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Measurement and evaluation of microbial contamination during orthopedic implant surgery

Master of Science Thesis

Master's Degree Program Biomedical Engineering

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Division of Nano and Biophysics
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ABSTRACT

Background: According to the Swedish Association of Local Authorities and Regions, the cost for society related to healthcare-associated infections (HAI) is estimated to be 6.5 billion SEK annually. Furthermore, the cost is expected to increase, due to an aging and growing population and the increase in antimicrobial resistance. HAIs affect 1 out of 10 patients, were surgical site infections (SSI) is the second most common HAI, after catheter related urinary tract infections. If correct precautions are performed, 30-50% of HAIs can be prevented. By measuring bacteria levels inside the operating room (OR) and analyzing the correlation to contamination of the surgical wound, the project *Clean Care* aims to investigate risk factors and transmission pathways for SSI.

Purpose and objectives: The purpose of this thesis is to gather knowledge and deeper understanding of contamination pathways that may cause SSI. The objective is to gather data from measurements during ongoing surgeries in order to evaluate contamination pathways, such as direct airborne contamination, indirect contamination via the surgical instruments and endogenously contamination from the patient's own skin where the incision is going to be.

Method: Measurements were performed during ongoing hemiarthroplasty femoral neck fracture orthopedic surgeries in ORs equipped with either a conventional ventilation system (n=10), conventional ventilation system with mobile laminar airflow units (n=3) or laminar airflow ventilation system (n=11). The measurements were conducted via active air sampling through gelatin-filters with a Satourios MD8 device, surface sampling from instrument tables and sampling from the skin and wound collected with flocked nylon swabs. Bacterial samples were grown on agar for enumeration and determining of specie, both aerobic and anaerobic.

Results and conclusions: A statistically significant higher number of bacterial-carrying particles was found in the air of the conventionally ventilated OR compared to the OR equipped with laminar airflow ventilation ($p<0.001$). No significant correlation between the amount of airborne contamination (direct or indirect) and contamination of the surgical wound could be seen. However, trends suggest that endogenously contamination is an influencing factor of wound contamination ($R^2 = 0.8071$). Due to too few measurements, no statistical analysis could be performed on the influence of the mobile laminar airflow units on bacterial contamination.

Keywords: Healthcare Associated Infections, Surgical Site Infections, Colony Forming Units, Post-operative Implant Related Infections, Medical Devices, Biomedical Engineering

Mätning och utvärdering av mikrobiell kontamination under ortopedisk implantat kirurgi
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Institutionen för fysik
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SAMMANFATTNING

Bakgrund: Enligt Sveriges Kommuner och Regioner är den årliga kostnaden för vårdrelaterade infektioner (VRI) 6,5 miljarder kronor för samhället och kostnaden väntas stiga på grund av en åldrande befolkning och på grund av det ökande antalet antibiotikaresistenta bakterier. VRI drabbar 1 av 10 patienter, där postoperativ sårinfektion är den näst vanligaste formen av VRI, efter kateter relaterad urinvägsinfektion. I fall där korrekt förebyggande behandlingar utförs kan 30-50% av VRI förhindras. Genom att mäta bakterienivåer i operationssalar och analysera korrelationen med kontamination av det kirurgiska såret, har projektet *Smittfri vård* som mål att undersöka riskfaktorer och kontaminationsvägar för postoperativa sårinfektioner.

Syfte och mål: Syftet med denna uppsats är att samla kunskap och ökad förståelse för bakteriella kontaminationsvägar som kan vålla postoperativa sårinfektioner efter höftimplantatkirurgi. Målet är att samla data från mätningar under pågående operationer för att göra utvärderingar av kontaminationsvägar som direkt luftburen kontamination av såret, indirekt luftburen kontamination via kirurgiska instrument och endogen kontamination från patientens hud där snittet kommer läggas.

Metod: Mätningar utfördes under pågående ortopediska halv-protesoperationer i höftled i operationssalar utrustade med antingen konventionellt ventilationssystem (n=10), konventionellt ventilationssystem med mobila laminärt luftflödesenheter (n=3) eller ventilationssystem med laminärt luftflöde (n=11). Mätningarna genomfördes via aktiv luftprovtagning genom gelatinfilter med en Sartorius MD8-enhet, passiv luftprovtagning från instrumentborden, samt provtagning från huden och i operationssåret med flockade nylonfiber svabbar. Mätningarna innehöll både aeroba och anaeroba bakterieprover.

Resultat och slutsatser: Ett statistiskt signifikant högre antal bakterier i luften i operationssal med konventionella ventilationssystem kunde mätas jämfört med operationssalar utrustade med ventilation med laminärt luftflöde ($p<0,001$). Ingen signifikans kunde ses mellan luftburensmitta (direkt eller indirekt) och kontamination av operationssåret. Dock påvisar trender att endogen kontamination från patientens hud är en influerande faktor av kontamination av operationssåret ($R^2 = 0,8019$). På grund av ett lågt antal prover kan inga statistiska analyser utföras vid utvärdering av mätningar med konventionellt ventilationssystem med mobila laminärt luftflödesenheter av bakteriell kontamination.

Nyckelord: Vårdrelaterade infektioner, Postoperativa sårinfektioner, Koloniformande enheter, Postoperativa implantatrelaterade infektioner, Medicinska instrument, Medicinteknik

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Firstly, I want to thank and express my gratitude towards my supervisors. Josefin Caous from RISE Research Institute of Sweden who has answered all my questions regarding the project and helped my tremendously with the completion of this Master's Thesis. Linda Åhlström, Henrik Malchau and Peter Grant from Sahlgrenska University Hospital who have taught me about the importance of this project and its impact on the health care system. Anders Rehn from CRC Medical, who have helped me get started with the measurements, knowledge about ventilation systems in operating rooms and measuring techniques. I also want to thank Julie Gold who has been mine examiner from Chalmers University of Technology and have provided me with feedback and encouragement throughout the thesis work.

It has been a privilege to take part and work with incredible and dedicated people who work day in and day out with care and well being for people in need. The medical crew on site do an amazing work and I am proud to have worked alongside such enthusiasm and dignity and for the experience that I have received.

Finally, I want to thank my family and friends for their endless support.

Preface

This Master's Thesis project was conducted between January and June, 2020. The project corresponded to 30 credits and was examined under the Department of Physics and is a part of the Master's Degree program Biomedical Engineering.

This Master's Thesis is a part of the project *Clean Care*, which aims for better patient safety by reducing spread of infection at hospitals and minimizing risk of surgical site infections after orthopedic implant surgery. This part of the project, focusing on orthopedic surgery, is a collaboration between RISE Research Institute of Sweden, Sahlgrenska University Hospital, Toul Meditech, Getinge and CRC Medical and my role was to investigate the microbial contamination inside the operating rooms.

This Master's Thesis was also conducted when the new Corona virus was at its rise and pinnacle of the global pandemic. That meant that Sahlgrenska University Hospital went into state of readiness as this thesis prolonged and executed several unique actions to be prepared to handle the pandemic and the outcome that it might bring. Several of the operating rooms where the measurements were going to be conducted was reorganized for other surgeries or temporarily closed. As a consequence, the amount of planned surgeries heavily declined during the spring, the registered nurses and assistant nurses which normally worked at the surgery department were transferred to other wards. Due to the unusual circumstances, this Master's Thesis failed to measure the amount of surgeries that was initially planned and instead had to adapt to the changes that this situation brought along with it. Nevertheless, the work continued with these changes and eventually lead to the completion of this Master's Thesis. The aim is for this thesis to contribute to the importance of microbial free environments, as the situation around the world during the pandemic has shown that entire societies can be turned up-side-down.

Abbreviations

AMR - Antimicrobial Resistance

CES - European Committee for Standardization

CFU - Colony Forming Unit

CI - Confidence Interval

CoNS - Coagulase-Negative Staphylococci

FAA - Fastidious Anaerobe Agar

HAI - Healthcare Associated Infection

HB - Horse Blood

HEPA - High Efficiency Particulate Arresting

ISO - International Standard Organization

LAF - Laminar Air Flow

OR - Operating Room

PCA - Principal Component Analysis

QoL - Quality of Life

RISE - Research Institutes of Sweden

SD - Standard Deviation

SSI - Surgical Site Infection

SIS - Swedish Institutes of Standard

SU - Sahlgrenska University Hospital

TMA - Turbulent Mixed Airflow

UC - Ultra Clean

WHO - World Health Organization

Contents

1. Introduction

In the following section, a background introduces the problem and why this project needs to be conducted. The purpose and objectives of this Master Thesis are stated, as well as the limitation for the project.

1.1 Background

Healthcare-associated infections (HAI) are commonly occurring complications globally and affect 1 in 10 patients that have been treated at a hospital (A. E. Andersson, Bergh, Karlsson, & Nilsson, 2010). A. E. Andersson et al. (2010) continuous to argue that retrieving an infection during surgery is a excruciating discomfort for the patient and can lead to additional re-surgeries that are very stressful for the patient and the health care system. If the infection is severe enough, the patient's life could be endangered and be terminal. The annual health care cost for HAI in Sweden is estimated to be SEK 6.5 billion, but is expected to be even higher in the following years, due to a growing and aging population and because current antibiotic treatment for infections losing their potency as antimicrobial resistance (AMR) is an eminent problem. This cost is based on the amount of extra treatment days that the hospital needs to provide, but excludes other costs, such as sick leave for the patient and lost tax revenues, according to Karlsson (2016) report for Swedish Association of Local Authorities and Regions (SKR) about HAI. From the same report, it is estimated that between 30-50% of HAI can be prevented, if right interventions and precautions are performed. In 2011, the United States estimated to have over 720 000 cases of HAI which resulted in approximately 75 000 reported cases of death (10.4%) and the annual cost was ranged to be between USD 4.5-11 billion.

HAI can affect any patient that visit the hospital. However, patients who undergo implant surgery are especially a vulnerable cohort of patients and are very susceptible for HAI. HAI that occur during the surgical interventions are known as surgical site infections (SSI) and is the second most common HAI, after urinary tract infection (UTI). Overall, from orthopedic implant surgeries, the cases of deep incisional SSI has been constantly 1-2% between the year 2008 and 2017, according to Professor and MD Henrik Malchau (2020). The susceptible reason is due to that the wound is sufficiently large for bacteria to infiltrate and the implant's adhesive surface is a suitable environment for bacteria to colonize and spread upon. In events of a deep incisional SSI, complications have led to that the entire implant must be revised and changed.

Inside the operating room (OR), strict regulatory legislation, guidelines and recommendations are withheld in order to secure the patients' safety for infection control. The amount of colony forming unit per cubic meter (CFU/m^3) of air, is an international standard measurement for what is classified to be microbial clean air. According to the International Standard Organization (ISO), there are several different classifications of how clean the air can be, with respect to particle sizes that may carry microbes. In Sweden, recommendations for ultraclean (UC) air according to *Instruction of care* (*Vårdhandboken*),(von Vogelsang & Åkesdotter Gustafsson, 2018), is 10 CFU/m^3. To maintain this level, 5 CFU/m^3 should be established, to secure the limit in implant surgery where the level of the purity of the air quality is crucial to lower the risk of infections. However, in the case of hip-fracture surgery that is especially susceptible and the risk of infection is very high e.g total arthroplasty, the amount of CFU/m^3 should be less than 5 CFU/m^3.

ORs at Sahlgrenska University Hospital (SU) Orthopedic Department in Mölndal have two different ventilation types. Modern laminar airflow (LAF) ventilation systems (also referred as LAF-ceilings) and older conventional ventilation system. Some implant surgery, such as hemiarthroplasty hip fracture surgeries, still takes place in ORs which relies on conventional ventilation systems, when the LAF ventilated ORs are occupied. To investigate the risk of SSI in orthopedic implant surgery and find possible correlations between the amount of bacterial contamination in the air, on the instrument tables, on patient's skin before incision and inside the wound of the patient before suturing, have several partners, including Research Institutes of Sweden (RISE), SU, Toul Meditech, Getinge and CRC Medical set up a project to investigate the technological approach to tackle this problem. *Clean Care*, as this VINNOVA financed project is called, has the vision to provide solutions that can prevent the spread of infections within the healthcare environment, with focus on air quality and SSI. In close collaboration with the OR nurses, surgeons and other OR staff, data on CFU levels will be gathered on surgical site. The CFU levels will be analyzed and compared with different settings and environments.

The long-term goal for the *Clean Care* project is to improve the environment in the OR so that the risk of contaminating the surgical wound is reduced, and thereby diminishing the patients' risk of developing SSI. The gained knowledge regarding the importance of different contamination pathways and how to best measure them will furthermore be the foundation for a quality measuring system that in real-time gives continuously feedback and guidance to the OR staff on critical factors which can reduce the levels of CFU and therefore reduce the preventable cases of SSI.

1.2 Purpose and objectives

The purpose of the present study, as a part of the *Clean Care* project, which has been ethical approved, is to gain better understanding of the pathways for bacterial contamination of the surgical wound during orthopedic implant surgery. The focus is on the air quality and factors association with this, to ensure patients' safety in the operating room based on infection control This study will compare three types of ventilation settings and techniques for comparisons and evaluation of contamination pathways to gather knowledge about diverging levels of microbial contamination in ORs which have different prerequisites when it comes to air quality. Namely: i) The conventional turbulent mixed airflow (TMA) ventilation system, ii) TMA ventilation system, with a protective horizontal LAF barrier over the surgical instruments and surgical wound and iii) LAF ventilation system.

To answer the purpose of this study, following objectives have been set up:

- Investigate if there is any divergence in contamination levels for the different ventilation systems, for better understanding of the contamination pathways and which preventive actions can be made.
- Investigation to see if there is a correlation between the number of viable bacteria in the air, on the instrument tables, the skin of the patient and the surgical wound.
- Perform a meta-analysis to see if the working routines of the OR staff (number of door openings, the amount of people inside the OR and the clothing of the working staff) correlates with number of CFU in the OR.

1.2.1 Limitations

The clinical study is limited to measurements during one type of orthopedic surgery, namely hemiarthroplasty surgeries. The specific type of surgery is chosen due to the relatively long surgery time (allowing many active air samples) and large wound size (increasing the risk of airborne contamination).

The samples will only be prepared on agar-plates. The typing, counting of colony forming units and storing the samples in a biobank will be handled by the hospital's microbiology lab.

Due to the outbreak of the Corona virus in the spring of 2020, the number of planned measurement heavily declined as the hospital reorganized its resources and the ORs with the turbulent mixed airflow ventilation were not in use during this period of time.

2. Microbial contamination

This chapter is intended to give a brief introduction to cover typical bacteria types that is commonly present inside orthopedics ORs. This is to increase the knowledge of what kind of bacteria that could cause postoperative implant related infections and also the probability of their occurrence. The chapter will also include a section about preventive healthcare and the decrease effectiveness of antibiotics, to highlight the purpose of the *Clean Care* project in general.

2.1 General bacteriology

Bacteria is a simple, but yet complex unicellular organism. Their length is typically a few micrometer and they exists everywhere in our surroundings and environments, even the most extreme. Their shape, size, appearance and virulence can however be very diverse. For example, they could exist as small round cells, cocci (for example *Staphylococcus* or *Streptococcus*), or rod shaped cells, bacillus (such as *Cutibacterium*). Some exists in desolated, extreme environments, while other are essential and are in contact with human life every day. Their interaction with the eukaryotic domain is of vital importance in many circumstances and the evolutionary course has led to that prokaryotic and eukaryotic cells has developed an endosymbiotic relationship, to ensure survival of both the two branches of life. However, if a hostile bacteria gets in contact with a vulnerable biological system, an invasion and spreading of that bacteria could cause infections and have a negative impact on the affected's health. Bacteria reproduce by asexually binary fission and can, in a nutritious environment, multiply to a tremendous amount replicates in a very short period of time, meaning that an infection could spread fast and uncontrolled inside the body. The invaded host's only defense mechanism is the immune system which intermittently is not sufficient enough to handle a bacterial attack. Medication, such as antibiotics, can however kill the bacteria cell and be a supplement to the immune systems natural defense.

In a hospital environment, many types and species of bacteria are the main cause of UTI, pneumonia, sepsis and SSI. The source of the bacteria are often the personnel or even the patient itself whom is the carrier the cause of spreading within the hospital walls. The risk of getting an infection is, however, generally low if routines and protocols are followed, such as basal hygiene and aseptic techniques.

2.1.1 Coagulase-negative Staphylococci

Coagulase-Negative Staphylococci (CoNS) are a heterogeneous group of Staphylococcus that does not produce the enzyme coagulase and therefore cannot process fibrinogen into fibrin. If fibrin is formed, a blood clot is more easily formed and therefore creates other physiological conditions. CoNS are typically opportunists of hospital-acquired pathogens and are strongly associated with postoperative implant related infections, especially when a patient's immune system has been compromised (Murrell & Nall, 2018).

2.1.2 Gram-positive or negative bacteria

The membrane of bacteria can be composed in typical two ways and therefore classified as either gram-positive (G+) or gram-negative (G−), after the Gram-staining method. G+ bacteria has a thicker cell wall composed of peptidoglycan layers (nearly 90%) and teichoic acid. The vast amount of peptidoglycans makes G+ bacteria highly resistant to environmental impact. In contrary, G− bacteria has only approximately 10% of peptidoglycans, but instead compensates with a thinner outer layer of lipopolysaccharide (LPS). LPS affects the bacteria's virulence as it can cause a toxic reaction if entering the circulation system of the body, which eventually can lead to sepsis.

These different classes of bacteria are susceptible to different kind of medical treatments. For example, G− bacteria are more resistant to various types of antibiotic treatment, compared to G+, due to that many antibiotics inhibit important metabolic pathways which are used to form the thick cell walls of peptidoglycans, which G− bacteria does not exhibit.

2.1.3 Aerobic and anaerobic bacteria

Oxygen dependent bacteria (aerobic), as indicated, require oxygen to survive and to thrive. Their counterpart is an oxygen independent bacteria (anaerobic) that do not demand oxygen for its cellular respiratory system to produce energy and can, if exposed, even die of toxic reaction, in the presence of oxygen. However, there is a wide and broad range of bacteria that are tolerant and partially oxygen dependent, classified as facultative anaerobic organism. Facultative anaerobic organism, includes, for example, the bacterial genus form *Staphylococcus*. Aerobic and anaerobic bacterial species does not exhibit any known diverged properties when it comes to virulence or treatment options for possible infections.

2.2 Bacterial pathogenesis

Bacteria which are known to cause diseases are called pathogenic bacteria, or simply pathogens. Many threats by pathogens is neutralized since the discovery of antibiotic treatment was made, but still, pathogens are a constant threat to human health and recovery from infectious diseases, and with the decreased effect of antibiotics, the future of pathogens are a threat to modern medicine and human health.

Pathogens exists in several of forms, both as bacteria, fungi, parasites, protozoa or viruses. However, bacteria pathogens are the main cause of postoperative implant related infections. Due to different characteristics, the virulence may differ among species as they use different mechanisms to colonize, grow and spread on the expense of the affected patient. A few examples of the bacterial virulence mechanisms could be, according to Murray, Pfaller, and Rosenthal (2015), adherence, degradative enzymes, endotoxins, capsule, resistance to antibiotics and intracellular growth. The virulence is determining the ability and in what grade a pathogen can cause a certain disease and illnesses to an infected patient.

2.2.1 Opportunistic pathogens

Most of the human normal flora bacteria are harmless, as can be read in 2.3. However, when an open wound is exposed to the surrounding environment, some of these less harmful bacteria could enter the wound and become problematic in this nutrient rich environment without other bacteria to compete with. Due to the circumstances of a surgery or trauma, the immune system could be weakened and impaired by the stressful situation and therefore could even these bacteria be a threat. In the event of an orthopedic implant surgery, the bacteria could get attached to the surface of the material and find a good, steady and protective environment from the body's own defense, and start to colonize, causing deep incisional SSI.

Micrococcus luteus/lylae, *Staphylococcus epidermidis* and *Staphylococcus capitis* are typical bacteria that are a part of the human normal flora. However, if these bacteria, endogenously or exogenously, gets inside a wound, these normal bacteria could cause severe damage to the infected patient, and even have a fatal outcome if the immune system is impaired and cannot handle the physical load. Another bacteria who is notorious famous to cause implant related infections is the yellow colored *Staphylococcus aureus*, which can cause both skin diseases and biofilm formation (see section 2.4) on prosthetic surfaces. *S. aureus* is a global clinical issue as strain of the specie has developed resistance to some antibiotic types. These type of strain are typically known as Methicillin-resistant Staphylococcus Aureus (MRSA) (Adlerberth & Wold, 2007).

Adlerberth and Wold (2007) states in *Människans Normalflora* (Human microbiome) that any specie of bacteria from the human flora can cause severe infections, if they end up in the wrong place and if the colonization gets to massive for the body's immune system to handle. Therefore, under the certain conditions, nearly any bacteria can cause some type of infection, implant related infection or sepsis. However, the virulence may differ from these bacteria, which could affect the mortality rate.

2.2.2 Colony forming unit, CFU

When sampling, measuring and cultivating bacteria, colony forming unit (CFU) is the most common way to express the occurrence and distribution with a quantify measure. In theory, each CFU that grows in cultured environment (often in petri dish consisted of agar) corresponds to one ancestor and viable bacterial cell from the start of the cultivation, with the assumption of no contamination. The colony is visible with the naked eye, in contrast to solitary and planktonic bacteria, which makes it easier to count. A backtracked scenario can therefore be established in order to calculate the presence of bacteria, in a defined occasion, such at the time of the surgical site, which is useful for an estimation of acceptable bacterial level inside the OR. If the number of CFU are well over the acceptable limit, analysts can go back to see if anything unusual or extraordinarily events caused the CFU level to fluctuate.

A study made by Jordestedt (2015) showed that distinctive room has various levels of airborne contamination. For example, the preoperative transfer area had approximately 40 CFU/m^3 in the air, 60 CFU/m^3 in the nearby staff room, whereas the toilet and the locker room have 300 respectively 400 CFU/m^3.

2.2.3 Bacterial infections

Infections are caused by bacteria, or other pathological invaders, who intrudes an exposed area in the body. Normally, many infections resolve without medical treatment, as the body's own defense is primary resource for riddance of the intruders. However, infections can be more susceptible, severe and threatening if the patient's immune system is inadequate for the exposure. Infections appear in several different varieties and causes different types of outcome in relation to where the bacteria has entered. This study only focuses on HAI that occur in the OR, namely SSI.

2.2.3.1 Surgical site infections, SSI

Mainly, there are three types of SSI;

- Superficial
- Deep incisional
- Organ or space associated

Superficial SSI involves the epidermis and the subcutaneous tissue and is the most manageable SSI type. It can be treated by local incision care or superficial debridement. Deep incisional SSI is a more severe type and involve fascial or muscle tissue and if foreign material has been implanted there is often a need for extensive depridement and sometimes also implant removal. Organ or space SSI involves abscesses and can affect both organs and the spaces between organs that lies deeper than the fascial or muscle tissue. This type also includes infections that's located on implant surfaces and must be surgical removed for extraction (Johns Hopkins Medicine, 2020).

2.3 Human microbiome

Bacteria, and other types of microorganism, have during the course of evolution developed a symbiosis with multicellular organisms to maintain a beneficial, ecological system with it host. An average human carry $3.8*10^{13}$ bacteria, according to Sender, Fuchs, and Milo (2016). This microbiota has several functions for the host as e.g. gut bacteria assisting in the degradation of food, so that the nutrient is better accessed or harmless bacteria competing of the space keeping down the growth of pathogenic species. These harmless bacteria are also crucial in their hosts survival and health, as they fend off and kill more dangerous microorganisms. They are also educational subjects for the immune system and train the immune system to recognize their more dangerous, pathogenic cousins since they share similar markers and receptors which the body's adaptive immune system can recognize and kill in case of an invasion (Adlerberth & Wold, 2007).

Bacteria inhabit and exists everywhere in and on our bodies. Our skin, gut and bowel are only few examples where bacteria have developed a symbiotic relationship and complement with biological mechanism that our human genome yet has not evolved to be self-dependent. Nonetheless, even these regularly innocuous bacteria could pose a threat if they are exposed to otherwise sterile sites, such as the urinary bladder or an open wound.

2.3.1 Skin microbiome

In Tabel 1, common skin bacteria are listed to raise the issue of what may be found on the skin of the patient, but also in the air as many bacteria from the skin microbiome gets attached to loosening skin particles. These bacteria are part of the human normal flora and is therefore of great importance due to that they all are a part of our everyday lives and are expected to be found, if our surroundings are to be sampled and measured.

Table 1: *Common types of bacteria that resides on the skin, (Adlerberth & Wold, 2007).*

Phylum	Genus	Species
Firmicutes	*Staphylococcus*	*S. epidermidis*
		S. hominis
		S. capitis
		S. haemolycticus
		S. saprophyticus
		S. warneri
		S. aureus
Actinobacteria	*Micrococcus*	*M. luteus*
		M. lylae
	Propionibacterium	*P. acnes*[1]

[1] *Renamed to Cutibacterium acnes (C. acnes)*

The skin is the body's largest organ with a normalized area of 1.8 m^2 and is the habitat for various types of bacteria. The skin is a cool, dry and acidic habitat that forms a physical barrier which prevents microorganisms and other dangerous substances to penetrate and get inside the body, causing disruption to the homeostasis. The prerequisite for this barrier to withhold, is that it needs to be unbroken. However, in a surgical implant procedure, the skin barrier must be damaged and is therefore an opening for the outer environment to get inside the skin barrier.

According to Grice and Serge (2013), the skin provides a milieu that is beneficial for the bacteria, but also beneficial for the host as they protect from outer and more dangerous substances and microorganisms, as previously discussed in 2.3. The skin microbiome consists of several ecological unique settings that microorganisms are adapted to and have developed a niches for specific surroundings. All the bacteria on the skin does not have to be depend on the same respiratory mechanism. For example, the anaerobic bacteria specie *C. acnes* is most likely to be found in the subcutaneous layer of the skin, getting nutrients from fatty acids from sebaceous glands in hair follicles. From the other spectrum, *M. luteus/lylae* is an obligate aerobic specie that resides on the epidermis layer of the skin and can be transported by loosening skin particles. In the middle of the spectrum is *S. epidermidis* which tolerate both aerobic and anaerobic environments and is therefore a facultative anaerobic organism.

The epidermis, the outer most layer of the skin, is mainly composed of terminally differentiated keratinocytes, and as illustrated in Figure 1, the cornified layer, or stratum corneum, give rise to small fragments of particles that sheds from the surface of the skin when the skin is self-renewing itself with new keratinocytes. The particles could also be shed from the surface from the skin from simply scratching the skin or from heat convection, which constantly occur. The issue with these loosening particles, is that they often carry bacteria. The bacteria can therefore be transported with the skin particles and land in inappropriate places, such as in the wound of a patient or on the surgical instruments. Every minute, a person releases approximately 10^4 skin particles and of these particles are 10% contaminated by bacteria, according to Sandle (2011).

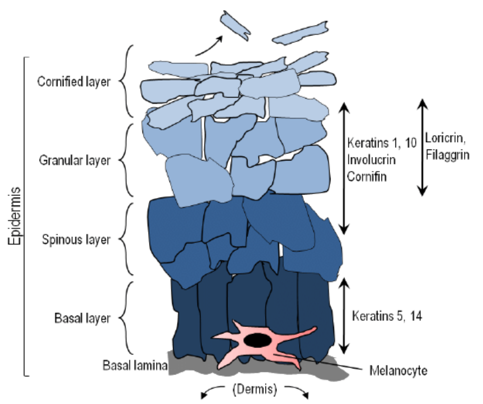

Figure 1: *Illustration of the epidermis, where skin particles are visible loosening (D'Orazio et al., 2013).*

2.4 Postoperative implant related infections

In orthopedic surgery, if an implant has been inserted into the patient, the risk of infection increases due to the foreign body which the bacteria can settle, colonize and spread upon. Postoperative implant related infections and SSI are complications that could have severe consequences for the patient, such as re-surgery, prolonged rehabilitation and increased risk for amputation or even death. From the hospitals' perspective, the unwanted complications could lead to increased usage of antibiotics, increased work load due to longer hospitals stay for the patient, extra demanding surgeries, continuously follow-up on the patient's health and unnecessary costs (Kumar et al., 2017).

If bacteria settle on implant, they can start to communicate with neighboring bacteria cells, an ability known as quorum sensing. Quorum sensing is a response to the population density and by communicating with neighboring colonies, they can change their physiological state and phenotype to get a more defensive barrier towards the body's immune system reaction, called a biofilm. Biofilms are a secrete of slime of polymeric matrix substance that adheres to a surface and consists of proteins and polysaccharides that are being produced and pumped out by the bacteria, called extracellular polymeric substances (EPS). Biofilms are a virulence factor and defense mechanism of complex architecture which often is composed of many different types of bacteria and can accumulate on an orthopedic prosthesis. Biofilms are not only successful in the protection against the infected patient's immune reaction as it induces a inflammatory cytokine response, but biofilms also exhibit a tenfold more resistance to antibiotic treatment than bacteria in secluded and planktonic state, as the EPS is very difficult for the antibiotic to penetrate to locate the target bacteria. If indications that leads to a suspected case of postoperative implant related infection, the implant may be surgical revised or removed.

Biofilms are a persistent clinical problem in orthopedic implant surgery and a problem that lacks a clear solution with modern material technology to stop the formation, as the material should not cause a toxic reaction to the body or an inflammatory reaction with would make the body to reject the implant. An orthopedic implant must show beneficial properties to its target cells (the surrounding tissue) and to design an implant with no aggressive characteristic that could be harmful for the patient's own tissue and cells. "Race for the surface" is a concept based on proper tissue integration instead of bacterial contamination on the surface, as the first one to adhere at the surface (cells or bacteria), has the advantages for dominance and can therefore steer the outcome of the implants successful integration, or possible rejection (Gristina, Naylor, & Myrvik, 1989).

As suggested from Figure 2, the biofilm formation starts with the attachment of secluded, planktonic bacteria on the surface. In this first stage, the bacteria uses quorum sensing to communicate, which is a response by gene regulation to the population density. Thereafter, they could change their phenotype into the most beneficial for the population, when it comes to survival and reproduction. Stage 2 in the figure illustrates the growing biofilm were bacteria have started to produce the protective EPS layer, forming a barrier towards both the immune system and medical treatment, such as antibiotics In stage 3, dispersion of bacteria from the biofilm take place, spreading bacteria to other locations of the body or, in worst case, into the blood stream, causing sepsis (Toole, Kaplan, & Kolter, 2000).

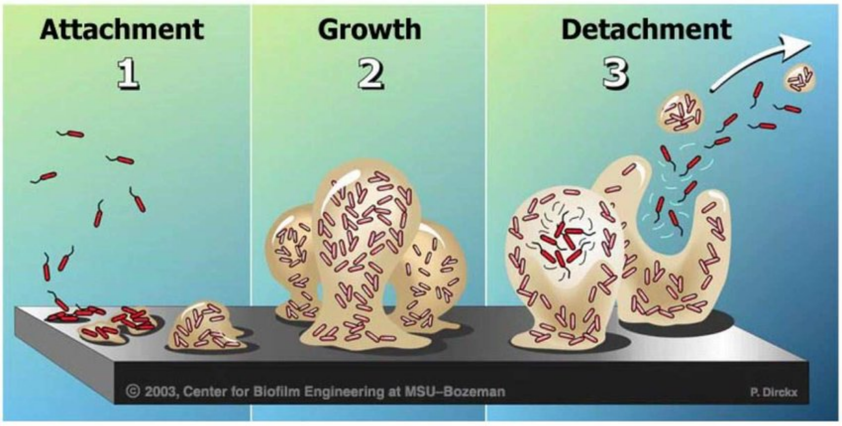

Figure 2: *The formation, development and dispersion of a biofilm, (Klapper & Dockeryt, 2010).*

It can take relatively long time for implant infections to develop and form and therefore difficult to find suitable treatment in time for. Symptoms can start to be visible first after several years after the implant has been inserted and is often diagnosed if the patient suddenly feels pain and discomfort from the implant. First then can an investigation and elimination of possible biofilm formation be conducted.

2.4.1 Hemiarthroplasty surgical intervention

In general, the typical trauma that elderly patients experience is by fall accidents, most commonly in their home when stumbling upon the edge of a rug, or on icy patches on the pavement. A common condition in elderly people is osteoporosis, meaning that the bone has been weakened due to a more porous bone structure, causing it to lose its former strength and be more fragile and susceptible for fractures. In Sweden, the amount of hip-fractures is estimated to be 17 500 annually. The average age of the patients is 82 years, where 69% of the cases are women, reported by Rikshöft (2014). All trauma resulting in hip-fracture should go through a surgical procedure within 24 hours for best outcome of the patient's recovery and improved quality of life (QoL). In the Orthopedics Department at SU, there are approximately 300 reported cases of hip fractures annually that get hemiarthroplasty surgeries, according to Henrik Malchau (2020).

For most hip-fracture patients in Sweden today, the typical surgical implant intervention is hemiarthroplasty. Hemi, meaning "half", and arthroplasty which refers to "joint replacement", is a less extensive surgery than total arthroplasty, where the entire ball and socket are replaced (namely the femoral head and acetabulum). Hemiarthroplasty only removes and replaces the femoral head, but still keep the acetabulum intact. Total arthroplasty is given to patients who are considered to be able to continue to live a more functional life, are cognitive aware and have good ability to walk. This due to that the hemiarthroplasty prosthesis may erode over time and therefore not suitable for young elderly patients. Total arthroplasty procedures are always performed in ORs with minimal number of staff members present and in ORs that are equipped with LAF-ceilings, as the surgery is regarded as more infection sensitive, compared to hemiarthroplasty surgeries. Even though the similarities in the intervention, hemiarthroplasty surgeries are considered to have slightly less operation time, not LAF-ceiling dependent and not as wide wound and is therefore more suitable to perform measurement upon in this present study.

Typically, the procedure is initiated by the orthopedic surgeon, performing an incision near the hip where the fracture is. Once the joint is visible, the femoral head is dislocated from the acetabulum by the surgeon with accessible tools. The femur canal is reamed and a metal stem is placed inside the femur after the femoral canal has been prepared. The metal stem is, in Sweden, most often fixed to the bone using bone cement of polymethyl methacrylate (PMMA). The artificial head is firmly fixed to the stem by a conical taper of the implant and can be put back into the acetabulum.

(a) *The body and head of the prosthesis.*

(b) *The prosthesis inserted into the acetabulum.*

Figure 3: *Images of Lubinus/Mega Head from Waldemar Link GmbH & Co. KG, used in hemiarthroplasty surgery.*

2.5 Preventive healthcare for infection control

There are multiple ways to increase the precaution in order to secure the patients' safety in the OR. In this section common types of modern approaches will be highlighted to understand its impact and its limitations for the health care system. Preventive healthcare, or prophylaxis, will come to play an important role in the medical field in the future, as certain existing methods of post-operative actions has begun to show a decline effectiveness and usefulness.

2.5.1 Antibiotic prophylaxis

The discovery of penicillin in 1928 by Alexander Fleming was the start of a new era of modern medicine. Diseases and infections we today regards as easy to cure or manage, could a century ago lead to certain death. The usage of antibiotics to cure and prevent infection has increased rapidly, not only at the hospital site, but also in agriculture where a healthy livestock is important to maintain, in order to process high quality food. The overextended usage of antibiotics used in agriculture is however a debated issue, due to the rise of antibiotic resistance in bacteria. In medicine, antibiotics have become a necessity to prevent infections to spread and cause harm to the patient. In orthopedic implant surgeries, the risk of infection is imminent, and precautions has been made to ensure the patients' safety. After the surgery, a medical evaluation has to be performed and staff with the right medical expertise needs to state the dosage for the patient to maintain low bacteria proliferation and therefore a decreased risk of developing an SSI.

However, the course of modern medicine is rapidly changing as the increased usage of antibiotics induces a decreased effectiveness of the drugs, as more bacteria tend to develop a resistance to the current drugs we today classify as common and important to fight infections (for example penicillin). In many countries, antibiotics can be found and be purchased in markets, shops and pharmaceuticals over the counter, meaning that prescription are not a requirement. The vast usage of antibiotics in unnecessary and uncontrolled applications is one of the major causes of that AMR is rapidly spreading, according to the World Health Organization WHO (2020).

AMR occurs as an evolutionary response to adapt to the current environmental changes, but also through conjugation, transformation and transduction of the genetic material between bacteria. Antibiotics acts a toxic reactant to bacteria and could attack certain life upholding properties, such as the membrane or respiratory system. In the case of penicillin, some of the surviving cells from the encounters gave rise to genetic mutations that where transferred to the clone cells from binary fission which lead to the creation of the enzyme penicillinase. This enzyme could then be produced by the bacteria to stop the growth-inhibiting property of penicillin to take place and neutralize the threat, presented in an early article by Abraham and Chain (1940). Since then, more genetic mutations have been evolved as biological defense mechanisms which eventually will lead to a total ineffective usage of antibiotic treatments.

In 2015, the third objective in WHO Global Action Plan on Antimicrobial Resistance on page 9 states:

"Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures"

This objective aims to find, explore and develop suitable techniques that can be used to stop, or decrease, the usage of antibiotic treatment by scrutinizing key components of preventable cases. WHO implore the scientific community and medical field to look what can be done with already existing method to fight AMR, to decrease the usage of antimicrobial agents (WHO, 2015).

2.5.2 Staff routines

All medical staff members are educated and trained to be able to work with preventive care. However, in an article by S. Andersson (2015) for Nollvision, the Infection Prevention and Control Physician Birgitta Lytsy from Uppsala University Hospital explains that the lack of knowledge by the staff on why routines exist is a big risk in surgical interventions. Much of the education is based on conservative, social and cultural structures at the hospital, meanwhile the underlying cause of the education is often neglected. A consequence of this is that the patients' safety when it comes to infection control is not well taught to new students, causing unawareness and insufficient knowledge of infection spreading. Basic routines as hand washing and correct working gear are important factor for preventing opportunistic pathogens to spread.

2.5.3 Laminar airflow (LAF) ventilation system

Modern ORs often have a so called LAF-ceiling to prevent contamination of the surgical wound. The ventilation of a LAF-ceiling is designed to have UC clinical air that flows unidirectional downward with a velocity at 0.3 m/s to prevent particles, as described in section 2.3.1, to drift and land nearby or inside the wound. As seen from Figure 4, the unidirectional thus forms a protective curtain that also should protect against penetration of particles that are emitted from the surgical team. The air is then forced to the floor and then transported to the side of the rooms, where the air is collected and filtered in the outlet. The LAF-ceiling is state of art technology and studies have shown that LAF-ceilings can achieve very low CFU count in the operating field (<5 CFU/m^3), by letting unidirectional airflow pass through a high efficiency particulate arresting (HEPA) filter (Rehn, 2020).

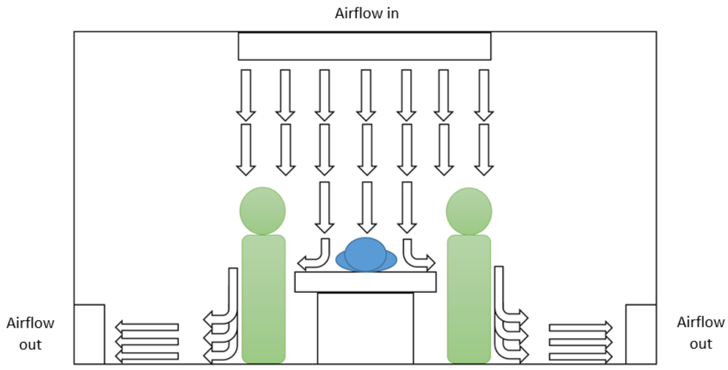

Figure 4: *Principle of the laminar airflow in ORs equipped with LAF-ceilings, creating a curtain of air around the patient. Illustration made by Frans Stålfelt.*

Newly built hospital often have installed modern LAF-ceilings in their OR instead of installing conventional ventilation systems, such as TMA ventilation. Older hospitals still have conventional ventilation systems in their OR and to convert existing ORs with conventional ventilation system to LAF can be both very complicating and expensive. Either can the roof be too low to for installment or not have the capacity to handle the demands which LAF ventilation require. Therefore must other options be regarded as suitable solutions to overcome a to high level of CFU/m^3, e.g applying laminar airflow with a mobile device on the instrument table, which is more described next in section 2.5.4.

WHO (2016) does not recommend LAF to be the solemn solution to prevent SSI. This is due to that scientific evidence that LAF ventilation systems contribute to a decrease frequency of patients' that develops an infection is "low to very low quality of evidence". However, a health technology assessment by Houltz et al. (2020) put forward evidences for fewer bacterial carrying particles near the surgical wound and thereby evidence for fewer bacteria. A very recent study by Langvatn et al. (2020), also exhibit clear evidence for a reduced amount of CFU near the surgical wound when LAF is applied, compared to conventional ventilated ORs. A constant debate between researchers and clinicians are whether LAF ventilation should be used or not and their opinions may be divergent about its prophylactic abilities to reduce SSI.

At SU there are in total 93 ORs in use, where 18 are equipped with LAF ventilation. The orthopedic surgery department at SU, Mölndal, there are 3 ORs with LAF-ceilings and 4 ORs with conventional turbulent mixed airflow ventilation systems. In the entire Region of Västra Götaland (VGR), approximatley 80% of all the hospitals ORs are equipped with conventional turbulent ventilation systems Houltz et al. (2020).

2.5.4 Toul MediTech's mobile LAF instrument tables

According to a study with measurement done by Aghaee, Erichsen Andersson, and Grant (2015), the levels of CFU/m^3 was evaluated when applying a LAF over the site of the surgical instruments. They could show a significant difference in air quality above the tables when using this technique, when used in an OR with conventional TMA ventilation. Approximately 17 times lower counts could be measured when the LAF instrument table was in use (findings showed 1.45 CFU/m^3 compared to 24.5 CFU/m^3, respectively).

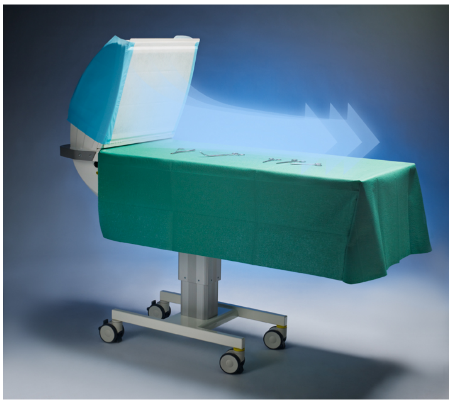

Figure 5: *Toul MediTech's (2020) Mobile LAF Instrument Table, Steristay.*

In this study, the LAF instrument table will also be used in order to compare conventional TMA ventilation system with LAF to strengthening the mentioned above study's claim. The Toul MediTech's Mobile LAF Instrument Tables are medical devices that are a possible solution where LAF technology needs to be applied, but when the OR setting is not optimal for installment of a LAF-ceiling and is dependent on other conventional ventilation.

Figure 6: *Toul MediTech's (2020) Mobile LAF Assistant Table, Operio.*

Toul MediTech has developed an instrument table that provides a LAF barrier over the instruments, as seen in Figure 5, called Steristay. The company has also developed an assistant table that are made to have commonly reoccurring surgical tools and instruments ready next to the operation nurse for easy access. This table is called Operio and can be seen in Figure 6. Besides the LAF barrier protecting the instrument and surgical tools has Operio has the purpose by letting the same barrier of horizontal LAF in the direction of the surgical wound of the patient and thus creating a protective barrier there as well.

2.5.5 Materials

To have a correct response of the implant and maintain a microbial free surface, the material itself could exhibit characteristic that could prevent the SSI to happen by having a protective attribute. This section is to highlight alternative preventable techniques that can be used to fight infections, although materials are not the main focus of this study.

As the decreasing efficiency of conventional treatment of antimicrobial medications and the growing concern of bacterial resistance to these therapies, materials and coating substances can reduce and limit the pathogens to spread on biomaterial, if the material surface has the ability to fend of bacterial settlement.

2.5.5.1 Biocompatibility

Materials that are used inside the body to interact with cell and tissue are commonly known as biomaterials. The biomaterial is used to make implants, such as prosthesis used in orthopedics, dental implants or ophthalmological lenses, as biomaterials integrate with the body and have functional properties for the patient. When developing and constructing a new material which purpose is to be used as an implant or prosthesis, specific criteria and regulation must be fulfilled to be used in clinical trials and put on the market. Biocompatibility is a complex concept and word which constantly is debated as it can be used incorrectly, stated by the initial user of the word and highly respected biological material scientist David Williams (2014). Williams discuss in his leading opinion article, *There is no such thing as a biocompatible material*, that biocompatibility is a mechanism that is not solemnly dedicated to the material's characteristics, but is the interaction between both the material and the surrounding tissue and that a material cannot be biocompatible by itself. In an earlier article conducted by Williams (2008), the author states that biocompatibility is important to fend off a foreign body reaction response by the body's immune system, but also microorganisms which could cause infectious responses.

2.5.5.2 Biomaterials in hip prostheses

Hip prosthesis stems used in orthopedic surgery are often made from Titanium (Ti-6Al-4V) alloys, stainless steel or Cobalt–Chromium–Molybdenum (CoCrMo) alloys, due to their relatively high mechanical tensile strength and have good interaction and performance in the body. Titanium alloys, CrCoMo alloys, polyethylene and ceramics are a commonly used materials as the bipolar head and bearing for the femoral stem upon the inner head, as seen in Figure 3. None of these materials are however resistant to biofilm formation.

2.5.5.3 Medical devices

"A medical device is something that is claimed by the manufacturer to be used combined or separately in humans to;

- *Prove, prevent, measure, treat or ease a disease*
- *Prove, prevent, treat, ease or compensate for an injury or disability*
- *Investigate, change or replace anatomy or physiologically process*
- *Control fertilization*

If the product achieves its main purpose with pharmaceutical, immunological or metabolically means, it is no longer consider to be a medical device according to this law"
\- Swedish Law (1993:584) (1993)

According to the law stated above, a medical device is in close contact with humans and is therefore, in most cases, important to maintain free from contamination during that time of contact. All the medical devices that are going to be used inside the sterile zone in OR must be sterile. Disposable items needs to be encapsulated by the manufacturer and usually has an expiration date. The sterilization methods can vary by different manufacturers, but autoclavation (steam sterilization), ethylene oxide and radiation is the most commonly used in industrial purposes. Products which can be used multiple times such as surgical instruments, are sent to the sterile technical unit where the products are cleaned and sterilized before they can be used again, usually by autoclavation. In cases of abnormalities and the medical devices are not properly sterilized, indirect contamination could lead to SSI, as described in section 3.2.

3. Contamination pathways

In this chapter, several of possible contamination pathways will be described in detail in order to understand important parameters under investigation for this study. The following contamination pathways plays important roles inside the OR and can be controlled or minimized to reduce the risk for the patient to develop a SSI.

3.1 Bacterial air contamination

A common pathway for bacteria to ascend into the wound of the patient is via air pollution, according to Andersson Erichsen (2013). Particles carrying bacteria in the air are mainly products from human activity, such as scratching on the skin or heat convection, described in 2.3.1. The airborne particles are moving across the OR as the airflow transports them, enabling them to reach the wound of the patient and possibly cause postoperative complications. In a study conducted by Whyte, Hodgson, and Tinkler (1982), the authors found indications that 98% of the bacteria that contaminated the patient's wound during hip and knee surgery (clean surgery in contrast to, for example, bowel surgery) in an OR equipped with conventional ventilation system could be linked to the air contamination. Air contamination of the wound appeared to occur both directly or indirectly by bacteria landing on other surfaces such as the surgical instruments and were then therefore transferred to the wound.

One factor that could affect the number of particles in the air is to the number of staff members inside the OR. When performing surgery, the number of participants should be as low as possible to decrease the risk of contamination of the patient. Normally for hemiarthroplasty surgeries, the working staff consist of one anesthetic nurse, one assistant nurse, one operating nurse and one surgeon, but the number can vary depending on if an assisting surgeon or OR nurse are present, or if students are present. The motion of the participants in the OR should also be low as possible to reduce movement of air near objects or the floor. Motion may cause dust particles to swirl upwards into the air and land near the surgical wound. More participants inside the OR are also the cause of more shedding of skin particles. The traffic flow in OR are a part of social and cultural heritage and differs between hospitals. According to a study regarding traffic flow in the OR by A. E. Andersson, Bergh, Karlsson, Eriksson, and Nilsson (2012), their conclusion were that by reducing the traffic flow overall inside OR and in the corridor, SSI could be prevented in a far greater extent, as much of the traffic flow is not focused on the patient or the ongoing surgery, but instead social encounters between the staff members.

In a study done made by Teter et al. (2017), the amount and length of door openings, corresponds to the increased amount of particles in the air inside the theater. Inside the OR, an over-pressure is built up to transport the particles outward and not inward in the occurrence of a door opening. However, if the door is open long enough or the width of the door opening is big, the door opening could destabilize the air pressure and the particles can move more freely as the draft transport the air through the room. Door opening can sometimes be crucial of getting necessary supplies to the patient, such as new necessary material for the operation, blood or anesthetic supplements, but many door openings during surgery could also be easily avoided, e.g commutation or consultation that instead could be over telephone or via visual aids, such as video camera.

The particle sizes also has also an effect on the microbial level, presented in a study made by Stocks et al. (2010). The particles surfaces can be adherent by bacteria and are therefore a transportation aid for the bacteria. The larger surface area equals more space for more bacteria to be attached to, which in order can cause more damaged if the particles end up in the wrong places (for example the surgical wound). A more detailed description about regulations of different particle sizes is mentioned in subsection 4.2.1.

3.2 Instrument table contamination

Before any orthopedic surgery starts, all the medical devices, instrumentation and implants which are going to be used on the patient must go through a sterilization event. To have a sterile working environment is crucial to secure the patient's safety, as contaminated instruments could have devastating consequences if contaminating normally sterile body cavities. Contaminated instruments could therefore be both a direct source (if the instruments are pre-contaminated) and indirect source (if contaminated inside the OR) of infection in the wound and the implant.

The instrument tables are prepared either in an adjacent room or inside the OR. The sterile instruments are laid out from trays that have been cleaned by the sterilization unit. After the instrument tables are set up, they are covered by a sterile drape before the patient is brought inside the OR or before multiple staff members from the surgical crew enters the room, to decrease the risk of the instrument table to be contaminated before the surgery begins. The preparation is often made by the operation nurse and assisted by an assisting nurse. Before preparing the instruments, the nurse must properly wash and disinfect hands and arms, to the elbow, followed by coverage of the OR clothes by surgical gowns and putting on sterile gloves, to prevent unwanted contamination. When the laying out of the instrument is done, the table is covered with many layers of sterile coverage, to ensure that no contamination takes place when the patient and rest of the staff enters the OR. These recommendations are stated by the Swedish organization *Protesrelaterade Infektioner Ska Stoppas* (PRISS, 2019) on precaution and prevention of prosthesis related infections.

It is impossible to detect if medical devices, such as surgical instruments, are safe to use from a microbial-free point of view, with just the naked eye. Therefore, a number of standards and regulations has been established to be guidelines, in order to direct the care givers and enlighten their responsibility to ensure that the medical devices have been thoroughly sterilized and are safe to use on a patient. There are strict restrictions that if anything sterile touches an object which is not sterile, the sterile object is immediately consider to be contaminated and must be removed and cannot be used further. However, if the air is "dirty" and contaminated, it is in constant contact with the instrument when they are not covered and this event cannot be avoided from happening. This could, however, be improved with certain techniques, such as LAF-barrier over the instruments, which prevent contaminated particles to fall on the tables.

3.3 Endogenous contamination

Endo-, meaning "within", and -genous, meaning "born from", is the term, as mentioned in section 2.2.1, where the patient's own bacterial flora can be the cause of an infection when a surgery takes place after a traumatic event. Therefore, before the surgeon begins to operate on the patient, the operating nurse clean and disinfects the patient where the skin incision is going to be. This is a critical procedure that is carried out in a structurally manner by protocol, to decrease the risk that bacteria are still present on the skin on the incision site.

Endogenous contamination is a very common type of HAI and very easy to prevent, if the cleaning is executed correctly. The cleaning takes approximately 10 minutes for the nurse to perform inside the OR when the patient is sedated. It's performed on the patient by the nurse which wears a sterile gown and uses 5 mg/ml chlorhexidine as disinfectant on the site where the incision is going to be placed. The patient is also showered and washed as ordinated in the protocol for preoperative action plan at the preoperative ward.

Exogenously contamination, in contrast, is when the bacteria descend from outside the endogenous biological system (outside the patient's own body). All personnel that are to be inside the sterile zone, often just the operating nurse and surgeon, are obligated to wash and scrub their hand and arms very thoroughly for approximately 2 minutes with aseptic soap and antibacterial agents before entering the OR. Whilst inside, a proper sterilized gown is dressed assisted by the assisting nurse onto the operating nurse and surgeon. Thereafter, the proper dressed personnel are now consider to be as sterile as possible and are granted access into the sterile zone next to the patient. However, circumstances could arise that somehow makes the personnel not entirely sterile, i.e they could touch or be touched by a non-sterile co-worker. If these events go by unnoticed, it can have a severe impact on the patient safety (Andersson Erichsen, 2013).

4. Clinical regulations and standards

This chapter is an introduction in clinical regulations and work manners used in the surgical environment in the Orthopedics department at Sahlgrenska University Hospital, Mölndal, in Sweden and in the European Union, as well as globally. The following factors play important roles in keeping the OR clean and noncontagious for the patient safety, but also secure for the working staff to operate in.

4.1 Clothing and surgical drape regulations: SS-EN 13795-1:2019

European Committee for Standardization (CES) and Swedish Institutes of Standards (SIS), has set up standard regulations on what clothes to wear and what draped material should be used inside the OR. According to SS-EN 13795-1:2019 (SIS & CEN, 2019), the requirements for clothes accepted in the OR environment should not only exhibit characteristics as, cleanliness, low particle release, and resistance to microbial penetration, but also provide a comfortable working gown for the personnel to use, which includes breath-ability, surface thickness, electrostatic properties, light reflection, odor and skin sensitivity.

Figure 7: *Mölnlycke Health Care (2017a) BARRIER® Clean Air Suit.*

Source strength is frequently used to indicate the amount of CFU that are emitted from a person's body per second (CFU/s). Source strength is an efficient measurement and evaluation tool when it comes to evaluating effectiveness of working clothes and garments, as CFU/s can give a more easily interpreted result rather than CFU/m^3, which instead indicates the air quality in total. When comparing different garments, ordinary working clothes generates approximately ≥ 5 CFU/s and special clean air suits ≥ 1.5 CFU/s. See Figure 7.

In the OR, the required clothes comprise of a scrub suit, gloves, protective hat, protective helmet over the hat, surgical mask and glasses or visor. Even though these regulations are followed and respected, the personnel's social and cultural view on how to wear these clothes properly are of great importance when it comes to the best outcome of reduced source strength. E.g the helm should be put inside the tunic to decrease the heat convection and therefore the spreading of skin particles. Also, the tunic should be put inside the trousers for the same effect as stated above. For best results to decrease the source strength, the personnel should wear clean air suits, which are equipped with cuffs around every opening. The clean air suit can reduce the level of CFU with 75% and the total particle count with 90% in the air, according to Reinmüller and Ljungqvist (2003). This result is valid for any ventilation system used inside the OR. With proper behavior, right ventilation and best working clothes available, the level of CFU/m^3 should be less than 5 CFU/m^3 during sensitive orthopedic implant surgery according to PRISS (2019).

In orthopedic implant surgery, after the positioning and preparations of the patient are completed, any clothing or garments that come in contact with the patient should be fully sterilized and is therefore unpacked from an enclosed package at the site at the operation. The package is opened and the surgical coat inside is dressed with assistance from the assisting nurse, creating a barrier from the sterile and neutral zone. The operating personnel (the surgeon and the operating nurse) should be wearing double pair of gloves to ensure that no contact between the skin and the wound takes place between the operators and the patient. Figure 8 shows what a properly dressed worker should look like (protective eye-wear is missing).

Figure 8: *Mölnlycke Health Care (2017b) BARRIER® Surgical Gown Universal.*

4.2 Cleanliness of air inside OR

In order to have a clean enough inside the operation room, several guidelines and regulations have been conducted by standard institutes, both domestic and international. They serve the purpose to protect the patient, as well as the staff members. According to a famous study made by Lidwell et al. (1987), the cleanliness of air and the ability to maintain UC air inside the OR have strong correlation with the number of patients that are infected and develop sepsis. Lidwell et al. (1987) results showed that the ventilation had an importance in the reported cases of sepsis and therefore the amount of bacteria contaminated particles that swirl in the air, see Figure 9. This figure illustrates the importance of choosing the right ventilation system and how this affects the cleanliness of the air, and by applying UC air, such as LAF-ventilation, the reported cases of infection could drastically be decreased.

To maintain UC air, the hospitals must adapt to several rules, work ethics and proper behavior. Door openings and traffic flow have negative impacts on the cleanliness of the air, as these factors contribute to diverse air pressure and increased concentration of particles in the air. To maintain an acceptable level of particle size inside the OR, different classification has been established, as can be read in section 4.2.1.

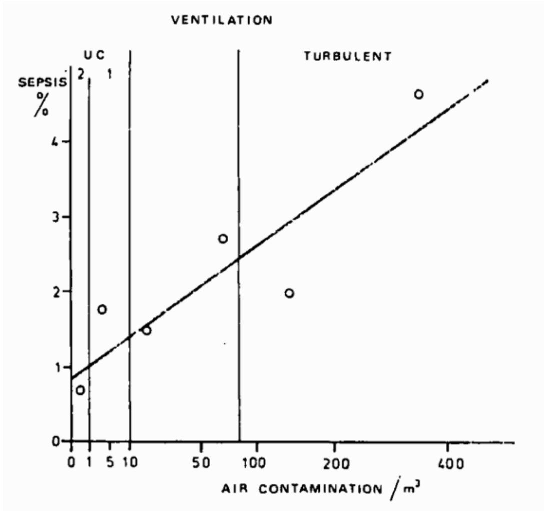

Figure 9: *Figure from Lidwells et al article about sepsis after joint replacement (Lidwell et al., 1987).*

4.2.1 ISO Standard 14644-1

To maintain and withhold an international standard of acceptable limits and classification of systems, the International Standard Organization (ISO), has set up certain criteria of the quality of air. These types of regulations are produced to have transnational guidelines that can be followed globally. As can be seen in Table 2, different classes are established to distinguish particle sizes and concentration. To reduce the particle concentration in the air can be a possibility to reduce the cases of SSI, as some of the particles can be inhibited by bacteria.

Table 2: *Classification of particle sizes versus the concentration, according to the ISO standard 14644-1.*

ISO Classification	$\geqslant 0.3\mu m$	$\geqslant 0.5\mu m$	$\geqslant 1.0\mu m$	$\geqslant 5.0\mu m$	$\geqslant 10.0\mu m$	$\geqslant 25.0\mu m$
Class 1	1	-	-	-	-	-
Class 2	10	4	-	-	-	-
Class 3	102	35	8	-	-	-
Class 4	1 020	352	83	3	1	-
Class 5	10 200	3 520	832	29	7	1
Class 6	102 000	35 200	8 320	293	69	10
Class 7	-	352 000	83 200	2 930	692	103
Class 8	-	3 520 000	832 000	29 300	6 920	1 030
Class 9	-	35 200 000	8 320 000	293 000	69 200	10 300

Class 1, as suggested by Table 2, is impossible to accomplish even with today's modern and highest technological developed tools, if the patients' and the staff members' health are going to be secure and safe, as a total particle free environment would take drastically methods to achieve. Conventional ventilation systems with TMA is typically rated as Class 6 system in the ISO-scale, and LAF-ceilings can achieve Class 5. To reduce the concentration further, a direct in- and outflow of air must take place, something which is difficult to manage in an operating environment (Terra Universal, 2018) as staff members and the patient constantly causes source strength.

Worth mentioning is that particle count in the OR is not directly correlated to CFU/m^3 in the air, as every particle is does not have bacteria adhered to the surface. However, the CFU/m^3 in the air is correlated to the particle count, as the bacteria needs transportation aid in the air. The ISO Standard 14644-1 is therefore a good estimation of acceptable limits.

4.2.1.1 SIS TS39:2015

SIS TS39:2015 is a standard directive developed by the Swedish Institute of Standards (SIS), produced to be tool to maintain equal OR environments throughout the country. Microbial cleanliness in the OR and how to prevent airborne caused infection is central in this standard and important to withhold and take actions towards. This standard is based on ISO 14644-1 classification on particle sizes and concentration and is consider to be a supplement to maintain a national legislation that is based on quality and patients' safety (SIS, 2015). This standard states methods to measure microbial contamination which are used in this study.

SIS is not an official authority and publishes and sells their sets of manuals and standards, as well as training and services for consultation. Therefore, it should be emphasized that there are no official recommendation about whether the usage of LAF ventilation system should be used inside the OR to reduce microbial contamination.

4.2.2 Turbulent mixed airflow (TMA) conventional ventilation systems

Ventilation systems are designed to provide fresh, clean air and remove particles which could carry dangerous substances. The polluted air is transported through HEPA-filters, which are then being trapped and refined in order for new, clean air to be accessible. The most commonly used ventilation system inside ORs is TMA ventilation, as can been seen as an illustration in Figure 10. In contrast to ORs equipped with LAF ventilation, described in section 2.5.3, this ventilation system provides TMA, which is less controlled compared to ventilation with LAF-ceilings and relies on even distribution of the particles in the entire room. However, with turbulent swirling of air, the possibility always exist that the air could find its way to the patient after being contaminated by the personnel in the room, or air being on the floor, picking up dust particles that can land in inappropriate areas.

As stated in section 4.2, the cleanliness of air correlates with the ventilation system. TMA generates turbulent swirls of air. Surgery that takes place with TMA can be correlated to reported cases of sepsis, compared to other types of ventilation systems, as seen from the study made by Lidwell et al. (1987).

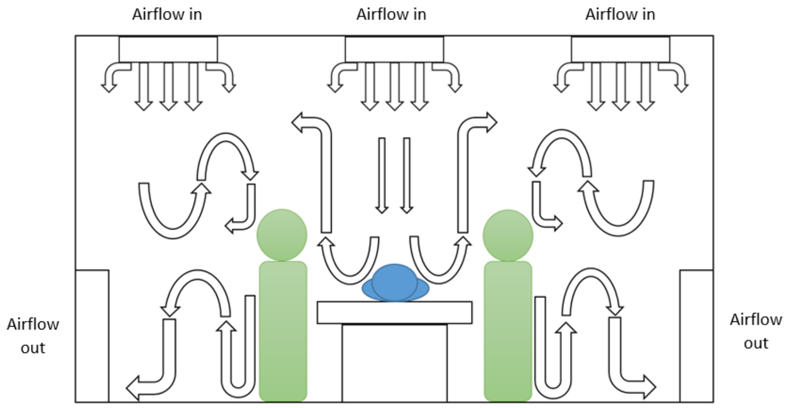

Figure 10: *Principle of turbulent airflow in OR equipped with conventional mixed airflow ventilation system. Illustration made by Frans Stålfelt.*

4.3 Sterilization and sterile zone

Sterilization is a central part in preparation for surgical interventions. To maintain a sterile zone, any microorganism present must have been eradicated in the process of sterilization. The so called "sterile zone" is the area with the patient's wound at the center, including the surgeons, operation nurse and the instrument tables. Everything around is the neutral zone and anything that enters the sterile zone from the neutral zone must be encapsulated in a sterile container which the assisting nurse delivers to the operating nurse by opening the container without touching the inside. An overview of a typical OR and the operating staff, and indication of the sterile zone, can be seen in Figure 11. Sterilization techniques are regulated by the standard SIS-TR 46:2014 (SIS, 2014).

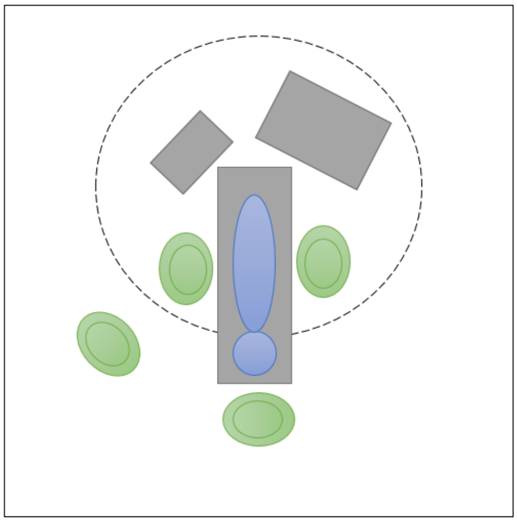

Figure 11: *Ordinary structure of an orthopedic hip surgery OR. The circled area indicates the sterile zone where the surgeon operates and the operating nurse aiding the surgeon. The assisting nurse and anesthetic nurse, who control the patient's vital signs, work in the neutral zone. Illustration made by Frans Stålfelt.*

5. Method

The experimental design and arrangement for this Master's Thesis will be conducted to gather scientific evidence on bacterial pathways inside the OR, as a part of the *Clean Care* project. The samples will be gathered and analyzed according to an ethical approved study with a cohort of patients from the orthopedics department at Sahlgrenska University Hospital, Mölndal. Included in the measurements are microbial sampling from air, the instrument tables, skin and wound of the patient as well as registration of number of door openings, number of personnel, protective clothing and ventilation system.

The samples will be prepared on agar-plates in a lab at the Orthopedic Department at SU, Mölndal. The plates will then be sent to the hospital's microbiology lab for cultivation, typing and counting of CFU. The samples will also be stored in a freezer, so that follow-up studies on patients that get SSI can be done via this biobank in the future. The CFU results will be analyzed for possible correlations and statistical methods used to determine its significance.

Possible abnormalities that occur inside the OR will be noted and recorded in order to find possible explainable outcomes of the results.

5.1 Experimental design

The number of CFU will be reported for the active air sampling with the Sartorius MD8, passive air sampling with sterile filter plates on the instrument and assistant table and swab samples that has been taken on the skin and wound of the patient. In total, 20 samples will be gathered for each measurement. Reference plates, from the same batch, will also be checked in order to reject pre-contamination of the plates. A summarized list of the samples for each measurement can be seen below.

1. Active air sample measurement performed on gelatin filters by Sartorius MD8 (placed on agar plates inside the OR after 10 minutes for each samples)
    - 3 measurements on aerobic HB agar plates
    - 3 measurements on anaerobic FAA plates
2. Passive air sample measurement on the instrument table and assistant table (placed on agar plates inside the OR afterward the suturing of the patient)
    - 4 samples collected with sterile filters placed on the instrument table
        - 2 filters placed on aerobic HB agar plates
        - 2 filters placed on anaerobic FAA plates
    - 4 samples collected with sterile filters placed on the assistant table

- 2 filters placed on aerobic HB agar plates
- 2 filters placed on anaerobic FAA plates

3. Swab samples from skin and wound of the patient (placed on agar plates outside the OR after being prepared in a lab)
   - Skin sample divided on
     - 400μl spread on aerobic HB agar plates
     - 400μl spread on anaerobic FAA plates
   - Wound sample with first swab
     - 400μl spread on aerobic HB agar plates
     - 400μl spread on anaerobic FAA plates
   - Wound sample with second swab
     - 400μl spread on aerobic HB agar plates
     - 400μl spread on anaerobic FAA plates

5.2 Experimental data gathering

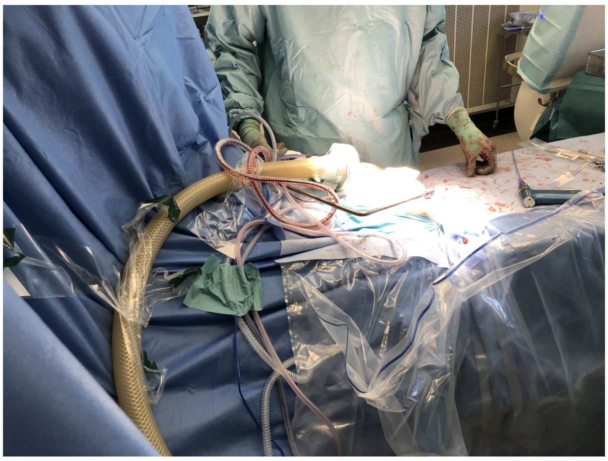

Figure 12: *Sample head and tube placed within 30 cm from the surgical wound for the active sampling measurement.*

The microbial sampling measurements will be performed in three different ways. The first measurement will be performed by gathering the concentration of bacteria in the air, within 30 cm from the wound, by active air sampling through 3 μm gelatin filters. $6m^3$ of air will pass through for an hour, which corresponds to 1 m^3 air passing through each filter during 10 minutes. The portable air sampler Sartorius MD8 will be used to perform the measurements (the Sartorius MD8 will be placed inside a sound isolated container in order to decrease the noise level for the working staff to maintain a proper working environment). After 10 minutes, the used filter will be transferred to an agar plate and replaced with a new one. 6 filters will be gathered from this measurement in total for each surgery, corresponding to 60 minutes of total measurement time. Every second filter is incubated aerobically and the other anaerobically. 3 of the filters will be transferred to horse blood (HB) agar plate for aerobic cultivation in 30°C for 5 days and 3 of the filters will be transferred to fastidious anaerobic agar (FAA) plates that are to be cultivated in an anaerobic environment, in 30°C for 5 days. The incubation temperature is limited to 30°C due to that the gelatin filters melting at 34-35°C, which would make them unreadable for further analysis. After cultivation, the number of CFU will be reported and registered for further analysis. The method for air sampling is in accordance with SIS TS39, mentioned in Chapter 4.

The second measurement also correlates with air quality of the OR but measures the deposition of contamination on the sterile surfaces of the instrument tables during the time of surgery. The measurement can be consider as a passive air sampling and is perform by placing membrane modified polyethersulfone sterile filters from PALL Corporation with a diameter of 47 mm on the instrument and assistant tables before the surgery begins and collecting and placing them on agar plates afterward the surgery is completed, for live count. Four of eight sterile filters will be transferred to HB agar plates where they are to be cultivated in aerobic environment in 35-36°C for 5 days. The remaining four will be transferred to FAA plates that are to be cultivated in an anaerobic environment in 35-36°C for 5 days. After cultivation, the number of CFU will be reported and registered for further analysis. Four filters will be placed on the instrument table and four will be placed on the assisting table, see Figure 13. Due to that each filter has the area 0.1735 dm^2, the CFU registered for every filter will be divided with the area to obtain the projected area of 1 dm^2.

Figure 13: *Instrument (right) and assisting (left) table with placement of filters marked in red circles. Two more on each tables are not visible in this picture.*

The third and last measurement will be performed with a flocked nylon fiber swab, seen in Figure 14, which can be snapped at the red marking to fit inside the transport container. One swab will be used on an area of 10x10 cm ($1dm^2$) with strokes both vertically and horizontally 15 times, to see if there still are any bacteria near where the surgical wound is going to be, after the patient has been washed and cleaned by the personnel. In the end of the surgery, before the suturing of the wound, the possible bacteria that may have landed inside the wound will be collected by swabbing the wound with two swabs. The entire wound will be swabbed first one time and then a second time with a new swab. Afterwards, the three used swabs for skin and wound sampling will be transferred to a lab where they each will be vortexed 10 s in supplied tubes containing 1 ml transport medium. The different medium samples will then be divided between 6 agar plates in total, where two will be used for the skin sample and four will be used the two different wound samples collected by the swabs. 400μl of medium will be spread on each agar plate for the skin sampling and 400μl of medium will be spread on each agar plate for the wound sampling. The samples will be incubated on 3 HB plates for aerobe and 3 FAA plates for anaerobe incubation. The agar plates are to be incubated at 35-36°C and counted after 5 days.

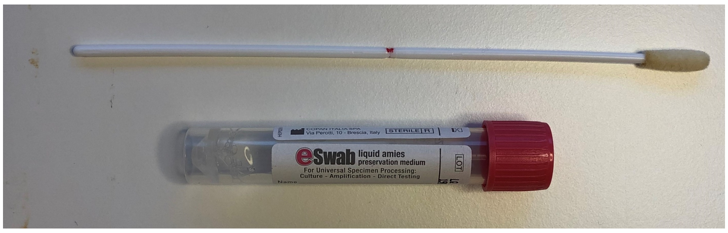

Figure 14: *The flocked nylon swab and transport medium used in this study, eSwab.*

A comparison between conventional ventilation and conventional ventilation with an addition of mobile LAF units will also be included in this study. Toul MediTech's mobile LAF instrument table Steristay and assistant table Operio protects the surgical instruments by creating an air barrier between bacteria-carrying particles and the surgical instruments. Operios will be directed so that the same protective laminar airflow over the instrument also protects the surgical site, as described in 2.5.4 ORs equipped with modern LAF-ceilings will also be evaluated and measured upon, to see if there is any difference from ORs equipped with conventional ventilation systems.

5.2.1 Statistical analysis

The analysis has been comprised to an average value from each sampling method for determination of the overall contamination level from each sampling method throughout each measurement/surgery. The statistical analysis has been performed with non-parametric Mann-Whitney U-test for determination of significance. The data evaluation has be done in MS Excel and the add-on XLstat has been used for the Principal Component Analysis.

5.2.1.1 Principal Component Analysis

Principal Component Analysis (PCA) is a exploratory, non-parametic, descriptive statistical tool that uses data sets with n dimensions (variables) to conduct a multivariate analysis and can be used on various types of numerical data sets. PCA is used to reduce the number of dimensions in order for the results to be more easily interpreted, but that still preserve the variability of the original data set as much as possible. To preserve the variability and still make the result accurate and reliable, new variables which are linear functions to the original data set with maximized variance by solving for eigenvectors, are found. The new dimensions (principal components (PC)) that can be displayed in a PCA-plot are linear combination of variables from the data set, thus makes it for easier interpretation. As each original variable from the data set generates a PC, the total representation of the variance can be seen in a Scree-plot (See Appendix B). By using the two PC which accounts for the largest variation combined, a two-dimensional plot of the two PC can create a good and accurate representation of the whole data. The active variables can be visualized as lines in a PCA plot, where the lines angels suggest whether the variables are positively or negatively correlated with each other (a angel of 0° between lines of two variables from the origin would suggest a positive correlation, whereas a angel of 180° would suggest a negative correlation). The measurements, which can be seen in a PCA-plot, which are close to these lines, have association with the active variables (Jollife & Cadima, 2016).

As this study consists of more than two active variables (air samples, instrument table samples, assistant table samples, skin samples, wound samples, door openings and people present), PCA is a useful, multivariate tool that was used to determine similarities, or clusters of measurements, between each of the different ventilation setting that are being examined. Therefore could predictive models of the different ventilation settings in the ORs be established.

5.2.2 Study cohort

The participants in this study consist of trauma patients which are in need of a hemiarthroplasty hip fracture surgeries at Sahlgrenska University Hospital, Mölndal. The Corona virus outbreak during the spring of 2020, when this study was conducted, forced the hospital to close down the TMA ventilated ORs in order to allocate equipment and resources to the intensive care units. Therefore, measurements performed during the surgeries in TMA ventilated OR (n=10) and TMA ventilated OR with the addition of mobile LAF units (n=3) were limited. In addition, LAF ventilated ORs was added to the study (n=11), generating results from a total of 24 surgeries. 67% of the patients were women and 33% were men. The mean age of the patients were 86.9 years old.

5.3 Ethical aspects

The ethical application has already been approved prior this study with reference number GU 2020/455, regarding the research project "Clean Care - The importance of cleanliness in the operating air for postoperative wound infections of orthopedic surgery".

6. Results

This chapter includes figures and tables over findings from the measurements from the air within 30 cm of the wound, on the instrument and assistant table, as well as on the skin and in the wound of the patient, taken during 24 operations. What the results means from a medical point of view will be covered in the next Chapter 7.

The occurrence rate presented below for each bacterial specie is based on the number of encounters at each performed surgery. The rate is not based on the CFU that was discovered for each species, but rather if 1 or more CFU was registered for each measurement. Therefore, the results from this illustrate the frequency of which species were found and which bacterial species that were most commonly reoccurring as percentage.

6.1 Active air sampling of viable airborne CFU

The CFU count for the TMA ventilation system dominates with a median of 17.85 CFU/m^3 (n=10, mean=17.25 CFU/m^3, SD=9.26) with a range between 5 and 31 CFU/m^3. For the measurements from the OR equipped with TMA ventilation systems, but with mobile LAF units as the assistant and instrument table, the median was 5.67 CFU/m^3 (n=3, mean=5.03 CFU/m^3, SD=1.64) and the highest and lowest value was at 6.25, respectively 3.17 CFU/m^3. For ORs equipped with LAF ventilation, the median was 0.33 CFU/m^3 (n=11, mean=0.47 CFU/m^3, SD=0.42) with highest value at 1.5 CFU/m^3 and lowest at 0 CFU/m^3. The three different settings are plotted in Figure 15. Significant higher CFU/m^3 was measured in the TMA ventilated OR compared to the LAF ventilated OR ($p<0.001$). Comparison with TMA ventilation with mobile LAF units cannot be conducted due to the small sample size. However, clinical significance suggests that this type of medical device exhibit better air quality near the patient's wound, as this is one of the aims of Toul MediTech's mobile LAF assistant table unit Operio, as stated in section 2.5.4. The results shows, based on the mean value, that operating in an OR equipped with TMA ventilation, the exposure of CFU/m^3 is 41 times higher, compared to LAF-ceilings. When operating with mobile LAF-units in a TMA ventilated OR, the CFU level in the air is 14 times less, compared to if no units where used.

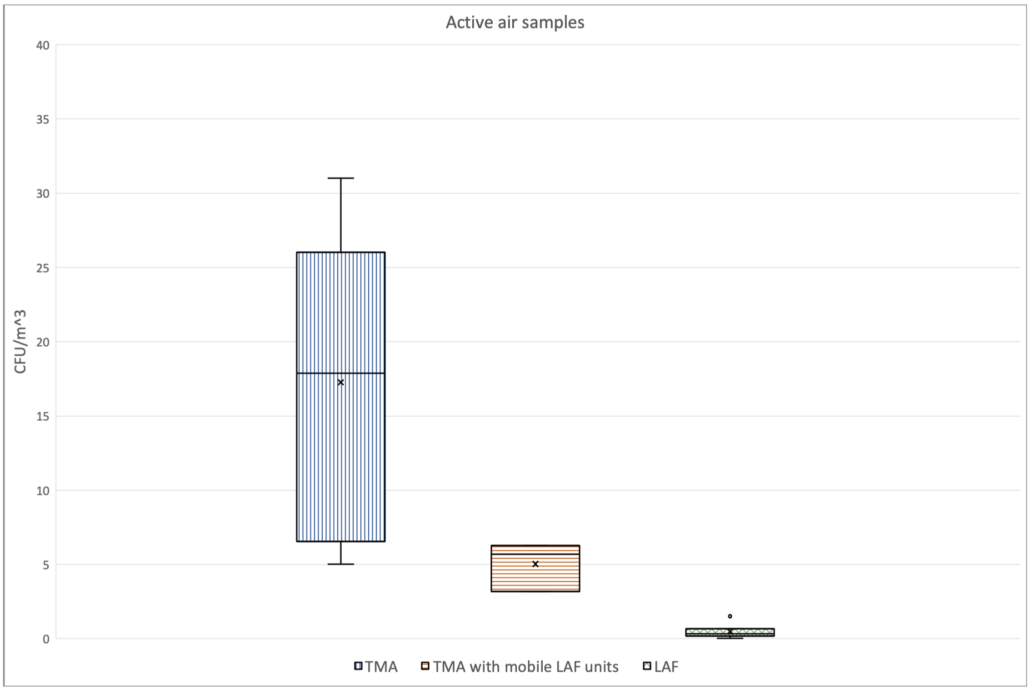

Figure 15: *TMA ventilation system (left, n=10) is significant different from LAF ventilation (right, n=11) (p<0.001), analysed with two-tailed Mann–Whitney U-test. Significance level with TMA ventilation with mobile LAF-units (middle, n=3) has not been conducted due to not sufficient measurements.*

6.1.1 Aerobic versus Anaerobic samples

The aerobic samples collected contained a higher portion of CFU/m^3 compared to anaerobic samples, for all types of ventilation settings and techniques. For TMA ventilation system, the median was 27.67 CFU/m^3 (n=10, mean=21.58 CFU/m^3, SD=13.4) for aerobic samples, compared to a median of 9.3 CFU/m^3 (n=10, mean=11.4 CFU/m^3, SD=8.12) for anaerobic samples. For aerobic samples measured with LAF-units present the median was 6.5 CFU/m^3 (n=3, mean=5.7 CFU/m^3, SD=1.8), compared to a median of 4.33 CFU/m^3 (n=3, mean=4.33 CFU/m^3, SD=1.67) for anaerobic samples. LAF-ceilings generated a median of 0.33 CFU/m^3 (n=11, mean=0.63 CFU/m^3, SD=0.67) and a median of 0 CFU/m^3 (n=11, mean=0.36 CFU/m^3, SD=0.79) for the aerobic, respectively anaerobic samples. The comparison between the groups, both within and between the samples and the settings respectively, can be seen in Figure 16.

The divergence of aerobic and anaerobic samples can been noticeable, as seen in Figure 16. Similar trend can be seen in all three different setting, suggesting that aerobic bacteria are present in a higher amount than anaerobic bacteria, in the air of the OR. However the bacteria *C. acnes* appeared in 100% of the anaerobic samples and were encountered in 25% of the measurements and reported 46 times, meanwhile *M. luteus/lylae* appeared in 99.56% of the aerobic samples (Total CFU=229, Occurrence rate=67%), indicating the importance of conducting two cultivation methods.

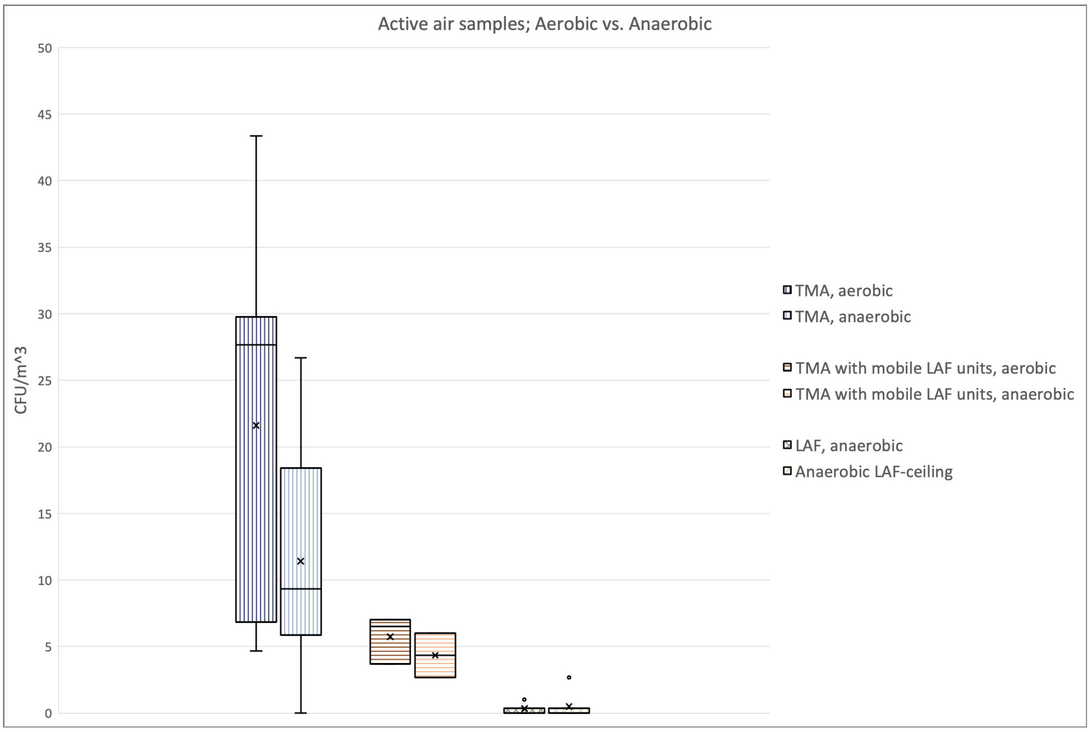

Figure 16: *Mean number of findings of aerobic samples (dark colored) compared to anaerobic samples (light colored) for each ventilation settings.*

6.1.2 Bacterial species encountered in the air inside the ORs

In Table 3, a summarized overview is presented of every bacteria that was found in the air within 30 cm of the surgical wound of the patients, for all three types of ventilation settings. The most commonly occurring bacterial species/group that was reported was "G+ coccus", which was found in 75% of all measurements that was performed (n=24). As this is not a specie, but rather an generalized group that was recorded and reported by the microbiologist, assumptions has to be made that these bacteria could be any from the G+ coccus bacterium (e.g. *Straphylcoccus*, *Streptocuccos* or *Micrococcus*).

The second most commonly sampled bacterial specie was *M.luteus/lylae*, which appeared in 66.67% of the samples (n=24) from the active air sampling. The species are obligate aerobes, which is confirmed by the occurrence for each samples of aerobic and anaerobic cultivation (99.56% compared to 0.44%). These bacteria appeared at the highest number, as shown in Table 3 where a total of 229 CFU/m^3 was reported for all measurements, in contrast to the second most common bacteria, *S. capitis* with 159 CFU. The third and fourth most commonly occurring species were *S. capitis* and *S. epidermidis*, which appeared in 62.5% respectively 58.33% of the measurements (n=24) performed. Both these species were found in both the aerobic and anaerobic samples almost equally, suggesting that they can survive both cultivation methods.

Table 3: *Tabular overview of bacteria groups/species that were encountered in the active air sampling.*

Group/Species	Total CFU[1]	Aerobic samples	Anaerobic samples	Occ. rate[2]
G+ coccus (unspecified)	96	89 (93%)	7 (7%)	75%
M. luteus/lylae	229	228 (99.56%)	1 (0.44%)	67%
S. capitis	159	84 (53%)	75 (47%)	63%
S. epidermidis	103	51 (50%)	52 (50%)	58%
G+ bacilli	62	59 (95%)	3 (5%)	42%
S. hominis	109	38 (35%)	71 (65%)	42%
S. saprophyticus	26	23 (88%)	3 (12%)	29%
C. acnes	46	0 (0%)	46 (100%)	25%
S. warneri	22	10 (45%)	12 (55%)	25%
CoNS	14	7 (50%)	7 (50%)	21%
G- bacilli	7	5 (71%)	2 (29%)	21%
S. caprae	10	1 (10%)	9 (90%)	17%
S. lugdunensis	9	8 (89%)	1 (11%)	17%
Bacillus species	50	1 (2%)	49 (98%)	13%
Corynebacterium	7	7 (100%)	0 (0%)	13%
S. haemolyticus	8	6 (75%)	2 (25%)	13%
Staphylococcus species	9	5 (56%)	4 (44%)	13%
Gramlabila	2	2 (100%)	0 (0%)	8%
S. mitis	7	4 (57%)	3 (43%)	8%
Actinomyces species	1	0 (0%)	1 (100%)	4%
E. faecalis	2	0 (0%)	2 (100%)	4%
G- coccus	1	1 (100%)	0 (0%)	4%
G+ bacteria	1	1 (100%)	0 (0%)	4%
Neisseria species	1	1 (100%)	0 (0%)	4%
Prevotella species	1	0 (0%)	1 (100%)	4%
S. aureus	1	0 (0%)	1 (100%)	4%
S. chonii	2	0 (0%)	2 (100%)	4%
S. simulans	5	2 (40%)	3 (60%)	4 %

[1] *Total CFU in this case is the total of CFU registered and added together from the air*
[2] *Occurrence rate = Percentage of operations that the bacteria was encountered in (n=24).*

6.1.3 Air contamination versus average staff members present in the OR

The relationship between the staff members present inside the OR and the level of CFU/m^3 can be visualized in Figure 17. When less than 6 staff members were present in the OR, low number of CFU/m^3 was measured. The average number of staff members inside ORs equipped with TMA was 6.9 (including TMA with mobile LAF units) and for ORs equipped with LAF ventilation the average was 6.05. This indicate that the staff members are more prone to follow the recommendation of max 6 person present for the, often more high risk, surgeries performed with LAF ventilation system compared to the TMA ventilated ORs. When counting, the observer is included, but not the patient. Therefore, under normal circumstances, with no observer present, the average number of staff members present can be expected to be beneath recommended level also in the TMA OR.

No significant difference can be seen between the groups. The blue squares (TMA) exhibit a scattered pattern with no indication of correlation between the number of staff members present in the OR and the level of CFU/m^3 in the air. The green dots in Figure 17 (LAF ventilation, n=11) maintain a low level of CFU/m^3, where some increasing level of CFU/m^3 occur at 7 staff member present inside the OR. The orange triangles (TMA with mobile LAF units, n=3) exhibit a small trend towards correlation between the number of people present and the CFU/m^3 level, but not sufficient measurements were performed to confirm this trend.

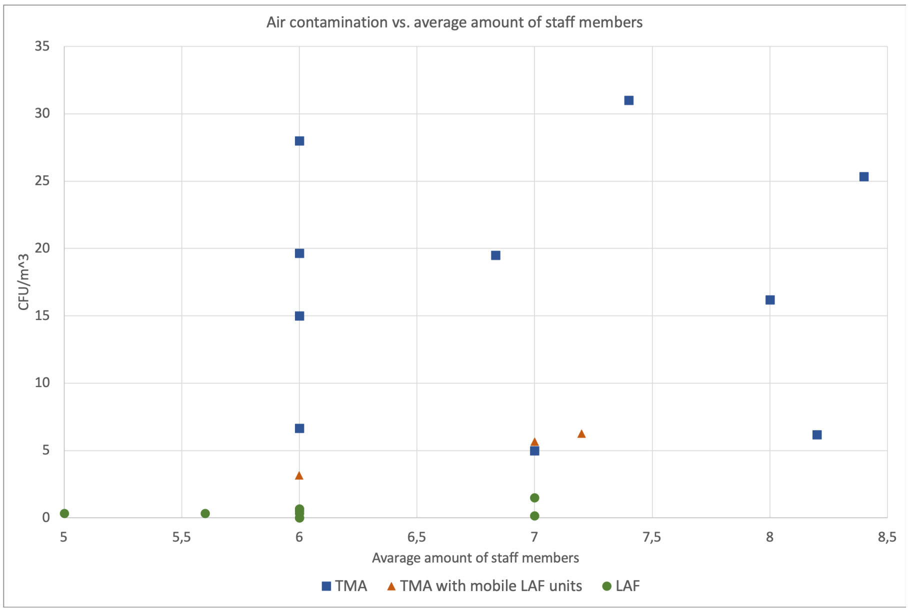

Figure 17: *Average number of people present in the OR versus CFU/m^3 in the air for each patient during ongoing surgery. The different groups indicate ventilation type of the OR.*

6.1.4 Air contamination versus CFU in the surgical wound

As seen in Figure 18, there is a trend towards a difference in level of CFU/m^3 depending on type of ventilation in the OR, where LAF has the lowest count of CFU/m^3 and TMA has the highest. However, one patient from each type of ventilation system stands out with higher CFU collected from the wounds compared to the rest of the samples patients (TMA: 74 CFU/wound, TMA with mobile LAF units: 65 CFU/wound and LAF: 22 CFU/wound), indicating more factors than ventilation be of equal or more important, and further analysis is needed to investigate what may have occurred at the time of these specific surgeries to determine the source of contamination (i.e. airborne contamination or endogenous).

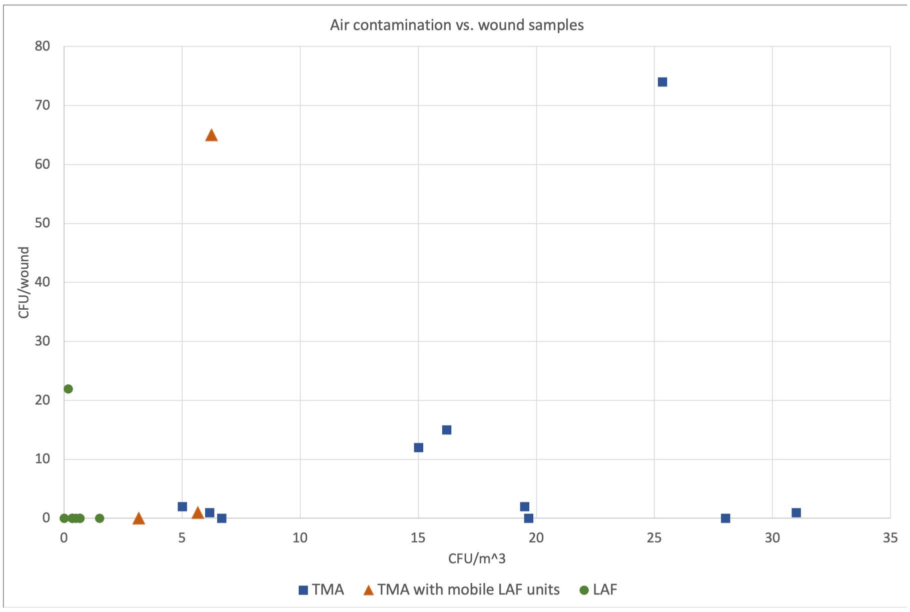

Figure 18: *CFU/wound versus CFU/m^3 in the air for each patient in the ongoing surgery. Symbols indicates which technique that have been used for each measurement.*

6.2 Passive air sampling of viable airborne CFU

To get a comparable value of CFU/dm^2, the number of sampled CFUs from each filter was divided with the area of the filter (0.17 dm^2), as described in the Method chapter. The results below are based on the average of the four filters placed on the instrument table and four filters placed on the assistant table, during each surgery.

The median CFU/dm^2 for TMA ventilation system was 3.6 CFU/dm^2 (n=10, mean=5.52 CFU/dm^2, SD=8.64) on the instrument table. When equipped with mobile LAF units in OR with TMA (n=3), 0 CFU/dm^2 was detected on the instrument table. For OR equipped with LAF ventilation, the median was 0 CFU/dm^2 (n=10, mean=0.13 CFU/dm^2, SD=0.48). This result can be seen from Figure 19. An outlier can be seen at TMA ventilation system at 28.8 CFU/dm^2. An outlier is also visible at 1.44 CFU/dm^2 for the OR with LAF-ceiling.

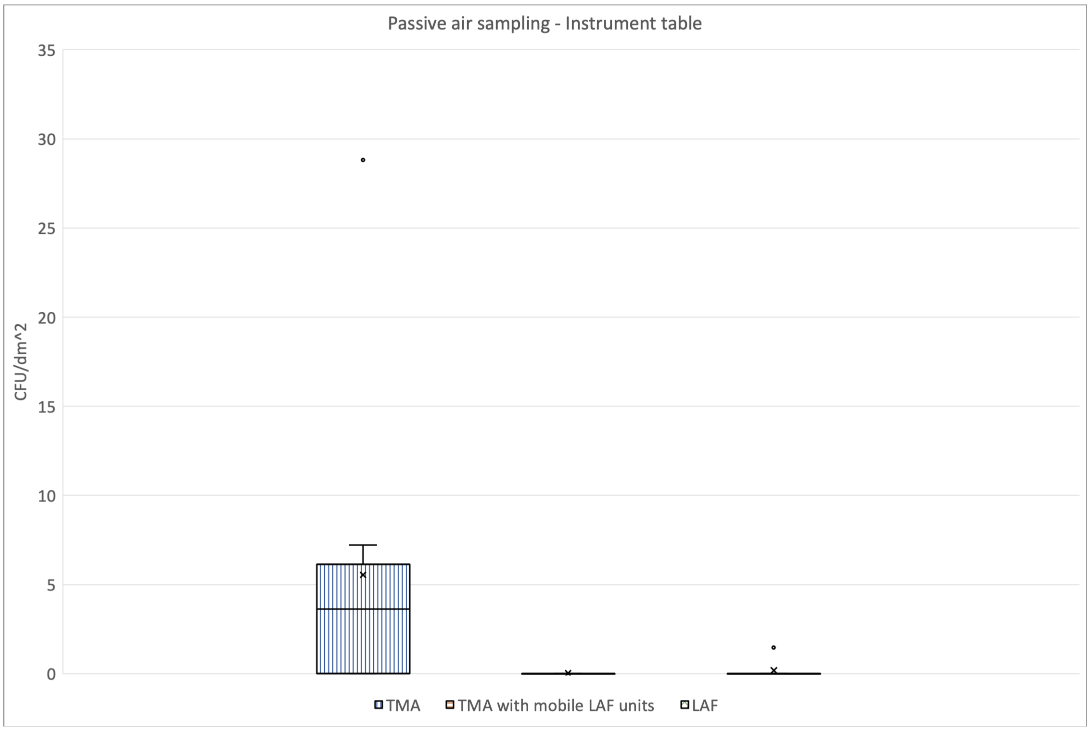

Figure 19: *Passive air sampling average value of CFU/dm^2 on the instrument table.*

The median CFU/dm^2 for conventional TMA ventilation system was 3.6 CFU/dm^2 (n=10, mean=5.33 CFU/dm^2, SD=5.12) with a highest value of 15.84 CFU/dm^2 on the assistant table. With the mobile LAF units, the median was 0 CFU/dm^2 (n=3, mean=1.92 CFU/dm^2, SD=3.33). For the ORs equipped with LAF-ceilings, the median was 0 CFU/dm^2 (n=11, mean=0.65 CFU/dm^2, SD=0.48). An outlier is visible at 5.76 CFU/dm^2 for LAF-ceiling. This result can be seen from Figure 20.

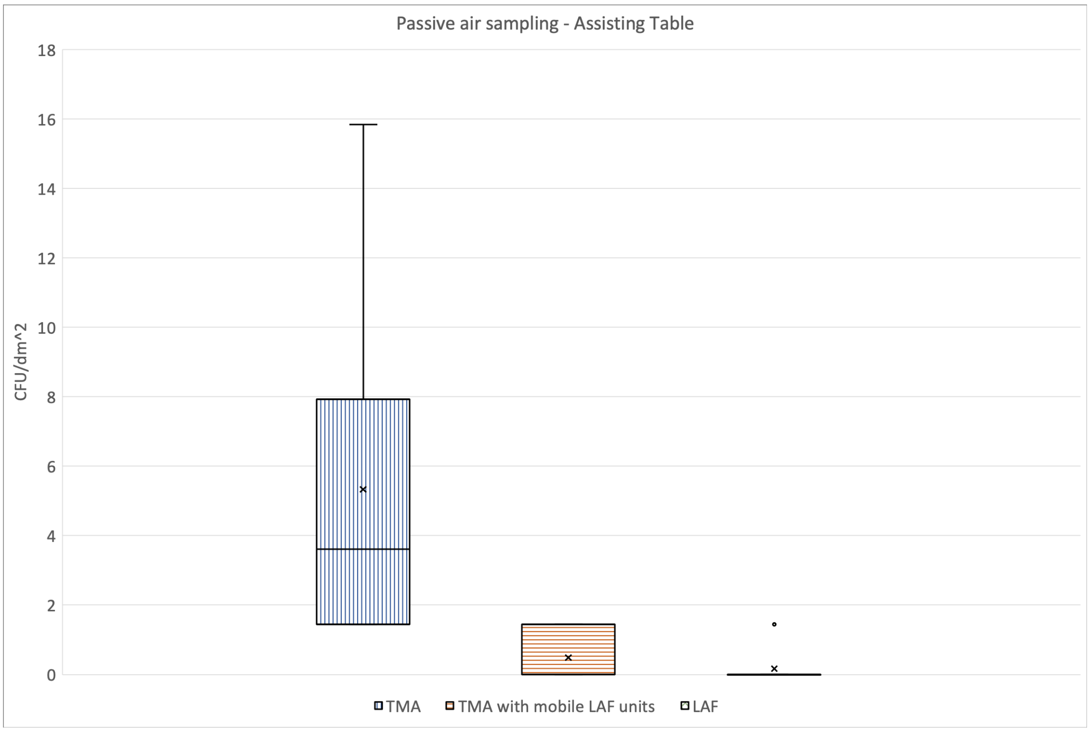

Figure 20: *Passive air sampling average value of CFU/dm^2 on the assistant table.*

6.2.1 Air contamination versus contamination on the instrument tables

Small trends of increasing CFU/dm^2 on the instrument tables can be observed as the increasing CFU/m^3 exist in the air, between the different ventilation settings, as illustrated from Figure 21. TMA ventilation has the highest value of CFU/m^3, which also corresponds to the highest value of CFU/dm^2 when combining the samples from the assistant table and the instrument table. Higher air contamination levels seem to have some effect on the CFU that ends up on the instrument table, where LAF-ceilings demonstrate the best ability to avoid both air contamination and contamination of the instrument tables.

6.2.2 Contamination of the instrument tables versus CFU in the surgical wound

No correlation could be seen between the amounts of CFU/dm^2 on the instrument tables compared to the CFU that was found in the surgical wound of the patient, as shown in Figure 22.

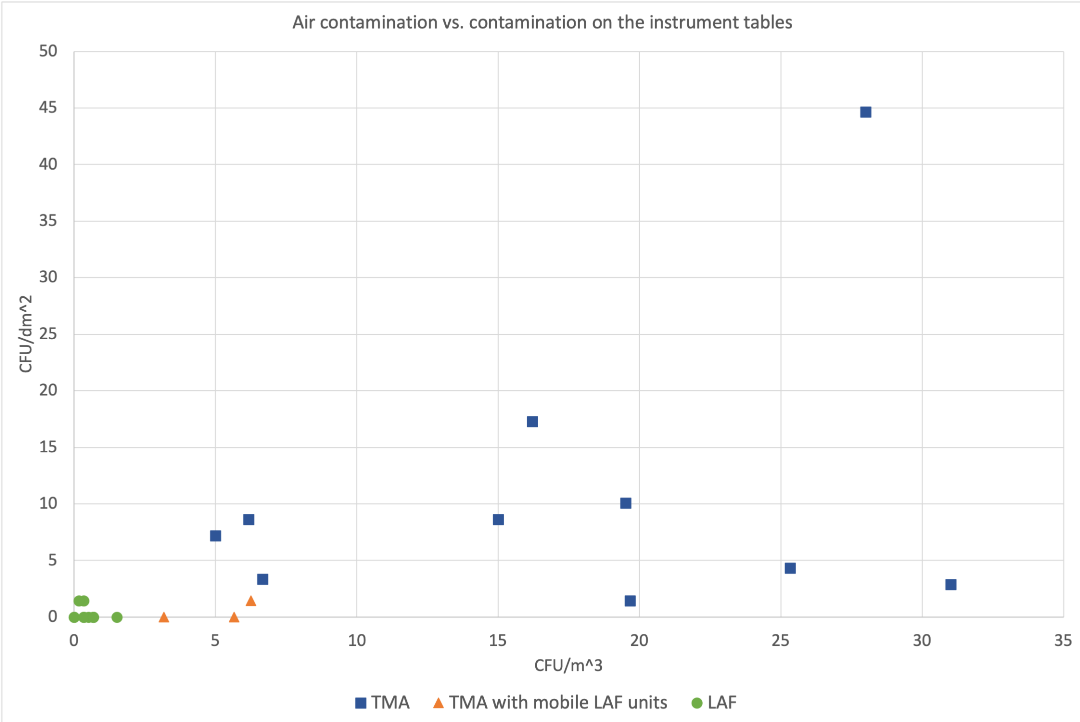

Figure 21: *Air contamination compared to contamination on the instrument and assistant table combined.*

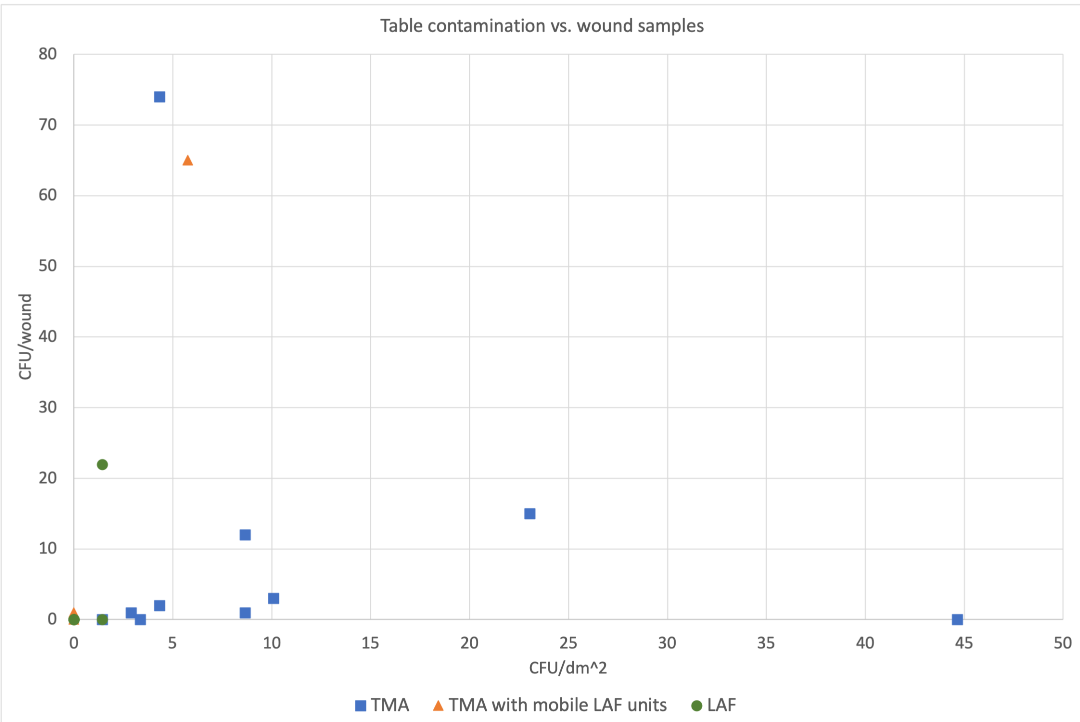

Figure 22: *Table contamination level (both instrument and assistant table) compared to the contamination of the surgical wound.*

6.2.3 Bacterial species encountered on the surgical tables

In Table 4, the most common occurring bacterial species and groups can be seen, from both the instrument table and the assistant table. The most common species found was *C. acnes* with an occurrence rate of 33.33 % and a total of 12 CFU was registered.

Table 4: *Tabular overview of bacteria groups/species that were encountered in the passive air sampling.*

Group/Species	Total CFU[1]	Aerobic samples	Anaerobic samples	Occ. rate[2]
C. acnes	12	1 (8%)	11 (92%)	33%
S. capitis	19	11 (58%)	8 (42%)	21%
M. luteus/lylae	8	8 (100%)	0 (0%)	17%
S. epidermidis	5	0 (0%)	5 (100%)	17%
S. hominis	15	9 (60%)	6 (40%)	17%
G+ coccus (unspecified)	5	5 (100%)	0 (0%)	13%
Bacillus species	2	1 (50%)	1 (50%)	8%
S. haemolyticus	3	2 (66%)	1 (33%)	8%
G+ staffs	1	0 (0%)	1 (100%)	4%
S. warneri	4	1 (25%)	3 (75%)	4%
G+ bacteria	1	0 (0%)	1 (100%)	4%
Bacillus cereus	1	0 (0%)	1 (100%)	4%
S. pettenkoferi	1	1 (100%)	0 (0%)	4%

[1] *Total CFU count, in this case is the total amount of CFU registered and added together from the instrument and assistant table*
[2] *Occurrence rate = Percentage of operations that the bacteria was encountered in (n=24).*

6.3 Skin sampling of viable endogenous CFU

The skin samples were taken with a swab, on an area of 1 dm^2 where the incision was to be placed as described in the Method chapter.

6.3.1 Bacterial species encountered on the skin

The most occurring bacterial species and groups are listed in Table 5. The most common group of bacteria was "G+ coccus" species that were encountered in 22% of the measurements, where a total of 47 separate CFU were extracted from the swabs from all the patients. Some of the most reoccurring bacterial species were *M. luteus/lylae* and *S. epidermidis* which were found in respectively 17% and 13% of the cases.

A broad range of unidentified bacteria was also registered in 9% of the cases with a total 13 CFU. A total of 33 CFU were reported for *S. capitis*, with an occurrence rate of 4%.

Table 5: *Tabular overview of bacteria groups/species that were encountered on the skin sampling.*

Group/Species	Total CFU[1]	Aerobic samples	Anaerobic samples	Occ. rate[2]
G+ coccus	47	31 (66%)	16 (34%)	22%
M. luteus/lylae	19	15 (79%)	4 (21%)	17%
G+ bacillus	5	1 (20%)	4 (80%)	17%
S. epidermidis	6	1 (17%)	5 (83%)	13%
Corynebacterium	2	1 (50%)	1 (50%)	9%
Unidentified bacteria	13	12 (92.31%)	1 (7.69%)	9%
C. acnes	7	0 (0%)	7 (100%)	4%
S. capitis	33	11 (33%)	22 (67%)	4%
S. hominis	3	2 (67%)	1 (33%)	4%
Bacillus species	1	0 (0%)	1 (100%)	4%
S. haemolyticus	1	0 (0%)	1 (100%)	4%
S. warneri	1	0 (0%)	1 (100%)	4%
G+ bacteria	1	1 (100%)	0 (0%)	4%

[1] *Total CFU in this case is the total amount of CFU registered and added together from the skin*
[2] *Occurrence rate = Percentage of operations that the bacteria was encountered in (n=23).*

6.3.2 Skin versus wound sampling

The correlation between amount of bacteria remaining on the patient's skin, after disinfection, and amount present in the wound before suturing, can be observed in Figure 23. When large numbers of CFU on the skin was found (45 CFU/dm^2 and 50 CFU/dm^2), the number of CFU in the wound was also higher than usual (74 and 65 CFU/wound, respectively). From these samples a correlation can be observed and linear regression suggest a trend between the data points (R^2=0.8019). To strengthen this result, more data points must be measured.

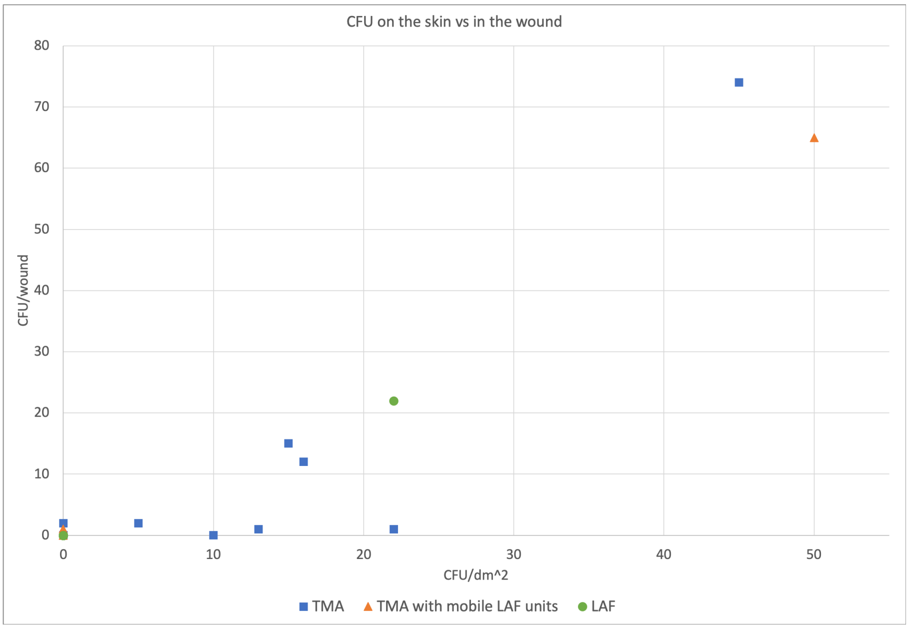

Figure 23: *The total number of CFU in the wound versus the number of CFU/dm^2 for each patient (n=23). Linear regression ($R = 0.8968$) is also included to see a trend line between the two factors ($R^2 = 0.8019$), suggesting a correlation.*

6.4 Wound sampling of viable CFU in the surgical wound

Before suturing, the surgical wound was swabbed using two swabs, as stated in the Method section. The two samples where then added together, and presented as one total throughout the Result chapter.

6.4.1 Bacterial species encountered in the surgical wound

The most often found bacterial group or species can be seen in Table 6. *M. luteus/lylae* was found in 21% of the reported cases and total 22 CFU was recorded. A total of 71 CFU was recorded for *S. capitis*, however it only occurred in 4% of the reported cases.

Table 6: *Tabular overview of bacteria groups/species that were encountered in the wound sampling.*

Group/species	Total CFU[1]	Aerobic samples	Anaerobic samples	Occ. rate[2]
M. luteus/lylae	22	20 (91%)	2 (9%)	21%
G+ staffs	3	0 (0%)	3 (100%)	13%
C. acnes	2	0 (0%)	2 (100%)	8%
Corynebacterium	5	2 (40%)	3 (60%)	4%
S. capitis	71	30 (42%)	41 (58%)	4%
Pseudomonas species	22	22 (100%)	0 (0%)	4%
Bacillus species	64	10 (16%)	54 (84%)	4%
G+ coccus (unspecified)	4	4 (100%)	0 (0%)	4%
S. epidermidis	2	0 (0%)	2 (100%)	4%

[1] *Total CFU in this case is the total amount of CFU registered and added together from the surgical wound*
[2] *Occurrence rate = Percentage of operations that the bacteria was encountered in (n=24).*

6.5 Principal component analysis (PCA)

In Figure 24, a multivariate analysis, displayed as a PCA-plot, can be seen. The principal components F1 (58.05%) and F2 (14.88%) covers 72.93% of the variance of the data set (See Appendix for Scree-plot). The biplot combines both the observations and active variables for the measurements, as well as door openings and average amount of people present in the OR. In Figure 25, the relationship between the air contamination, door openings and number of people present can be visualized. The principal components F1 (63.88%) and F2 (58.05%) covers 83.19% of the variance of the data set (See Appendix B for Scree-plot).

The active variables in the PCA plot represent the associating factors under investigation. The red lines which can be seen in both Figure 24 and 25, represent these associating factors and the direction and angels between the lines suggest that they are positively correlated with each other. Clusters of the different ventilation settings can be seen from both Figure 24 and 25 as the PCA can create predictive models for the measurements. For "TMA with mobile LAF units", the indication of a cluster is not as appearing as the other settings, due to too few observations. The measurement seems however to follow the pattern of being somewhere in between of LAF and TMA measurements. The difference in LAF and TMA ventilation makes a clear appearance, even in a multivariate analysis. As LAF exhibit low values from every category and samples for each measurements and TMA has high values, a clear distinctions can be made from the different measurements.

The PCA can also distinguish individual measurements, to highlight those patient who might be in the risk of getting an infection. The PCA visualized in Figure 24 and 25 detects that the measurement labeled "Patient 5" for the TMA-group presented very high values for each category and sample. Figure 24 also hints that "Patient 11" and "Patient 19" for the "TMA with mobile LAF units"-group and the LAF-group, respectively, have high values compared to the other measurements for each ventialtion setting.

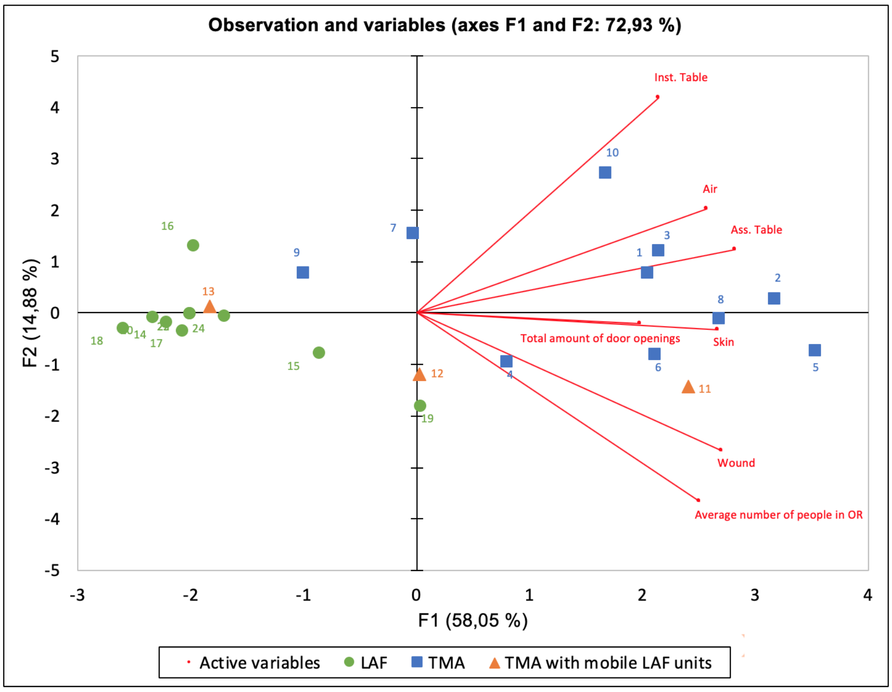

Figure 24: *PCA plot where the different active variables, including the two variables for door openings and number of people present, are visible as well as the observations from the different ventilation settings (n=23, CI=95%).*

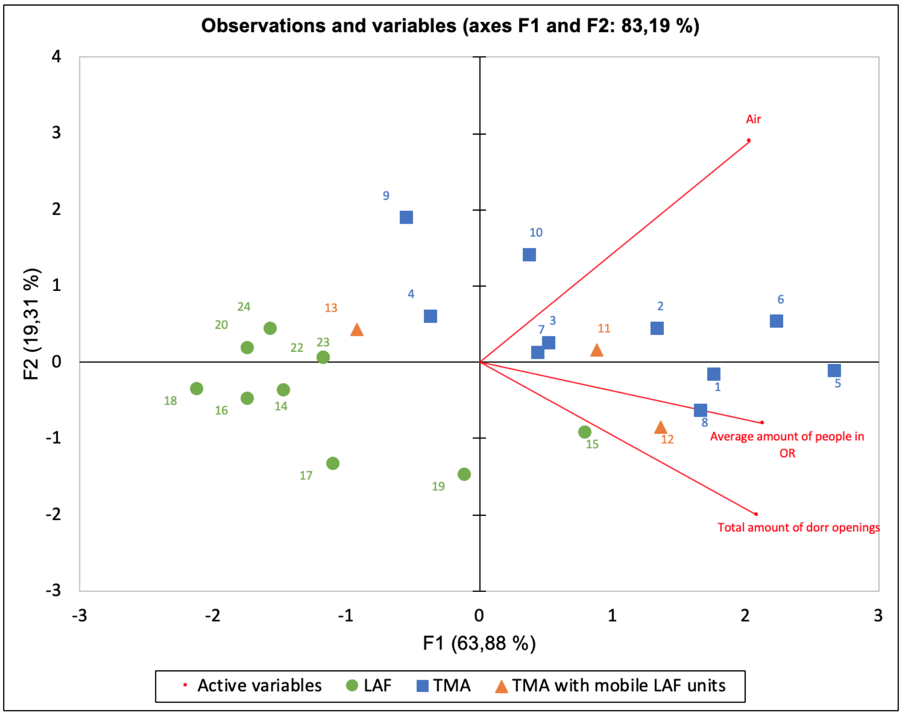

Figure 25: *PCA plot where the active variables of the three variables of air contamination, door openings and number of people present visible as well as the observations from the different ventilation settings (n=23, CI=95%).*

6.6 Correlation between variables

Figure 26 contains a heat-map table generated from the PCA and displays correlation between the active variables. The correlation matrix indicates what associated factors intervene with the contamination pathway the most. For instance, average number of people present in the OR seems to inflict with the wound contamination. The correlation matrix includes as the measurements for each ventilation setting that were used in this study.

Variables	Air	Inst. Table	Ass. Table	Skin	Wound	Total amount of door openings	Average number of people in OR
Air	**1.00**	**0.55**	**0.72**	**0.58**	**0.43**	**0.43**	**0.46**
Inst. Table	**0.55**	**1.00**	**0.61**	**0.46**	0.33	**0.48**	0.15
Ass. Table	**0.72**	**0.61**	**1.00**	**0.70**	**0.65**	0.28	**0.53**
Skin	**0.58**	**0.46**	**0.70**	**1.00**	**0.65**	0.22	**0.60**
Wound	**0.43**	0.33	**0.65**	**0.65**	**1.00**	**0.50**	**0.77**
Total amount of door openings	**0.43**	**0.48**	0.28	0.22	**0.50**	**1.00**	**0.49**
Average number of people in OR	**0.46**	0.15	**0.53**	**0.60**	**0.77**	**0.49**	**1.00**

Figure 26: *Heat-map generated from Spearman correlation matrix (n=23), where values in bold are different from 0 with a significance level α=0.5. A greener color indicating a higher correlation, whereas a redder color indicates less correlation.*

6.7 Reported cases of infections

As of this date, two reported cases of infections has been confirmed for "Patient 11" and "Patient 19". For these measurements, the patients had 65, respectively 22 CFU in the wound, which in comparison with other measurements are very outstanding. Whether the infection arose from the bacterial species that was located in the surgical wound or not is uncertain at this point in time.

7. Discussion

The Discussion chapter is divided into sections to highlight the importance of the results and what the results may contribute to. The sections includes the analysis of microbial levels for the different settings, the bacterial species that where encountered and discussion about observations that was made during the study. A brief evaluation of the methodology will also be reviewed in the end of the chapter, as well as comments about the clinical importance and future research.

7.1 Microbial contamination levels inside the ORs

The level of CFU/m^3 inside ORs equipped with TMA is significantly higher compared to the level of CFU/m^3 in LAF ventilated ORs. The TMA ventilated ORs with mobile LAF units also exhibit a lower CFU count compared to only TMA ventilated ORs (see Figure 15). Our findings are in accordance with previous studies reviewed by Houltz et al. (2020), indicating that LAF ventilation generally accomplishes higher air quality compared to TMA ventilation, due to the high amount of air exchange in the OR. However, as described above, LAF ventilation cannot be installed in all cases, hence mobile LAF units could be a valuable complement. Technical solutions in form of mobile LAF units, such as the medical devices developed by Toul MediTech that are used in this study, displays a decline of the aerial contamination level inside the OR. Although just 3 measurements were performed, and therefore no statistically based comparison could be established, the preliminary results indicate towards that lower CFU levels in the air can be accomplished when operating with these mobile LAF units. Besides type of ventilation, other factors that may affect air quality was documented and observations were made about e.g. that a decreased number of staff members are present when operating under LAF-ceilings and the door is shut more often, compared to TMA ventilated ORs. As humans are the prime source of bacterial contamination and door openings inflicts and disturbs the air pressure, the less of these activities are the most optimal prerequisites for a possible, contaminated free environment. Discussions with nurses and staff confirm these results and admit that there is a more relaxed attitude when it comes to door openings in TMA ventilated ORs. Stricter rules about door openings are followed in LAF ventilated ORs, where the door only opens when necessary materials must be transported in or out of the room (such as blood supplies and medical equipment).

As stated in section 2.5.3, the WHO does not recommend the usage of LAF-ceilings as a prevention for SSI, as the current evidence is of poor quality. Along with Langvatn et al. (2020), mentioned in section 2.5.3, this study can put forth evidence that the CFU level in the air is in fact lower when operating under LAF ventilation and is therefore a better choice than performing surgery under TMA conditions. However, this study cannot claim that LAF ventilated ORs can be used as a prophylactic precaution for SSI as the amount of CFU/m^3 does not exhibit a correlation with what end up in the wound, as seen from Figure 18, which Langvatn et al. (2020) study can.

The divergent levels of CFU/m^3 for the aerobic and anaerobic bacteria samples are also an interesting discovery from this study, as can be seen from Figure 16 from the air. All three settings exhibited the same results, where the aerobic bacterial levels of CFU/m^3 was higher compared to anaerobic samples, If bacteria uses aerobic or anaerobic respiration, clues can reveal where these type of bacteria's originates from. As information about some bacterial species in section 2.3.1, bacteria can only survive under certain conditions and exists in different environments of the human flora. If this contamination source (both endogenous and exogenous) can be mapped, this gathered knowledge of aerobic and anaerobic bacterial presence can give possible hints and directives on how to avoid contamination from these sources.

No correlation could be established between the amount of CFU/dm^2 that lands on either of the instrument tables, compared to the CFU that was found in the surgical wound of the patient. As this indirect contamination source is hard to define as the samples are gathered from the corners of the tables as seen in Figure 13 in the Method chapter and a projected area have been established. No samples were gathered from the surgical instruments in use and as the instruments are the source of indirect contamination and not the tables themselves, the amount of CFU that descend into the wound is hard to measure and estimate. This method gives a approximation of what falls down on the table and therefore an approximation of what may land on the instruments. In further studies, cultivation of bacteria from the instruments directly can promote results which can give more justification about if the instruments are the contamination source. However, the tables are an indirect contamination source of interest and more measurements must be gathered to be able to determine possible correlation with the risk of wound contamination.

Slightly higher numbers of CFU was found on the assistant table compared to the instrument table, for all ventilation settings (Figure 19 and Figure 20). The assistant table is a place for instruments which often is in contact with the wound (i.e. scalpels and haemostatic forceps) and this table is in constant use by the operation nurse and the surgeon, meaning that instrument causally move around on the table and activity and movement are commonly occurring due to this. The activity caused by the constantly moving of the instrument could be the reason of the higher levels of CFU/dm^2, as the airflow is disturbed by the movement of hands. The airflow over the instrument table however is not as much disturbed, as movement only occur here when specific instruments (such as implants and cementing) are needed for the surgical procedure. This knowledge contributes to the fact that CFU levels can be decreased if less movement take place over the instruments and that a laminar flow of air (both vertically and horizontally) can protect the surface. The movement is however crucial for the personnel to perform their work, but can perhaps be less intensive if this knowledge becomes common.

Figure 23 shows that a correlation exist between the bacteria that was located on the patient's skin and what was located in the patient's wound. Table 5 and Table 6 shows that in some cases, the same species and groups of bacteria was recorded and registered for the skin and for the wound, indicating that they were responsible for the wound contamination. However, to be certain, more measurement from surgeries needs to be performed as well as more detailed evaluation of the bacterial phenotype. Since most bacteria found are common skin bacteria that can have come both from the staff and the patient, a genetic comparison will be needed in order to identify a certain source. Since all bacterial samples are stored in a biobank, this can be performed in the future. *Clean Care* will continue to gather data for this reason, in order to map the contamination pathways. The fact that bacteria is found on the patient's skin is an interesting discovery. Endogenous contamination is not a widely explored research field. Chlorhexidine agent is the aseptic technique used in the washing and cleaning routine to disinfecting the patient in the OR. Cleaning and showering is also done in the pre-operation department to ensure that as low count of bacteria exist on the patient's skin before the incision takes place. However, a patient with a hip fracture is often in unbearable pain and is therefore difficult to perform these cleaning routines on and can sometimes be less thoroughly performed in a attempt to ease the patient's pain. Despite this, the fact remains that this study have had measurements that showed skin samples with up to 74 CFU, which is very alarming. These routines may be reevaluated in future studies to improve the quality of the cleaning and compare the results to see any difference from endogenous contamination.

Interesting enough, as can be seen in Figure 23, there were more CFU on the patient's skin on the TMA (both with and without mobile LAF unit)-group, compared to the LAF-group. The reason behind this might be that the air is so contaminated as the results from the active air sampling revealed near the site of the skin, causing it to land on the skin right after the cleaning is done. Another reason could be that mere chance could be the factor or that the pre-op cleaning procedure were more thoroughly performed on patients aimed for surgery in LAF ORs. To investigate this further, every patient's journal must be followed to see if abnormalities had occurred for those patient which had very high number of CFU on their skin and thereby draw conclusions. As no analysis has been done whether the bacteria found are endogenous species, or exogenous only speculations can be done where these bacteria's originate from for now.

For the multivariate analysis of several factors, clusters can be seen from the TMA-group and LAF-group from Figure 24 and 25. "TMA with mobile LAF units"-group cannot be clustered due to only 3 measurements. With this interpretation of the measurements, models can be established by what factorial association is common for each type of ventilation settings. Also, with the PCA, abnormalities can easily be detected and therefore be of usage when locating surgeries that are of risk to give cases of SSIs, such as high values of CFU in the surgical wound and in that way having a followup dialogue with the patient for possibilities to stop the possible SSI. As can be seen from Table 7, 8 and 9 in Appendix A, patient specific scenarios can be followed to notice any trends or abnormalities that occurred for specific surgeries, with multivariate analysis. For instance, "Patient 5" from Table 7, exhibited relatively high values for nearly every column in the Table, especially wound contamination. The plots should however be used as a interpretation tool to predict patterns between the measurements and what factors are the most dominant when it comes to the different ventilation settings. From this study, the PCA exhibits that TMA have high values for most of the factors, whilst LAF has very low values for the same factors.

"Patient 11" and "Patient 19" were those patient who developed an SSI at the time that this study was conducted. Abnormal values from each category could be discriminated from these measurements, where the samples from the wound especially stood out, compared to the other measurements. This does in fact proof that these surgeries failed to provide infection controlled patient safety. "Patient 5", which was another patient where abnormal values of CFU in the wound were measured, has not developed any infection (at this time point). Interestingly enough have none of the patients from the TMA-group (without mobile LAF units) developed any infections, meaning that despite all the high values of CFU from the various contamination pathway (i.e the direct and indirect air contamination pathway), no evidence exist that TMA ventilated OR causes more infections, compared to LAF. However, considerably more CFU was located in the wound of patient who had surgery in TMA ventilated ORs, meaning that the risk of developing SSI is, in fact, higher in TMA ventilated ORs. This assumption is based on that CFU in the wound does cause SSI, which evidence for "Patient 11" and "Patient 19" suggest.

7.2 Bacterial species found in the OR

As stated in 2.2.1, bacterial species like *M. luteus/lylae*, *S. capitis* and *S. epidermidis* are all known opportunistic pathogens and are often reported to be the causes of HAI and SSI. *M. luteus/lylae* is a commonly occurring species as an air contaminant and have also been found in many of the surgical wound samples. They are also commonly inhabitants of the human skin microbiome. It is therefore expected that these bacterial species would be found in the air and on the patient's skin samples as they are constantly reoccurring. From Table 3, the registered bacterial species or groups can be seen from the air inside the OR. Due to that a vast amount are registered as unidentified "G+ coccus", no certainty can be confirmed whether these bacteria correlates to other define bacteria that exist in the surgical wound, as presented in Table 6. Assumptions can be made that the "G+ coccus" from every type of sampling is also some type of the stated bacterial species above, but was not identified by the microbiologists. The reason for this can be that common characteristics was not recognized. The possibility that unknown bacteria has been found is extremely low. Another remark is that *S. aureus* was only found in one sample for one measurement for the entire study period. Adlerberth and Wold (2007) states that *S. aureus* is one of the most notorious bacteria to cause HAI. Therefore could one assumption be that some reported "G+ coccus", are in fact *S. aureus*, but was not identified. This is however unlikely, as *S. aureus* is a very characteristic bacteria species and is not hard to detect or misinterpret when studying.

7.3 Observational study

At the orthopedics department in SU, Mölndal, clean air suits, as described in Chapter 4, are not in use by the working staff. Instead, simpler scrubs are used which does not have cuffs on the open areas around arm or waist. The scrubs could be tugged in by the waist, but this is not practiced by the personnel and is therefore a possible pathway for bacteria to descend from heat convection. Interviews with nurses and other working staff revealed that the reason for this is mainly comfort and that clean air suits may cause rashes and eczema due to the tight cuffs around arms and waist. Comfort is an important factor when working and is regulated by the Swedish Work Environment Authority. This study does not include a comparison between different garments that is used by the working personnel. However, the importance of the working cloths remains an unsolved question that needs to be investigated to evaluate the effect of source strength and contamination of the air and the surgical wound.

As each measurement was performed, observations were also made on site where several of notes and comments was taken for each measurement/surgery to detect abnormalities. Measurements from "Patient 5" and "Patient 11" gave abnormal high values of CFU located in the patient's skin. After closer examination and evaluation of these specific surgeries, some important factors was singled out as potential causes for these high numbers of CFU. Firstly, for both "Patient 5" and "Patient 11", a student was performing the cleaning of the patients, meanwhile a supervisor instructed the student on how to perform the cleaning. This event did not only included more personnel inside the OR, but also the possibility of less thoroughness and exactitude of the cleaning, compared to if a more experienced co-worker had performed the cleaning. As seen from Figure 23 the skin was very contaminated and the same bacterial species were found on the patient's skin and inside the surgical wound for one of the measurements, namely *S. capitis*. Secondly, measurements on "Patient 5" experienced a change of surgical team in the middle of the surgery. Team switching is a very common event and is performed when the starting team has finished working their shift and must be replaced with a new team, even though the remaining time of the surgery is estimated to be short. This replacement includes the operating nurses, assisting nurses and anesthetic nurse, but not the surgeon or surgeons. At the switch for this measurement, a total of 12 participants were in the OR simultaneously, which is 5 more than what is normally allowed. Discussions can follow whether team switching should include this many people simultaneously or if other routines can be established which can decrease the number of people at the time of the switch.

The amount of people present in the OR have tendency of correlation with the CFU count that was found in the wound of the patient, as can be seen from the heat map in Figure 26. This is in accordance to results that previous studies has shown, for example the study by A. E. Andersson et al. (2012) mentioned in Chapter 3. When there are more people inside the OR than normally during a surgery, team switching, as stated above, or education of new nurses or surgeons are often the reasons. As earlier discussed, education and students present in the OR can result in less thoroughly cleaning and therefore higher CFU that ends up in the surgical wound via endogenous contamination. It is therefore crucial to determine the association of these factors and their relationship on the wound contamination to better understand this correlation.

7.4 Methodology evaluation, clinical implications and future research

The method to place the sample head and tube 30 cm from the wound was followed as good as possible, but the discommodious placement of the head was for some measurement to close to the surgeon's hand that it affected their ability to work. The sample head was therefore directed orthogonal from the wound for some measurements, as well directed upward or downward for other measurements. To have a continuous and consistent measurements in the future, a tube and sample head holder should be applied to ensure that the air near the wound is sampled, as well as it can give a better and more sustainable working area for the surgeons to operate on and be less disturbing for the surgeon.

A potential source of error could be that the samples that were gathered from the measurements could be pre-contaminated, either by the sampler performing the measurements or some other factor that could impact the agar plates beforehand or during the transportation to the incubation. For each measurement that was performed, references agar plates (both HB and FAA plates) from the same batch of agar plates used in the measurement were sent to the microbiologist for incubation, to determination of pre-contamination of the plates from the batch. None of the reference plates from this study exhibited any high number of CFU. In events of registered CFU on the reference plates, exclusion of the measurement data points must be evaluated, to evade suspected samples of pre-contamination which could be a possibility of unreliable results.

According to Henrik Malchau, professor and chief of orthopedics at SU, the request for new and modern ORs equipped with both better x-ray possibilities and LAF ventilation in the ORs has been circling around since 2004, and no formal decisions have been made by the city's counsel to this date. He states that the political lack of beneficial decisions could threatening the patient's safety. Project *Clean Care* and this study can be the foundation for evidence based fact that can show the beneficial effects of a contamination free environment in the OR, both economically and increase the patients' quality of life.

The future of the *Clean Care* project will be to gathered more data by continue with the measurements and include more variables which can be of interest, such as temperature, humidity and air pressure inside the ORs. This is due to that there has long be an interest and suggestion that these factors could inflict with the microbial contamination present in the OR. Antibiotic prophylaxis is, as stated in the introduction, not going to be a reliable treatment option in the future as AMR outspreading threatening human health and modern medicine. It is therefore crucial to be able to halt the infection before it can start to progress, and not afterwards when the damage is done. This study can acts as a supplement to future studies and the significantly results (both statistical and clinical) can be used by the hospital as evidence and scientific guidelines of which ORs and technical approaches that can be used to reduce the amount of bacterial contamination and thus limit the risk of SSI. These evidence together with other studies, such as Langvatn et al. (2020) study, can be a guidance for better patient's safety.

For future studies, investigation of how the air moves inside the ORs with the different ventilation setting can be a key to understand where the contaminated air is most likely to be during a surgery. To do this investigation, a mock-surgery could be setup in the OR, where a smoke propagation analysis of the air could be a possible way to determine the transportation and distribution of the air in the ORs. This knowledge could contribute to the discovery of previously unknown contamination pathways. Further studies from where the bacterial species originates from is also of key interest to increase the knowledge of the contamination pathways. As *Clean Care* project stores the gathered samples in a bio-freezer, the possibility to see if the bacteria that was found in the patient's wound is the same one that caused the infection in the end, can be very important to strengthen the evidence that certain investigated contamination pathways is in fact the underlying cause of the infection.

Most importantly, to achieve high quality surgical interventions that can reduce the risk for a patient to develop SSI, a close collaboration between the health care system and the technological sector must be well established. By working together and have mutual agreements on the problem at hand, we can ensure that bacterial contamination levels can be measured continuously and thereby be a tool for the surgical staff to use as an risk indicator of SSI. The mutual understanding between technology and the health care system is something that also is concluded by Sadrizadeh (2016) in his doctoral thesis about OR ventilation design.

8. Conclusion

To connect back to the purpose of this study, LAF-ceilings exhibit a significant lower contamination level in the air compared to TMA ventilation system. Thus, conclusions are that ORs equipped with LAF-ceilings is presumably the best option to perform surgery as it can reduce the risk of aerial contaminated particles to reach the surgical wound. Mobile LAF units also exhibited promising results to reduce the contamination in the air when applied in ORs equipped with TMA.

From this study, correlation between the CFU level on patient's skin and the surgical wound indicates that endogenous contamination is an important pathway that may cause SSI. To draw strong, evidence-based conclusions, more measurements must be perform to strengthen the result and thereby be able to verify the correctness from this study.

From the measurement points gathered from this study, no strong correlation can be seen between the effects on whether the number of personnel that are present in the OR, or the number of door openings has on the level of air contamination. However a tendency of correlation could be noticed from the number of people in the OR and what was found in the patients' wound. As earlier clinical studies have proven fluctuating values of airborne microbial contamination in the OR when it comes to association of these factors, such as traffic flow in the OR described in Chapter 3, these results are expected. Thus, more measurements and research are needed to be performed understand these pathways better and to draw evidence-based conclusions.

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A. Summarized data from measurements

Table 7: *Summarized data from ORs equipped with TMA ventilation systems.*

Patient	Ventilation type	Air contamination samples (CFU/m^3)	Inst. Table samples (CFU/dm^2)	Ass. Table samples (CFU/dm^2)	Skin samples (CFU/dm^2)	Wound samples	Total amount of door openings	Average people in OR
1	TMA	19.5	4.32	5.76	0	3	11	6.8
2	TMA	16.2	7.2	10.08	15	15	2	8
3	TMA	15	5.76	2.88	16	12	4	6
4	TMA	5	0	7.2	3	2	0	7
5	TMA	25.33	2.88	1.44	45	74	5	8.4
6	TMA	31	0	2.88	13	0	4	7.4
7	TMA	6.67	1.91	1.44	0	0	4	6
8	TMA	6.17	4.32	4.32	17	0	4	8.2
9	TMA	19.67	0	1.44	0	0	0	6
10	TMA	28	28.8	15.84	10	0	2	6
Mean		17.25	5.52	5.33	11.9	9.81	3.6	6.98

Table 8: *Summarized data from ORs equipped with conventional TMA ventilation system, with mobile LAF units.*

Patient	Ventilation type	Air contamination samples (CFU/m^3)	Inst. Table samples (CFU/dm^2)	Ass. Table samples (CFU/dm^2)	Skin samples (CFU/dm^2)	Wound samples	Total amount of door openings	Average people in OR
11	TMA with mobile LAF units	6.25	0	1.44	50	65	2	7.2
12	TMA with mobile LAF units	5.67	0	0	0	1	5	7
13	TMA with mobile LAF units	3.17	0	0	0	0	1	6
Mean		5.03	0	0.48	16.67	22	2.67	6.73

Table 9: *Summarized data from ORs equipped with LAF ventilation systems.*

Patient	Ventilation type	Air contamination samples (CFU/m^3)	Inst. Table samples (CFU/dm^2)	Ass. Table samples (CFU/dm^2)	Skin samples (CFU/dm^2)	Wound samples (CFU)	Total amount of door openings	Average people in OR
14	LAF	0.33	0	0	0	0	1	6
15	LAF	1,5	0	0	0	0	4	7
16	LAF	0.33	1.44	0	0	0	2	5
17	LAF	0	0	0	0	0	3	6
18	LAF	0	0	0	0	0	0	6
19	LAF	0.17	0	1.44	0	22	3	7
20	LAF	0,5	0	0	0	0	0	6
21	LAF	0.33	0	0	- †	0	0	5.6
22	LAF	0.67	0	0	0	0	1	6
23	LAF	0.67	0	0	0	0	1	6
24	LAF	0.67	0	1	0	0	0	6
Mean		0.47	0.13	0.65	0	2	1.36	6.05

† *Sample was not taken due to miss*

B. Scree plots for PCA

The appendix contains supplementary information to the Result Chapter.

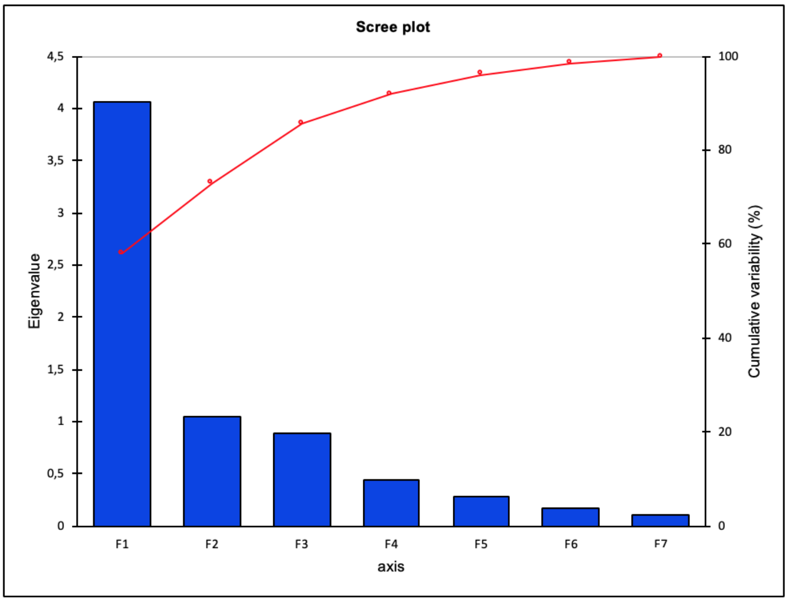

Figure 27: *Scree plot for the PCA analysis from Figure 24, showing the most important components to analyze.*

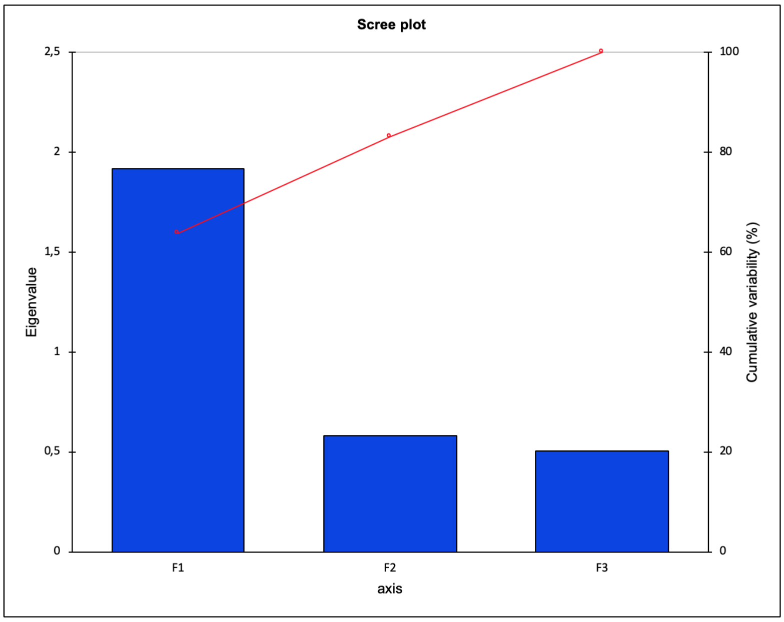

Figure 28: *Scree plot for the PCA analysis from Figure 25, showing the most important components to analyze.*