an ICU is of great concern as it poses a threat to all patients in the ward and this threat is not just theoretical.

It also has been shown that patients residing in a room which before had been occupied by patients who were positive for antibiotic resistant bacteria such as MRSA or VRE, were 40% more likely to acquire these pathogens even though these rooms were cleaned after the previous patient had left (130). The most plausible explanation for this transmission route is that these bacteria can survive on certain surfaces, which are not sufficiently cleaned, for a long time, and thus be transmitted to the next patient. This poses the threat of potentially infecting the new patients.

3.7.2.3 Resistome of the hospital and ICU microbiome

The microorganisms present in hospitals and especially in ICUs have to withstand many selective pressures. Frequent cleaning and disinfecting as well as the use of numerous antibiotics paired with low nutrient supply and other stresses allow only a few microorganisms to survive. These microbes are resistant to many of these stresses and are able to survive under harsh environmental circumstances. Many of the microorganisms able to do so are bacteria that are pathogenic or at least opportunistic pathogenic (126).

These factors lead to the situation that hospitals and especially ICUs are considered to be reservoirs for MDR bacteria. Resistances to antibiotics and antiseptics and acquiring of HAI with MDR pathogens have been rising for the last decades (131).
However, general statements about the resistance levels of bacteria found in hospitals and ICUs are very difficult to make as the resistance levels depend on numerous factors. Regional variability e.g. an increase in resistances from north to south can be observed and local outbreaks of certain MDR bacteria in individual hospitals or ICUs are regularly reported. Moreover, correct cleaning, architectural design and many other additional factors all influence the individual resistome of a specific hospital and ICU microbiome and can be changing over time.
Most research performed on the resistances of hospital and ICU bacteria only studies the resistances of bacteria responsible for HAI.
One US study found that on average almost 50% of HAIs caused by *Staphylococcus aureus* were caused by MRSA and nearly 30% of HAI caused by *Enterococcus* spp. were vancomycin resistant species. Moreover more than 50% of *Acinetobacter baumanii* and ~16% of *Pseudomonas aeruginosa* associated with HAIs were multi drug resistant (132).
These numbers differed depending on the length of stay, the hospital and also the type of infection.
The 2017 ECDC report for HAIs stated that in ICUs 23.5% of *S. aureus* isolates were MRSAs and almost 64% of *A. baumanii* isolates were carbapenem resistant. *Enterococcus* spp. showed a vancomycin resistance in nearly 10% and resistance levels in *P. aeruginosa* were about 26% to ceftazidime as well as to carbapenem

(133). When the ECDC looked at acute care facilities they found that on average 31.6% of HAIs are caused by MDR organisms. However, a great variability depending on the exact country was observed with rates ranging from 0% in Iceland to 68.9% in Romania (134).
When comparing these numbers to data from the EARS-NET data, which includes not only HAIs but also community acquired infections outside of hospitals, it was shown that in hospitals the proportion of resistant bacteria causing hospital associated infection is about 36% higher than the proportion of resistant bacteria causing health care associated infections outside of hospitals (134). This supports the theory of hospitals and ICUs being a reservoir for resistant bacteria.
A study which looked at the general indoor microbiome of CBs including ICUs (unrelated to HAI) and compared them to that of unconfined buildings (UB) found that the microbiome of confined buildings had 20% higher diversity of resistant features than the microbiome of UBs (112). An increased number of intrinsic bacterial resistance genes that coded for efflux pumps (*mexK, mexB*) was found in this study. Especially in ICUs, higher abundance of stress tolerance was present and CBs in general showed higher resistance to fluoroquinolones than UBs. Furthermore, the CBs microbiome also showed more elements like flanking repeats which are associated with HGT (112).

In conclusion it can be said that the hospital and ICU microbiome is generally less diverse but more resistant than the microbiome of normal private & public houses which do not undergo as strict cleaning procedures and pose lower selective pressures.

3.7.3 Cleanrooms

Cleanrooms are quite modern infrastructure facilities and have only been in use for roughly 70 years. A cleanroom is defined by the International Organization of Standardization (ISO) as a "*room within which the number concentration of airborne particles is controlled and classified, and which is designed, constructed and operated in a manner to control the introduction, generation and retention of particles inside the room."* (135).
Cleanrooms are important facilities in many different industrial fields such as semiconductor industry, food industry, pharmaceutical industry, microelectronics, and medical processes. The aim behind cleanrooms is to prevent the contamination of certain products that must be uncontaminated in order to function correctly or to ensure their safety, and therefore cannot be produced in regular industrial manufacturing facilities.

There are a couple of mechanisms involved in the function of cleanrooms. First, cleanrooms are built of materials which do not shed any particles themselves, and which thoroughly separate the inside of the room from the outside to prevent any particles to get in. The materials used in cleanrooms are generally easy to clean and cleaning protocols are very harsh and frequent. Secondly there is always a continuous stream of efficiently filtered air flowing into the room. This continuously applied high amount of particle free air flowing through the room removes particles and microorganisms that are shed into the air by workers, machines and other sources. Moreover, the high amount of air supply is used to pressurize the inside of cleanrooms and thus prevent particles from flowing into cleanrooms (136).
Furthermore, people working in cleanrooms must wear special protective suits to minimize the number of microorganisms and particles shed and to lower the chances of contamination of the products and cleanrooms through the workers. Studies have shown that the use of protective gear minimizes the particles shed by people 13.6-fold for microbe carrying particles (MCP) and 8.9-fold for particles with more than 0.5 µm and thus is a very effective way to reduce dispersion of particles. MCPs shed from people wearing protective garment reduced from an average 2,400 to 177 per minute (137).

However, environments cannot be entirely free of any particles, as there are always some points of entry into a system that is not completely sealed off of its surroundings. These entry pints can be e.g., the people working in the cleanrooms by transferring a few particles with them into the room. Thus, there are certain limits for the number of particles that are allowed in the air of cleanrooms and depending on the ISO class of the industrial facility there are different maximum levels of airborne particles. These different ISO classes divide cleanrooms into different levels of cleanliness ranging from ISO 1 to ISO 9 depending on the number and size of particles in a room. An ISO 1 class facility is the cleanest cleanroom with a maximum of 10 particles bigger than 0.1 µm per cubic meter and maximum two particles bigger than 0.2 µm. In contrary an ISO 9 class cleanroom allows up to 35,200,000 particles bigger than 0.5 µm per cubic meter (136).
Most cleanrooms in industrial use are level 8 to 9. However, if more sterile conditions are necessary, lower classes of ISO cleanrooms should be used as these have lower numbers of particles in the air. Cleaner areas are often only accessible through higher ISO cleanroom classes to decrease the risk of contamination through staff entering and transporting microbes directly from the outside with them. Access to cleanrooms is often only possible through airlocks or sluice systems which seal the cleanroom from the outside (136).
However, even though cleanrooms try to ensure the highest amount of cleanliness they are never completely sterile. A complete absence of all particles and microorganisms is not possible in a room connected to the outside. But what kind of particles and microorganisms exist in such harsh environments? And which particles are capable of entering these rooms and which can survive in them?

Most data available for microorganisms present in cleanrooms comes from spacecraft assembly facilities. These facilities also are cleanrooms due to the planetary protection requirements. These rules are supposed to protect other solar system bodies from contamination through spacecraft and to protect Earth from life forms that might be brought to Earth back from other planets (138).
The reason for this is, on the one hand to protect other planets from potential changes to their environments and to guarantee the integrity of the search for extraterrestrial life and on the other hand to prevent the contamination of the Earth with possibly harmful life forms or bioactive molecules through returning

spacecraft. Thus, high efforts are made to produce as uncontaminated spacecraft as possible and lots of research is done afterwards to measure the outcome of these efforts.

The Viking spacecraft that were sent to Mars as part of NASA's Viking program in the 1970s were the first spacecraft to be microbiologically assessed during their construction in cleanrooms. Regardless of the efforts to eradicate all live forms on the spacecraft, microorganisms were still detectable on the surfaces (139).
Since then, many new methods, especially cultivation independent methods, have been developed and given us new insights and a better understanding of the abundance and diversity of the microorganisms and particles found in cleanrooms.
The microbial air contamination has been shown to be a good indicator for the contamination of the surfaces in the same room. In samples taken from the spacecraft surfaces on average one colony-forming units (CFU) and 0.1 spores were found per cm^2. Phylogenetic 16S rRNA gene sequencing showed that the microorganisms found in the cleanrooms were Gram-negative and Gram-positive in approximately the same proportion. The bacterial classes most found in the sequencing were Alpha, Beta- and Gammaproteobacteria as well as Firmicutes and in the cultivation dependent methods *Bacillus* species and *Staphylococcus* species were most frequent to grow (140,141).
Up to 75% of bacterial species can be associated with humans, the rest was mainly associated with soil and dirt and thus probably transmitted into the cleanrooms from the outside (142). This study also detected a significant number of spore-forming microbes, which are considered especially potent to contribute to extraterrestrial forward contamination due to their high number of resistances.
Another study compared the different NASA space craft assembly facilities and their microbiomes. It was found that each facility, located in different geographic regions in the US seems to have its own unique microbiome that differs quite significantly from the other locations (141).
However, the indoor microbiome of the cleanrooms also showed to be independent of its geographic outdoor microbiome. This leads to the conclusion that the main factor influencing the indoor microbiome of cleanrooms is not their surrounding microbiome, but rather factors like humidity and temperatures inside the facility as well as human activity (141).

It described Proteobacteria along with Firmicutes to be the most common bacteria in cleanrooms. The bacteria found in almost all facilities were Staphylococci, Sphingomonadaceae, Methylobacteria, Caulobacteriaceae*,* Commamonadaceae*,* Moraxellaceae *and* Acinetobacter*.* Whereas staphylococci are most likely transmitted by humans, the other species probably originated from the surrounding air (141).

Another study also tested the resistance of cleanroom isolates and exposed the bacteria found in cleanrooms to even harsher stresses like UV-C radiation, extreme heat, pH extremes or hydrogen peroxide, to simulate the extreme conditions microorganisms would have to survive in space. It showed that certain extremotolerant bacteria can even survive these extreme conditions, among them spore forming members of Bacillaceae family and Alpha- or Betaproteobacteria *or Acinetobacter* (140).
The number of the bacteria collected in different spacecraft assembly facilities is, as expected, associated with the certification level of cleanroom. It has been proven that cleanrooms are an effective tool for lowering the number of microorganisms and that microbes cannot survive well and proliferate under such harsh conditions. However, although the abundance of microorganism in cleanrooms has been shown to be greatly reduced, the diversity of microbes found in these facility does not decline in the same extent as the abundance does (143). Contrarily the diversity of spore forming bacterial species and certain archaeal species even seemed to be enriched in cleanrooms. Species like *Ammoniphilus, Bacillus, Clostridium, Cohnella* or *Geobacillus* and human associated archaeal species such as *Nitrososphaera* and *Haloferax* are all species that can survive well under harsh conditions and can all be found in cleanroom facilities (143). Moreover, it is described that in higher classified cleanrooms (lower number of air particulates allowed) the diversity of microbes is higher than in lower classified ones (141). The theory behind this is that the lack of nutrients enables numerous slow-growing, spore forming bacterial species to survive rather than only a hand full of fast-growing bacteria, which would outcompete the slower ones. However, also other factors such as humidity and temperature could contribute to a change in diversity.

The effect of this is yet unclear, but these species are also the ones best suited to endure and survive the stresses of interplanetary space travel.

Especially anaerobic microbes are considered to be a potential threat to survive on other planets, as all of the planets surrounding us and which are reachable at our current technological level, have a very low amount of oxygen in their atmosphere (e.g. Mars 0.13% oxygen) which would most likely make it almost impossible for aerobic microbes to thrive under these conditions (144).
This makes it even more concerning that also obligately anaerobic bacteria of the genus *Clostridium* or *Propionibacterium* have been found in spacecraft assembly facilities. And even though to our current knowledge there are no organic compounds on planets like Mars that are needed by these microorganisms, other anaerobic bacteria that metabolize nitrogen and CO_2 such as *P. borealis* could potentially be able to survive on Mars. And hence, these chemolithotrophs produce organic compounds they could be the first piece in a chain that enables other anaerobia to also thrive on other planets (145).

Archaea are also microorganisms that can be quite resistant to environmental stresses like UV- radiation, desiccation, and temperature extremes. Thus, making them a potential threat to survive interplanetary space travel and contaminating other planets. Especially methanogenic archaea, which are known to be able to thrive under anaerobic conditions and need no or only few organic compounds, are considered to potentially be able to survive on certain extraterrestrial planets such as Mars. Although archaea seem to be even less abundant in cleanrooms than bacteria, with only one archaeal cell for every 100 bacterial cells, archaeal 16S rRNA gene sequences have been detected in cleanrooms including gene sequences from methanogenic species (146).

Furthermore, newer methods like next generation sequencing in combination with the application of propidium monoazide, which masks the DNA of dead cells were introduced. These studies have shown that 99% of the DNA in cleanrooms is from dead cells and made it possible to detect gene sequences from less abundant living microorganisms such as viruses and eukaryotic cells like amoeba e.g.

Acanthamoeba or fungi like *Leotiomyceta,* which have been recently found in the cleanrooms of spacecraft assembly facilities (2,147).

This leads to the conclusion that even though cleanrooms are highly controlled and harsh environments aiming to be as microorganism free as possible, there is still an occurrence of many different microbes ranging from bacteria to eukaryotes. Moreover it has been shown that the unfavorable conditions in cleanrooms select for bacterial survival specialists such as spore forming bacteria or anaerobic microorganisms which are also more likely to withstand the stresses of interplanetary space travel such as UV-radiation, temperature extremes and desiccation. Therefore, these bacteria are also the ones most likely to contaminate and survive on other planets, hence posing a threat to the planetary protection requirements.

Although the occurrence of other microorganisms such as archaea, viruses and eukaryotes in cleanrooms is not yet as well researched, it is also probable that the environmental conditions of cleanrooms also favor survival specialists among these microorganisms.

3.7.4 Space Stations

Space stations are probably the most confined habitats that mankind has ever built. There is currently one space station in operation, the International Space Station or short ISS, which orbits the earth at an altitude of approximately 400 km above sea level. The ISS has been continuously occupied by astronauts since the year 2000. The ISS is a very interesting research facility regarding microorganisms, as it is in no direct contact with surrounding environments that harbor microorganisms. The only vectors of microbial life are the astronauts and their associated microbiomes and the materials that get shipped to the ISS regularly. Astronauts usually spend about six months on the space station before returning to earth, with so far more than 200 astronauts that have visited the ISS (148).
Furthermore, also the extreme environmental conditions on the ISS, e.g. the increased radiation, the microgravity and the low range of nutrients accessible under which microorganisms have to grow make it an incredibly interesting research facility. It gives insight on how microorganisms thrive in space and which microbes are able to survive under these conditions and which of them might be harmful to astronauts. Moreover, it also gives information about which microorganisms might be able to survive interplanetary space travel and be a danger to planetary protection efforts (149).

The first evidence of microorganisms living on space stations was found on the former Russian space station *Mir* which was in operation from 1986 to 2001. A study by Novikova et al. found a total of 108 bacterial species and 126 fungal species among them opportunistic pathogens or microorganisms which were damaging structural materials of the space stations by degrading or corroding them. Bacterial species most often found on *Mir* were *Staphylococcus, Bacillus, Micrococcus* and *Corynebacterium*. The most common fungal species were *Aspergillus, Penicillium* and *Cladosporium* (150).

Thus, to prevent potential damage to the spacecraft itself on the one hand, and to the crew living on it on the other hand, the space agencies operating the ISS (NASA, Roscosmos, JAXA, ESA and CSA) decided to introduce strict measures(151).

These included a strict cleaning and disinfecting schedule, usage of HEPA- filters or similar filters to filter particles out of the air and continuous microbial monitoring. There are certain limits for the concentrations of bacteria or fungi in the air or water of the ISS, namely 1,000 CFU/m³ air and 100 CFU/m³ air for bacteria and fungi, respectively. The maximum levels for surface contamination are 10,000 CFU/100 cm² and 100 CFU/100 cm² for bacteria and fungi, respectively. The bacteria limit for potable water is 50 CFU/ml. Thus, the limits for airborne and surface microorganisms are not too strict, they are higher on the ISS than they are for example in food or health care facilities and the drinking water standards match the recommendations of the WHO for drinking water (151).

The cleaning procedures consist of regular cleaning of surfaces and disinfection with quaternary ammonium or quaternary ammonium with hydrogen peroxide on the US and Russian module, respectively. The cleaned surfaces are then microbiologically monitored and evaluated. Therefore, on the US segment two surfaces per module, one for bacteria one for fungi, are sampled every 90 days and then analyzed aboard, whereas in the Russian segment cosmonauts swab certain surfaces before returning to earth and taking the samples they collected with them for analysis on ground. The potable water on the ISS consists of partly water that is shipped there from earth and partly of water that is recycled from either fuel cells, humidity condensate originating from mostly sweat and the breath or urine distillate. As water is also a potential habitat for many microorganisms and a place of predilection for the formation of biofilms, the addition of silver and temporarily iodine to the water resources is supposed to prevent it from becoming microbially contaminated. Furthermore the water is regularly tested for heterotrophic bacteria and coliforms (151).

3.7.4.1 ISS Microbiome

To determine the number of microorganisms multiple studies have been conducted on the ISS. One looked at the debris and lint collected in vacuum cleaner bags on the ISS and it was shown that the total number of microbial signatures on the ISS measured was up to 8.9×10^8 16S rRNA gene copies per gram with up to 12% of them being viable (152).

The most common bacterial phyla on the ISS surfaces and in the air seem to be Actinobacteria, Firmicutes, Proteobacteria and Cyanobacteria with Bacilli as the predominant class and on the level of bacterial families Staphylococcaceae, Neisseriaceae and Enterobacteriaceae were most frequently found (152–154). Cultivation dependent methods have shown that the most common species repeatedly found on the ISS are *Bacillus, Micrococcus* and *Staphylococcus* which was also reported for the former Russian space station *Mir* (155).
Other methods also showed that different species of *Corynebacterium, Streptococcus* and *Propionibacterium* are among the most abundant on the surfaces and in the air of the ISS (152,156).
In water samples the most common bacteria were typical water associated bacteria among them *Pseudomonas, Burkholderia, Methylobacterium and Ralstonia.* And even though the water had been treated with silver before, some samples showed too numerous CFU/100 ml water to count when cultivated (149,157).
Concerning the fungal microbiome on the ISS these studies showed that the most abundant ones are different fungal species of the *Penicillium* and *Aspergillum* family. Other frequently found fungi are *Cladosporium, Hyphomycetes* and *Malassezia*.
Moreover, also archaeal organisms have been found on the ISS, these were mostly human associated species of the *Woesearchaeota, Thaumarchaeota*, and *Euryarchaeota* phyla (158).

The vast majority of the microorganisms found on the ISS are human associated organisms, especially human skin and gut associated species. They are most likely shed by the astronauts or transported to the ISS via spacecraft and then transmitted to the surfaces. The only place on the ISS that is not predominated by human microbiome associated species is the water with which the astronauts have little direct contact. However there are also other mechanisms of transmission possible for bacteria and fungi and also for non-human associated species, e.g. microorganisms that survive on the cargo transported to the ISS (2,156).

3.7.4.1.1 Resistances of the ISS Microbiome

Another question that is raised concerning the microbiome on the ISS is the abundance of pathogenic microorganisms and the evolution and development of antimicrobial resistances. This is especially interesting and important as it has been shown that space, radiation and microgravity have several effects on the human body and immune system. And as access to health care institutions is non-existent on the ISS, it is especially important to prevent diseases and infections, hence the approach to keeping the ISS as free from pathogens as possible. Changes to our body include reduction of muscular mass, changes to our cardiovascular system and respiratory system, hormonal changes and dysregulation and reduced function of our immune system making us more susceptible to infections (159,160).
Furthermore, new investigations have also shown that space also has effects on several pathogens, e.g. some organisms are boosted in their virulence and others have a reduce virulence. Also the resistances and susceptibility to antibiotic drugs might be altered in space leading to an increased risk of infection and less treatment options (161,162).
Among the bacteria and fungi onboard the ISS many are opportunistic pathogens such as *Staphylococcus aureus, Staphylococcus epidermis or Bacillus fusiformis*, which normally are members of our skin, respiratory or gut microbiome. They do not harm us but can become a danger to us if our immune system is weakened as it is in space or if their virulence is boosted. Also, the treatment of diseases caused by these bacteria can become more difficult if their susceptibility to antibiotics decreases in space. And since there is also a limited number of antibiotics available on the ISS it is important to know the resistances of the present bacteria, to provide the knowledge for the right treatment if necessary. Thus, the monitoring of pathogens and their resistances is important to evaluate their danger and the necessity to take measures (162).

However, not only the resistances to antibiotics play an important role, also the resistance against environmental stresses such us radiation, low nutrient supply or desiccation are important in terms of capability to survive on a space station.
A study analyzing old dust samples from the Russian segment of the ISS found

several species of extremotolerant bacteria (158). All of them were resistant to desiccation; all the spore forming bacteria showed heat-shock resistance and many showed a non-species-specific reduced susceptibility to antibiotics. Other studies also showed resistant species against UV- light, x-rays or species that could grow under harsh pH conditions. Although these resistances are not concerning in terms of a possible health hazard for crew members, these resistances can pose a threat to planetary protection strategies (158).

The antibiotic resistant bacteria mostly showed resistances against different β-lactam antibiotics such as penicillin, ceftriaxone and cefotaxime. One species was resistant to almost half of the antibiotics (158).
Other studies showed that 75% of bacteria found on the ISS have at least one resistance to an antibiotic among them also common opportunistic pathogens such as *Staphylococcus aureus* or different *Enterococcus faecalis* which showed resistances to common antibiotics like erythromycin, tetracycline and streptomycin (154).
A 2018 study found more than 60 resistance genes encoding for 28 different antibiotics among the ISS bacteria (163). And even though not all of the resistance genes were expressed in the bacteria a lot of them showed resistances to common antibiotics such as penicillin, rifampin or oxacillin, among them were also multi-resistant species like *Enterobacter bugadensis.* Resistance genes like *ermA*, *blaZ,* tetR, *macA* or *oqxA* that encode for resistances to antibiotics were detected, but also resistance genes to certain metals such as copper and zink and resistance genes encoding for efflux pumps were found (163).
Furthermore plasmids, which are the most common mechanism for horizontal gene transfer have been found in more than 85% of the ISS-bacteria, and the same number of bacteria also showed that they had relaxase and transferase genes necessary for conjugation encoded on their plasmids (154).
The level of resistances to various antibiotics in different bacterial species even opportunistic pathogens is concerning as it shows that resistant microorganisms exist on the ISS. Further, it also holds the potential that these resistant bacteria transfer their resistance genes via horizontal gene transfer to other facultative or obligatory pathogens, enabling pathogenic bacteria to become resistant to antibiotics available on the ISS.

However, even though resistances against antibiotics and environmental stresses exist on the ISS, it has also been shown that the resistance pool of ISS bacteria does not exceed the resistances and extremotolerances of ground control microbiomes (156).

3.8 Biocontrol

Over the last 200 years we have discovered and learned a great deal about the microorganisms surrounding us. From their existence to their functions and the role they play as commensals as well as pathogens, we have come to have a good understanding of these microorganisms. Especially the understanding of infectious diseases and their pathogens has led to many new measures and therapies that have greatly benefited our society over the last century. From simple procedures like hand washing and hand disinfection, surface cleaning and disinfection and sanitation to more sophisticated measures like sterilization or antibiotic treatment, many new strategies to prevent or treat infectious diseases have been introduced and lowered the number of deaths caused by infections over the last century tremendously. However, there is still a lot to learn and discover about the microorganisms surrounding us. When looking at the rise in hospital-associated infections and the number of infections caused by multi drug resistant bacteria we see that there is a long way to go to understand our surrounding microorganisms and that certain measures that are being taken are leading to new problems.
One field that helps us to understand microorganisms is studying their microbiomes and observing how measures that are taken like cleaning, disinfection or antibiotic treatment influence and change the microbiome. As described above these measures often lead to a less numerous but more resistant microbiome, as more resistant bacteria are more suited to survive under such conditions, thus leading to more infections with multi resistant microorganisms (112).

Therefore new strategies which aim to control our surrounding microbiome, especially the indoor microbiome in hospitals or ICUs, are being discussed. There are many different approached to achieve this goal. These strategies include new disinfecting methods, innovative architectural design or usage of parameters like humidity or temperature to control the microbiome.
Other strategies follow a rather new approach, they aim to make our surroundings, for example indoor surfaces, more habitable for non-pathogenic microorganisms and thus to reduce pathogens by making them compete with other

microorganisms (164).
Most of these strategies aim to reduce the number of pathogenic bacteria present in certain microbiomes surrounding us and thus to reduce the probability for the transmission of infectious diseases through these surfaces.

3.8.1 Improvement of Cleaning Strategies and Cleaning Agents

Places like ICUs in hospitals already have very strict cleaning protocols in place. However, these methods show insufficient results in terms of infection control, as already discussed. One approach to reduce the number of pathogenic microorganisms in these places is to improve cleaning methods and strategies to achieve better results in terms of hygiene and pathogen free surfaces.
These strategies to improve our cleaning success rate range from basic measures like more frequent disinfection of high-touch surfaces and better education of cleaning staff to more sophisticated measures such as monitoring of cleaning sufficiency, use of new cleaning detergents and antimicrobial surfaces, which are easier to clean (165).
To ensure good everyday cleaning practices in places like hospitals it is important to follow guidelines like the CDC guidelines to ensure the adequate cleaning or, when necessary, disinfection with the correct detergents and disinfectants as not every surface should be disinfected and as the type of disinfectant can play a vital role for achieving correct decontamination (36).
Another method that might be able to improve the success of cleaning is to monitor the contamination of surfaces. New methods do not rely on the subjective visual observation for cleanliness of surfaces but rather try to provide objective data for whether a surface is still contaminated or not. Monitoring cleanliness with ATP markers or fluorescent markers to provide feedback on the level of residual organic compounds left on surfaces after cleaning them are examples of said new methods. However, there are no objective criteria yet at what level a surface is to be considered clean and the technique needs improving regarding sensitivity and specificity (165).

Another hope in fighting HAIs is the introduction of new cleaning agents and products. One promising approach is to develop agents that are able to dissolve biofilms or can prevent biofilm formation. As described above biofilms are a major problem when talking about HAIs, as they enable bacteria living in them to survive harsh conditions and procedures such as disinfection and dehydration.
There are several approaches being researched currently that could help battle this problem. One of them is the coating with addition of special enzymes like DNase or dispersinB to disinfectants to inhibit the formation of biofilm and help to dissolve biofilms. It has been shown in studies that these enzymes are effective in dissolving biofilms (101,102).
Other promising substances currently being researched are quorum sensing inhibitors, antimicrobial peptides or bacteriophages (92).
Bacteriophages are viruses that do not use eukaryotic cells as host but use bacteria as host cells. New methods try to take advantage of bacteriophages or certain mechanisms they use, such as the endolysin *PlyC* which first has been found encoded in certain bacteriophage genomes and functions as a hydrolase that can destroy biofilm formation and raise the susceptibility to antibiotics in biofilm forming cells (166).

Another completely different approach is the introduction of new cleaning detergents, so called microbial-based cleaning detergents.
The idea of microbial based cleaning is to use microorganisms that are not harmful to us in order to prevent the growth of infection causing microorganisms on surrounding hard surfaces. As described above often cleaned and disinfected surfaces tend to select for multi-resistant microorganisms that are suited better for surviving under harsh conditions and as other non-resistant microbes are more susceptible to these cleaning and disinfection procedures, the multi-resistant pathogens have much less competition and thus can grow more easily on these surfaces. The theory behind the idea of microbial cleaning is to add non-pathogenic bacteria to cleaning detergent which is then used to clean surfaces. By this the previously disinfected and cleaned surfaces should get inhabited with non-pathogenic bacteria, thus making it tougher for resistant microorganisms to grow and thrive on these surfaces (167).

Studies have found that the regular usage of microbial based cleaning detergents (e.g. *Bacillus* based cleaners) leads to a significant reduction of almost all hospital-associated infections causing pathogens on hard surfaces especially bacteria like *Staphylococcus*. Compared to commonly cleaned and disinfected surfaces, the surfaces treated with microbial cleaning detergent showed up to 90% less abundance of HAI-causing pathogens. Furthermore, also the number of drug resistance genes detected after the treatment was lower than before, and the biocontrol bacteria showed no sign of acquiring resistance genes themselves (167,168).

This approach relies more on the principle of controlling the microbiome and what it is composed of rather than trying to eradicate all its microorganisms with the unwanted effect that only the most resilient ones survive and that it is re-contaminated by pathogens quickly. Moreover, it also seems to have a positive effect in terms of resistances, as there appear to be fewer resistant species of bacteria.

However, more research in these field is necessary, especially whether this also leads to an actual decrease in hospital-associated infection in patients or to a decrease in infections with multi drug resistant species.

To what extent these measures can contribute to the prevention of HAIs remains yet unclear and would need further investigation in the future.

But it shows that new inventions with the aim to prevent infections are made on many different levels and together with other measures might contribute to reduce the threat of infections.

3.8.2 Architectural design

Other elements influencing the microbiome of built environments is the structural and architectural design of the rooms, as well as the conditions present in the room and what kind of habitat the built environment presents for microorganisms. This includes for examples factors such as materials that are used for the surfaces in the rooms, the furnishing of the rooms or the temperature and humidity in the rooms. These are factors that can be influenced and regulated by the inhabitants, thus giving us a chance to control the microbiome and maintaining it in a way that is beneficial to us by lowering the abundance of pathogens and multi resistant microorganisms and making the environment more inhabitable for them.

As already described above, factors like temperature, humidity, and air flow influence the microbiome in built environments. These are all factors that can change the composition of microbiomes. It has been shown that a higher temperature generally leads to less airborne bacterial survival across all bacterial types. Concerning relative humidity, the effects are less universal and more complex, as some bacteria tend to thrive worse at intermediate others at high relative humidity (169). However in a different study the proportion of bacterial sequences closely related to pathogens in indoor environments correlated negatively with relative humidity and positively with temperature, meaning a higher relative humidity and a lower temperature led to less pathogenic sequences in indoor environments (20). It was also shown that ventilation and air flow play a vital role in bacterial composition of the microbiome, with higher air flow leading to lower abundances of airborne pathogens in the rooms.
Other microorganisms such as viruses and fungi are also influenced by temperature and humidity. Viruses seem to survive best at low and high relative humidity depending on the type of virus, but generally thrive worst at medium relative humidity (169).
It is however still unclear and requires more research to fully understand and predict how exactly microorganisms react to changes in humidity, temperature and other factors that can be regulated in rooms.

Other structures in built environments that can be influenced are the materials used to build these environments. Many different studies have investigated and experimented with different materials and structures in the search for surfaces that prevent biofilm formation, the adhesion of microorganisms to these surfaces or are themselves antimicrobial in general. One idea is to coat surfaces with biocides. Many medical devices like catheters, stents and prosthetics nowadays are already made of or coated with material inhibiting biofilm formation (92,170).

In recent years several different studies have discovered that indoor plants influence the indoor microbiome of built environments. It was shown that indoor plants in rooms lead to an increase in the abundance of microorganisms like bacteria, archaea and fungi. And not only does it increase the abundance of microorganisms, but it also increases the diversity of bacteria present (171). And although the rhizosphere of plants has been described to be a reservoir for opportunistic pathogens (172), plants also harbor many beneficial microorganisms that would contribute to enriching the diversity of microbiomes and preventing infections. Their beneficial factors might even outweigh the risks posed by them, so scientist think about them potentially not only benefiting private or public house indoor microbiomes but also to be overall beneficial in hospitals in ICUs.

4 Discussion

Over the last decades new sequencing technologies have made it possible to study the surrounding and commensal microorganisms in our environments more deeply than ever before. Microbiome research has revealed a lot of new information and new discoveries have been made in different microbiome research fields such as gastrointestinal microbiome, skin microbiome or indoor microbiome. These discoveries have led to a new understanding of microbiomes, how they influence us and how they are influenced by us.

As people spend about 90% of their time indoors (1), the study of the indoor microbiome is essential to understand how the indoor microorganisms affect the people living in these environments, especially concerning their effects on health. Furthermore, studying the indoor microbiome shows light on how actions that are taken to create a harmless and non-pathogenic microbiome change the indoor microbiome, its composition, and especially its resistances.

One major health challenge closely related to indoor microorganisms is the topic of anti-microbial resistant organisms. The WHO has declared the problem of anti-microbial resistances as one of the thirteen most urgent health concerns facing humanity in the next decade (4).

Infections caused by anti-microbial resistant pathogens lead to fewer treatment options and thus longer hospital stays, higher costs, and higher mortality (30).

As these resistances have the potential to reverse many of the achievements and progress of modern medicine and infection control, the WHO urges its member countries to take immediate action against antimicrobial resistances. The WHO lists, among others, following actions as essential for tackling the problem of AMR in their ‘Global action plan on microbial resistances’: effective sanitation, hygiene and infection prevention as well as optimized use of antimicrobial medicine and it also emphasizes the importance of further research and developing new antimicrobials (173). The challenge of antimicrobial resistances is also closely related to the problem of health care acquired infections as many HAIs are caused by resistant microorganisms (29).

The study of the indoor microbiome and its resistome helps to understand the compositions and functions of microbiomes in different locations like hospitals, cleanrooms, or the ISS. Furthermore, it aids in our understanding about what

effects factors like confinement, temperature, ventilation, geography, or cleaning have on the different microbiomes. Finally, microbiome research provides important insight on how the resistome of the microbiome changes based on these factors and which factors make the microorganisms less or more resistant.

As described before, indoor microbiome research showed us that with increasing confinement the microbiome usually gets more similar to the human microbiome (112). In places with few influences other than people, microorganisms that live in and on humans will be transferred to their surroundings and predominantly inhabit these environments. This effect can be counteracted by creating contact with other microbiomes such as outdoor or plant microorganisms, e.g. by opening windows to let outdoor microorganisms in or putting up pot plants which have their own plant microbiome (11,119). Such measures lead to additional, different microorganisms living in the indoor environments and thus to a more diverse microbiome (12).

The principle behind cleaning, disinfecting and hygiene is to eliminate pathogens. Over the last century, the improvements in sanitation and hygiene especially water and food hygiene have led to tremendous improvements in infection prevention. This and the development of antibiotics and vaccines have led to a substantial decrease in infection mortality (174). But the rising problems of antimicrobial resistances have the potential to reverse these achievements.
It has been shown that in locations like hospitals, ICUs or cleanrooms the increased confinement and the strict cleaning protocols lead not only to a less diverse microbiome but this loss in diversity also leads to more resistant microorganisms inhabiting these environments (112). And when these microorganisms are pathogens or opportunistic pathogens, as often emitted by patients in hospitals, more resistant species can cause diseases that withstand standard treatment options. This does of course not mean that cleaning, disinfection and hygiene principles in hospitals should be neglected because they have a great impact on the abundance of microorganisms and thus also on the number of pathogens and have been a viable part of infection prevention for the last decades. However, the achievements of the past should also not stop us from trying to further improve our methods and also to rethink them.

A problem with the strict surface cleaning and disinfecting regiments in places like hospitals is that it leads to a harsher environment for microorganisms to survive in. Thus, even though selective pressures (e.g. excessive cleaning and disinfecting) lead to a lower abundance of microorganisms, they also lead to a less diverse microbiome and generally select for more resistant and resilient microorganisms which can survive under harsher conditions among them pathogens or opportunistic pathogens such as MRSA or VRE (98,112).
The strategies to tackle this problem are often to try to create even harsher environments by introducing even stricter cleaning protocols and new disinfectants and new materials which make it more difficult to survive for microorganisms. Although these strategies can bring promising and important developments like biofilm degrading cleaning detergents which destroy environments that are especially favorable for microorganisms, the idea of creating a completely pathogen free environment is unrealistic.
Even in much more confined environments than hospitals such as cleanrooms, where the number of particles in the air is held under a certain threshold and workers wear protective suits, it has been shown that there are still many microorganisms living in these environments. And again, in these environments the microorganisms surviving are the ones that are the most resistant (2). And while new inventions often show good short-term results in reducing the pathogen burden on surfaces, it is questionable whether in the long run they can prevent that microorganisms develop mechanisms to survive under the now even harsher conditions as they have often shown.
Furthermore, contact with different harmless microorganisms is essential for an adequate function of the human immune system and to prevent it from overreacting. By eliminating interaction with microbial life, the normal development of the immune system is hindered, potentially leading to an increase of chronic immune diseases such as atopic diseases (106,164).

Thus, scientists have been thinking of new strategies to tackle these problems. One approach is to not try to eradicate all pathogens and with them all other microorganisms but to use the harmless and beneficial members of the microbiome to prevent infections, by competing with these pathogens (175). It has been shown that an enriched diversity of different microorganisms can prevent

infections. Research has also shown that human associated microbiomes usually also harbor more pathogens than other microbiomes (176,177). Taken together these findings lead to new ideas that could help us to tackle the problem of HAI and AMR. These ideas range from simple ones like opening windows, changing temperature and humidity or putting up pot plants to enrich the diversity of the indoor microbiome to more complex ideas like introducing probiotic cleaning agents that actively spread harmless microorganisms on surfaces (11,119,167). The advantage of these new approaches could be that they might be able to break the vicious circle of killing pathogens, thus lowering the diversity which leads to more resistances and then again to have to kill the newly resistant pathogens. However, the exact effects of these new approaches and whether they would really reduce the amount of HAI and infections caused by AMR microorganisms has yet to be elucidated.
Moreover, increased contact with harmless microorganisms could not only help developing a normally functioning immune system and thus help to prevent overreacting immune systems and diseases associated with it, but might even contribute to a patient´s recovery (164,178).

This approach, however, might not be suited for certain other indoor environments such as cleanrooms or spacecraft sent to other planets. Here the focus does not rely on the prevention of infections but on creating sterile items or items that are as microbe-free as possible. In these fields, inventions to lower the abundance of microorganisms such as self-disinfecting materials or the development of new and better disinfectants that ideally do not select for resistant microbes could be essential developments. However, since research also shows that in these areas the strict protocols again select for more resistant microorganisms, ones that would be especially fit to survive space travel for example, a shift in the principles of strategies could also be debated (2). When looking at space travel it perhaps would be better to send more ‘normal’ microorganisms that have low chances of surviving the hazards of space travel like increased radiation, desiccation and heat, than to unwillingly select for resistant and extremotolerant microbes that have higher chances of surviving the flight. However, this most likely would be a very difficult balancing act that would require much further research beforehand.

In terms of the microbiome on space stations such as the ISS, it has been shown that most of it is associated with the human microbiome (2). This is as expected because humans are the almost only influencing factor up there. The only place with predominantly non-human associated microorganisms is the water resources. The reason why it is important to know about the microbiome of the ISS is not only scientific interests but foremost the health of the crew, especially as many factors such as resistance, virulence but also functions of the immune system have been shown to change in space (160). And even though some pathogens and opportunistic pathogens can be found on the ISS, in terms of resistances, it has been shown that although some of them have resistances even to antibiotics the percentage of resistant species and the diversity of resistance features does not exceed ground control microorganisms (156). This also tells us that human-inhabited space environments, even though they are a harsher place to survive than other indoor environments, do not select for more resistant microorganisms.

Although new methods and techniques in the field of microbiome research have led to an enormous amount of new knowledge, it has also raised many new questions. Methods such as 16S rRNA gene sequencing have revealed the sheer abundance of microorganisms in the environment, even in places that were believed to be scarcely inhabited as many of the organisms were not being culturable. Even though modern research also studies the occurrence of archaea, fungi and viruses in the different indoor microbiomes, the majority of these studies is focused mainly on bacteria. Thus, to further understand the indoor microbiomes it is important to intensify the efforts to increase the research of the other microorganisms and their resistome as well. This would not only include knowing the composition of the indoor microbiomes in detail but would also allow to better understand the interactions between the microbes, for example how certain fungi or bacteriophages influence bacteria and vice versa and what their effects on resistances are. This could also lead to new insights on how to influence surrounding indoor microbiomes to make them more beneficial to humans and what a beneficial microbiome would even look like.
Furthermore, additional research on the factors influencing the microbiome such as temperature, humidity or ventilation would be of great importance, as these

factors are easy to change in modern indoor environments and would allow for more control over indoor microbiomes.
Other important future research fields might be the further improvement of new cleaning detergents. Detergents that destroy biofilms might prove to be vital, as biofilms provide better protection and are locations with higher horizontal gene transfer.
Additionally, research on products and strategies that focus on creating an indoor microbiome that is beneficial for the humans living in it and that help to prevent infections and resistances might be essential in the future.

5 Conclusion

In conclusion, this literature review highlights that indoor microbiomes are shaped by many different factors, the most important one being humans who spread their associated microorganisms in these indoor environments. However, indoor microbiomes are also influenced by many other different factors that can alter their composition and characteristics. The effects of increasing confinement and harsh environments not only lead to a lower abundance of microorganisms, but generally also create less diverse and subsequently more resistant microbiomes which cause numerous problems in different scientific fields. Thus, rethinking the current principles and strategies of hygiene by enriching the diversity of beneficial microbiomes in indoor microbiomes could aid in preventing the future spread of antimicrobial resistance mechanisms and lead to healthier indoor environments.

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