Transmission of Infectious Bioaerosols

Sources, transport and prevention strategies for airborne viruses and bacteria

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ERGONOMICS AND AEROSOL TECHNOLOGY | LTH | LUND UNIVERSITY

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Sources, transport and prevention strategies for airborne viruses and bacteria

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LUND UNIVERSITY

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Transmission of Infectious Bioaerosols: Sources, transport and prevention strategies for airborne viruses and bacteria

Abstract

Infectious diseases that can be transmitted via air often spread rapidly, sometimes causing large epidemic and pandemic outbreaks. As an increasing number of people live in crowded urban environments, and with frequent and long-distance traveling across the world, infectious diseases can spread even faster. Yet, our knowledge of how much airborne transmission (here defined as aerosol particles <100 µm that contain infectious agents) contributes to the spreading of diseases is scarce and frequently debated. The aim of this thesis was to increase knowledge about the sources and airborne transport of infectious bioaerosols in order to prevent diseases from spreading via air.

To identify possible sources of infectious bioaerosols, we collected air samples in hospitals for detection of bacteria (in operating rooms) and norovirus (in hospital wards) and correlated the results with possible source events. To study bacterial viability and viral infectivity after airborne transport, we developed an experimental setup in the laboratory where aerosolized model organisms were examined. The setup was also used to evaluate the particle collection efficiency of a novel bioaerosol sampler. In addition, three types of high-airflow ventilation systems for operating rooms were compared for their ability to maintain clean air during ongoing surgery.

The median bacterial concentrations measured in operating rooms ranged from 0 to 22 CFU m^{-3} (colony forming units) depending on the sampling point and ventilation type. However, no correlations were found between bacterial concentrations and the number of door openings or the number of people present in the room. Based on the comparison of three types of ventilation, we concluded that the two ventilation techniques with the incoming airflow above the operating table, directed downwards, resulted in lower bacterial concentrations close to the wound than the ventilation based on turbulent mixing.

We detected norovirus RNA in air samples collected in hospitals during outbreaks of the winter vomiting disease. Our results showed a significantly higher risk of finding norovirus RNA in the air within a short time (3 h) after a patient vomited. From size-separated sampling, norovirus was detected in aerosol particles >4.5 µm and <0.94 µm, indicating that airborne norovirus has the potential to remain infectious for hours and spread in indoor environments. To evaluate the infectivity of airborne norovirus, murine norovirus was used as a model organism in a laboratory study. The infectivity of murine norovirus relative to the virus genome copy number was reduced by two orders of magnitude when aerosolized by either twin-fluid nebulization or bubble bursting. We proposed that aerosol droplet drying from a low-solute solution caused the loss of viral infectivity. A similar experimental setup, was used to study the viability of *Pseudomonas syringae* in air with varying levels of relative humidity. The bacterial survival was higher when aerosolized into air with low relative humidity, corresponding to rapid drying.

For detection of bioaerosol sources in the field, we evaluated the particle collection efficiency of a novel electrostatic bioaerosol sampler. Owing to the small liquid collection volume of ~0.3 mL, the new bioaerosol sampler had higher sample concentrations than a commonly used impinger when collecting microspheres of sizes >1 µm.

Airborne transmission of infectious diseases has long been neglected; however, as new infectious diseases emerge, knowledge that can be generalized across organism types is highly valuable. With this research, I highlight its importance, in particular for nosocomial infections, by showing that sufficient concentrations of bacteria and viruses are present in hospital air that can trigger new infections, and that bacteria and viruses aerosolized under controlled laboratory conditions remain viable and infectious. Finally, I also show that by choosing appropriate preventive measures, such as room ventilation, airborne microbial concentrations can be significantly reduced, limiting transmission of airborne disease.

Key words bioaerosol, infectious disease, norovirus, air sampling, operating room ventilation, surgical site infection, Pseudomonas syringae, bubble bursting

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Aerodynamically the bumble bee shouldn't be able to fly,
but the bumble bee doesn't know it so it goes on flying anyway.

~ Mary Kay Ash

Table of Contents

Abstract

Infectious diseases that can be transmitted via air often spread rapidly, sometimes causing large epidemic and pandemic outbreaks. As an increasing number of people live in crowded urban environments, and with frequent and long-distance traveling across the world, infectious diseases can spread even faster. Yet, our knowledge of how much airborne transmission (here defined as aerosol particles <100 μm that contain infectious agents) contributes to the spreading of diseases is scarce and frequently debated. The aim of this thesis was to increase knowledge about the sources and airborne transport of infectious bioaerosols in order to prevent diseases from spreading via air.

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Populärvetenskaplig sammanfattning

Infektionssjukdomar är en naturlig del av vår miljö och vårt samhälle. De cirkulerar ständigt bland människor, djur och växter, och de har stor påverkan på både individ- och samhällsnivå eftersom de gör oss och våra närstående sjuka. Ibland ställer de till med storskaliga utbrott – epidemier eller pandemier. Infektionssjukdomar som kan spridas via luft är ofta svåra att kontrollera och riskerar att spridas snabbt. Några exempel på sådana är lungtuberkulos, pest (digerdöden), mässling, influensa och SARS. Troligtvis också covid-19.

Idag vet vi att smitta orsakas av bakterier och virus och att dessa kan spridas via direktkontakt med en smittad person eller via smittämnen som denne avgett till miljön, till exempel på ytor, i vätskor eller i luft. Smitta som sprids via luften är speciellt svår att få stopp på eftersom vi inte kan avstå från att andas luften vi har omkring oss. Partiklar som svävar i luften kallas aerosolpartiklar och de är så små att vi inte kan se dem – mindre än en tiondels millimeter. Det är därför svårt att veta när smittsamma aerosolpartiklar finns i luften omkring oss.

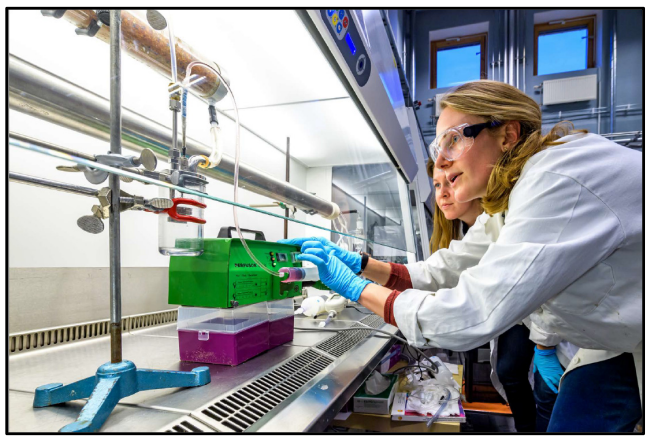
Biologiska aerosoler kallas bioaerosoler, och exempel på dessa är bakterier och virus i luften. Generellt sett är luften en otrevlig miljö för bakterier och virus eftersom den är torr, näringsfattig, och öppen för skadligt UV-ljus. Många bakterier och virus är därför inte längre smittsamma efter att ha varit i luft. För att en infektionssjukdom ska kunna spridas via luft krävs det först och främst att virus eller bakterier på något sätt blir luftburna – att de aerosoliseras. Aerosolisering kan ske genom att en smittad person nyser, hostar, pratar eller andas, eller också när någon spolar i en toalett efter en diarré, eller via hud- och hårfragment som vi människor avger naturligt (ca en miljon partiklar i timmen!). Sedan måste de smittsamma partiklarna transporteras i luften utan att förstöras och nå fram till en ny person. Slutligen krävs det också att en tillräckligt stor dos av de smittsamma bakterierna eller virusen når den plats i kroppen där personen är mottaglig för infektion.

I arbetet som lett fram till denna avhandling har vi studerat 1) möjliga källor till smittsam bioaerosol på sjukhus, 2) hur virus och bakterier överlever aerosolisering och transport i luften genom experiment i laboratorium, och 3) metoder för att minska luftburen smitta: effektiv ventilation och effektiva mätmetoder.

Ett exempel på ett väldigt smittsamt virus är det som orsakar vinterkräksjukan – norovirus. Det kan räcka med så lite som några tiotal virus för att orsaka en infektion och i en kräkning finns det över en miljon virus per milliliter kräkvätska. Vinterkräksjukan anses vanligtvis inte smitta via luft, men vi lyckades samla in luftprover på sjukhus och identifiera norovirus i dessa. Resultaten visade att en stor andel av proverna som samlades in en kort tid efter att en smittad patient kräkts var norovirus-positiva. Vår slutsats var följaktligen att kräkningar kan vara en källa till luftburet norovirus. I tidigare fallstudier beskrivna i litteraturen har man också sett

samband mellan kräkningar och utbrott av sjukdom. Nästa steg var därför att se om de virus man kan samla in från luften är smittsamma.

Norovirus som smittar människor (humant norovirus) är svåra att odla i ett laboratorium och vi gjorde därför en studie på norovirus för möss (murint norovirus). Vi aerosoliserade virusen i en experimentuppställning (se bild) och kunde sedan samla in dem efter en kort tid – ca 10 sekunder – i luften. Genom att infektera cellodlingar med de insamlade proverna kom vi fram till att de murina norovirusen fortfarande var smittsamma efter experimentet, även om smittsamheten minskat 100 gånger. Man kan anta att smittsamheten för humant norovirus också minskar i luften, men att någon andel behåller sin förmåga att infektera.



Experimentuppställning för aerosolisering av virus och bakterier i laboratorium. Foto: Kennet Ruona.

Vinterkräksjuka, tillsammans med många andra infektionssjukdomar dominerar under vintern, och man har i århundraden undrat varför. Några studier har sett en koppling mellan torr luft och bioaerosolers smittsamhet. Under vintern värmer vi upp luften inomhus vilket gör den torrare. Därför undersökte vi hur luftburna bakterier påverkas av olika luftfuktighet. Vi aerosoliserade miljöbakterien *Pseudomonas syringae* (bakterier från samma släkte kan orsaka lunginflammation) i vår experimentuppställning och såg att bakterierna överlevde i större grad i låg luftfuktighet jämfört med hög. Något som ändras vid olika luftfuktighet är torktiden för de aerosoliserade dropparna som innehåller bakterierna. Vid låg luftfuktighet torkar droppar fortare än vid hög luftfuktighet. Samma sak borde gälla om man varierar storleken på dropparna. Vi jämförde därför bakteriernas överlevnad efter den korta torktiden i aerosol – några sekunder, med en längre torktid – någon timme, genom att torka större droppar deponerade på en yta. Resultaten visade att bakteriernas överlevnad var ca 100 gånger större efter den snabba uttorkningen i aerosol jämfört med den långsamma uttorkningen på en yta. Vi drog slutsatsen att en kort torktid ökar *Pseudomonas*-bakteriernas förmåga att överleva.

Ett kritiskt moment då man vill ha så lite bioaerosol som möjligt är under en kirurgisk operation. Man använder därför avancerade ventilationssystem för att minimera risken att bakterier i luften deponeras i det öppna såret och orsakar en postoperativ sårinfektion. Postoperativa sårinfektioner vållar ofta stort lidande för patienten och leder till ökade vårdkostnader eftersom behandlingstiden är lång. Vi studerade tre olika ventilationssystem för operationssalar genom att mäta koncentrationen bakterier i luften under pågående operationer. Vi kom fram till att de två ventilationssystemen som introducerade den rena luften ovanför operationsbordet, med ett neråtriktat luftflöde, var bättre på att minimera koncentrationer av luftburna bakterier nära det öppna såret än omblandande ventilation. Vi genomförde dessutom en enkätundersökning om hur arbetsmiljön upplevdes som visade att personalen uppskattade ventilationssystem som hade låg ljudnivå, mindre kalldrag och behaglig temperatur.

För att minska luftburen smittspridning behövs, utöver effektiv ventilation, också bra metoder för att detektera bioaerosoler i luften. Koncentrationen av bioaerosol i luften är generellt låg, så man använder instrument med höga luftflöden för att provta en så stor volym luft som möjligt. Höga luftflöden riskerar att skada känsliga strukturer på bakterierna och virusen, och då kan det bli svårt att analysera proverna. Vi utvärderade därför en nyutvecklad provtagare som samlar in bioaerosol med ett lägre flöde, men till en väldigt liten volym vätska, 0,3 milliliter. Den lilla vätskevolymen gör att koncentrationen i provet blir hög, vilket underlättar för analysen. Insamlaren skulle kunna användas för att samla ta prover på till exempel sjukhusluft eller utandningsluft från misstänkt smittsamma patienter.

Smittspridning är ett komplext problem med många komponenter att ha hänsyn till: den smittbärande personen, virusens eller bakteriernas egenskaper, och förhållandena runt den friska personen som smittas. För att förstå hur det går till krävs fältmätningar där smittan sker, kontrollerade laboratoriestudier och teoretiska förklaringsmodeller. Och detta är inte ett enmansjobb utan något som kräver tvärvetenskapliga samarbeten med expertis från läkare, sjuksköterskor, mikrobiologer, virologer, och teoretiska och experimentella aerosolfysiker – en kombination av dessa är vad som lett fram till denna avhandling.

Papers included in this thesis

I. Temperature-controlled airflow ventilation in operating rooms compared with laminar airflow and turbulent mixed airflow

Alsved, M., Civilis, A., Ekolind, P., Tammelin, A., Erichsen Andersson, A., Jakobsson, J., Svensson, T., Ramstorp, M., Sadrizadeh, S., Larsson, P-A., Bohgard, M., Šantl-Temkiv, T., Löndahl, J. *Journal of Hospital Infection*, 98, 181-190 (2017).

II. Sources of airborne norovirus in hospital outbreaks

Alsved, M.*, Fraenkel, C-J.*, Bohgard, M., Widell, A., Söderlund-Strand, A., Lanbeck, P., Holmdahl, T., Isaxon, C., Medstrand, P., Böttiger, B., Löndahl, J. *Clinical Infectious Diseases*, 70 (10), 2023-2028 (2020).

III. Effect of aerosolization and drying on the viability of *Pseudomonas syringae* cells

Alsved, M.*, Holm, S.*, Christiansen, S., Smidt, M., Rosati, B., Ling, M., Boesen, T., Finster, K., Bilde, M., Löndahl, J., Šantl-Temkiv, T. *Frontiers in Microbiology*, 9, 3086 (2018).

IV. Aerosolization and recovery of viable murine norovirus in an experimental setup

Alsved, M., Widell, A., Dahlin, H., Karlson, S., Medstrand, P., Löndahl, J. *Submitted manuscript.*

V. Natural sources and experimental generation of bioaerosols: Challenges and perspectives

Alsved, M.*, Bourouiba, L.*, Duchaine, C.*, Löndahl, J.*, Marr, L. M.*, Parker, S. T.*, Prussin II, A. J.*, Thomas, R. J.*. *Aerosol Science and Technology*, DOI: 10.1080/02786826.2019.1682509.

VI. Efficient electrostatic sampling of bioaerosols into liquid.

Ladhani, L., **Alsved, M.**, Yasuga, H., Wollmer, P., Löndahl, J., van der Wijngaart, W. *Submitted manuscript*.

*Authors contributed equally to the manuscript.

Author's contributions to the papers included in this thesis

Paper I
I took part in data collection and air sampling at the hospital. I summarized and analyzed the data, and I was the major contributor in writing the article and producing the figures. I responded to the reviewers' questions in the publication process.

Paper II
I was a main contributor to planning the study and the measurements. I was one of three who collected air samples at hospitals and I had a major role in the data analysis. The other first author and I shared the responsibility of writing the article and responding to the peer-review comments.

Paper III
I had a major role in planning, developing and performing the aerosolization experiments with the sparging liquid aerosol generator (SLAG). I carried out the analysis of the flow cytometry data from all experiments together with the other first author. I performed the statistical data analysis and I produced the article figures. The other first author and I wrote the article and the peer-review response together.

Paper IV
I had a major role in designing and planning the study. I performed the majority of the experiments, and supervised a Bachelor student in one part of the experiments. I did essentially all data analysis and wrote the article.

Paper V
I contributed to this literature review article by carrying out literature search, writing, constructing a table and producing a figure, mainly in the part regarding laboratory generation of bioaerosols.

Paper VI
I had a major role in planning and executing the experimental parts about radioactive aerosol and microsphere particles. I carried out the analysis of the microsphere particle collection efficiency and wrote the corresponding part of the manuscript.

Peer-reviewed publications not included in this thesis

Arctic sympagic and pelagic ecosystems enhancing cloud seeding aerosols

Dall´Osto, M., Šantl-Temkiv, T., Finster, K. W., **Alsved, M.**, Löndahl, J., Massling, A., Skov, H. *Submitted manuscript.*

Airborne allergens from dogs – quantity and particle size

Wintersand, A., **Alsved, M.**, Jakobsson, Sadrizadeh, S., Grönlund, H., Löndahl, J., J., Gafvelin, G. *Manuscript in preparation.*

Conference abstracts as lead author

M. Alsved, A. Civilis, P. Ekolind, A. Tammelin, A. Erichsen Andersson, J. Jakobsson, T. Svensson, M. Ramstorp, T. Santl Temkiv, P.A. Larsson, M. Bohgard, J. Löndahl. Airborne bacteria during surgery in hospital operating rooms with different ventilation systems (poster presentation). *Nordic Society for Aerosol Research symposium*, Aarhus, Denmark, 2016.

M. Alsved, A. Civilis, P. Ekolind, A. Tammelin, A. Erichsen Andersson, J. Jakobsson, T. Svensson, M. Ramstorp, T. Santl Temkiv, P.A. Larsson, M. Bohgard, J. Löndahl. Airborne bacteria in hospital operating theatres during surgery (oral presentation). *European Aerosol Conference*, Tours, France, 2016.

M. Alsved, A. Civilis, P. Ekolind, A. Tammelin, A. Erichsen Andersson, J. Jakobsson, T. Svensson, M. Ramstorp, T. Santl Temkiv, P.A. Larsson, M. Bohgard, J. Löndahl. Airborne bacteria in hospital operating theatres during surgery (oral presentation). *Ulmer Symposium Krankenhausinfektionen*, Ulm, Germany, 2017.

M. Alsved, T. Svensson, P. Medstrand, A. Widell, M. Bohgard, J. Löndahl. Aerosolization of a model virus for studies of human winter vomiting disease (oral presentation). *Nordic Society for Aerosol Research Symposium*, Lund, 2017, Sweden.

M. Alsved, J. Löndahl, K. Finster, T. Šantl-Temkiv. Online measurements of biological aerosols along the Greenland west coast (poster presentation). *Nordic Society for Aerosol Research Symposium*, Lund, Sweden, 2017.

M. Alsved, A. Widell, P. Medstrand, C-J Fraenkel, K. Lovén, C. Isaxon, T. Svensson, T. Holmdahl, B. Böttiger, M. Bohgard, J. Löndahl. Field sampling and laboratory studies of airborne norovirus (oral presentation). *Bioaerosols – Aerosol Society Focus Meeting 10*, Bristol, United Kingdom, 2017.

M. Alsved, Šantl-Temkiv, S. Holm, T. Svensson, P. Medstrand, A. Widell, M. Bohgard, J. Löndahl. Experimental set-up for studies of viability of aerosolized model organisms for infectious diseases (oral presentation). *European Aerosol Conference*, Zürich, Switzerland, 2017.

M. Alsved, A. Civilis, P. Ekolind, A. Tammelin, A. Erichsen Andersson, J. Jakobsson, T. Svensson, M. Ramstorp, S. Sadrizadeh, P-A. Larsson, M. Bohgard, T. Šantl-Temkiv, J. Löndahl. Airborne bacteria in hospital operating rooms during ongoing surgery (poster presentation). *German Society for Hygiene and Microbiology*, Bochum, Germany, 2018.

M. Alsved, A. Civilis, P. Ekolind, A. Tammelin, A. Erichsen Andersson, J. Jakobsson, T. Svensson, M. Ramstorp, S. Sadrizadeh, P-A. Larsson, M. Bohgard, T. Šantl-Temkiv, J. Löndahl. Airborne bacteria in hospital operating rooms during

ongoing surgery (oral presentation). *14th Kongress für Kranhenhaushygiene*, Berlin, Germany, 2018.

M. Alsved, C-J. Fraenkel, A. Widell, R. Lange, A. Gudmundsson, C. Isaxon, K. Lovén, M. Ramstorp, T. Holmdahl, P. Lanbeck, B. Böttiger, M. Bohgard, P. Medstrand, J. Löndahl. Detection of airborne noroviruses in hospitals and lab experiments (poster presentation). *Aerosols 2018 – Workplace and Indoor Aerosols Conference*, Cassino, Italy, 2018.

M. Alsved, A. Widell, M. Bohgard, P. Medstrand, J. Löndahl. Viability of aerosolized murine norovirus in experimental setup (poster presentation). *International Aerosol Conference*, Saint Louis, MO, USA, 2018.

M. Alsved, H. Dahlin, A. Widell, P. Medstrand, J. Löndahl. Experimental assessment of aerosolized murine noroviruses (oral presentation). *Swedish Virology Meeting*, Smögen, Sweden, 2019.

M. Alsved, C-J. Fraenkel, M. Bohgard, A. Widell, A. Söderlund-Strand, P. Lanbeck, T. Holmdahl, C. Isaxon, A. Gudmundsson, P. Medstrand, B. Böttiger, J. Löndahl. Airborne winter vomiting disease virus detected in particles <1 μm in hospital outbreaks (oral presentation). *European Aerosol Conference*, Gothenburg, Sweden, 2018.

M. Alsved, H. Dahlin, A. Widell, P. Medstrand, J. Löndahl. Experimental assessment of aerosolized murine noroviruses (poster presentation). *Swedish National Infection Biology Meeting*, Aronsborg, Sweden, 2019.

List of abbreviations and acronyms

APS	aerodynamic particle sizer
BANG	bioaerosol nebulizing generator
CFU	colony forming units
CoV	coronavirus
CPE	cytopathic effect
ESP	electrostatic precipitation
HEPA	high efficient particulate arresting (used for filters)
INP	ice nucleation particle
LAF	laminar airflow
LIF	laser-induced fluorescence
MNV	murine norovirus
NoV	human norovirus
OPS	optical particle sizer
PBS	phosphate buffer saline
PCR	polymerase chain reaction
RH	relative humidity
RT-qPCR	reverse transcription quantitative PCR
RNA	ribonucleic acid
psRNA	positive sense RNA
nsRNA	negative sense RNA
SARS	severe acute respiratory syndrome
SMPS	scanning mobility particle sizer
SLAG	sparging liquid aerosol generator
SSI	surgical site infection
T_CAF	temperature-controlled airflow
TCID	tissue culture infection dose
TMA	turbulent mixed airflow
VBNC	viable but non-cultivable

1 Introduction

1.1 Airborne infectious diseases

Aerosols (Greek *aer*=air, *solutio*=solution) are solid or liquid particles suspended in gas (often air). Bioaerosols are aerosol particles that are living or that originate from living organisms and they are ubiquitous in the environment. Infectious diseases that can transmit as bioaerosols often spread rapidly. Many of these diseases have caused pandemics through history and have had an important impact on our society, for example:

- the Spanish flu (influenza) that infected one third of the world's population and killed ~50 million people in 1918 and 1919 [1];
- tuberculosis, that caused 25% of all deaths in Europe in the 19th century and still kills more than a million people every year [2];
- the recent epidemics of coronaviruses causing 8000 Severe Acute Respiratory Syndrome (SARS-CoV) infections in Asia in 2002 and 2003 with a 10% death rate [3], and the ongoing covid-19 pandemic that has infected millions of people and paralyzed societies [4].

Urbanization and globalization are two risk factors when it comes to the spread of airborne diseases. In many cities, especially in low- and middle-income countries, people tend to live close to each other and sometimes in close contact with animals, resulting in a breeding ground for infections. Globalization entails fast and frequent traveling and today, one can travel to any place in the world within the incubation time of our most infectious diseases. This is something that became very obvious in January 2020 when the SARS-CoV-2, causing covid-19, emerged in a food market in the city of Wuhan, China, forcing a complete lock down of the county and traveling restrictions over several big cities. During February, SARS-CoV-2 continued to spread and developed into a pandemic with cases in almost all countries across the world [5].

Today we know that bacteria and viruses cause infectious diseases. Our main prevention strategy against the spread of diseases that cannot be prevented by vaccination is social distancing and hygienic practices like washing our hands. Handwashing is an efficient tool for limiting contact spread of infectious diseases, but when it comes to airborne spread, other strategies need to be in place, especially

in indoor environments, such as hospitals. A main problem is that we have limited knowledge on when and where potential infectious bioaerosols can be generated, how long they can remain airborne, how far they can spread and if they are still infectious after having been airborne. This thesis contributes with new results that address these topics, and new insights on how infectious bioaerosols can be prevented.

Infectious diseases that target the respiratory system are commonly airborne, as we breathe microorganisms into our lungs, and expel them through breathing, talking, coughing and sneezing. It may seem more farfetched to think of microorganisms that cause gastrointestinal diseases as being airborne. But microorganisms in feces and emesis are likely to be aerosolized from toilet flushing or vomiting, respectively [6]. Viral respiratory diseases, such as influenza, and acute gastroenteritis (vomiting flu) appear annually, reaching their peaks during the winter months in temperate regions [7-9]. Nobody has so far been able to fully explain the seasonality of these infectious diseases, but several studies have tried to link viral infectivity to environmental factors such as temperature and humidity [10-13], or to human behavior and health status. A question that needs to be addressed if we want to conquer these diseases is:

- Can environmental factors during the winter months increase the spread of infectious bioaerosols?

In today's hospitals and many institutions, there are thorough hygiene routines and the awareness of potential cross-contaminations is high. Nevertheless, the winter vomiting disease caused by noroviruses (NoV) spreads successfully and causes outbreaks in hospital wards, resulting in tough working conditions for the staff. A second question to ask is therefore:

- If accurate handwashing and other prevention strategies for contact spread are followed and the disease still spreads and causes outbreaks, could the microbial disease agents spread via air?

Airborne bacteria in hospitals can cause nosocomial infections, which lead to suffering, prolonged hospitalizations and high economical costs [14]. Hospitalized patients often have a weakened health status, which makes them more susceptible to getting an infection. During open wound surgery, the first defence of our immune system – the skin barrier – is opened, which increases the risk for surgical site infections (SSI). Especially vulnerable for SSIs are joint surgeries (*e.g.*, hip or knee replacement surgery). This is because joints are poorly vascularized, and there is thus a very limited immune defence that can fight off intruding microorganisms. In the 1960s, a study showed a decreased incidence of SSIs from 8.9% to 1.3% after improvements of the ventilation in operating rooms, proving the importance of clean air [15]. Since then, short-term antibiotic prophylaxis has been introduced as common practice in surgery. However, with increasing occurrence of antibiotic

resistant bacteria that cause SSIs [16], other prevention measures such as the ventilation need to be as efficient as possible.

Infection prevention strategies in hospitals should be evaluated with a holistic perspective, including not only the infection rate, but also energy efficiency and working environment factors. Functional and applicable infection prevention guidelines that also provide a good working environment are particularly important; otherwise, the staff may not work according to the guidelines.

Infectious diseases may sound trivial to us who live in high-income countries, as we are vaccinated for many of them and have access to good health care and medication if we get sick. Nevertheless, the covid-19 pandemic has revealed how efficient viral diseases to which we have no immunity or vaccines can spread, resulting in high mortality in rich countries as well. In low-income countries, infections in the lower respiratory tract and diarrheal diseases are the two leading causes of death [17] (Figure 1). Infected children who are also suffering from malnutrition are especially vulnerable; they may account for 50% of the 4.5 million childhood deaths in sub-Saharan Africa and Asia [18-20].

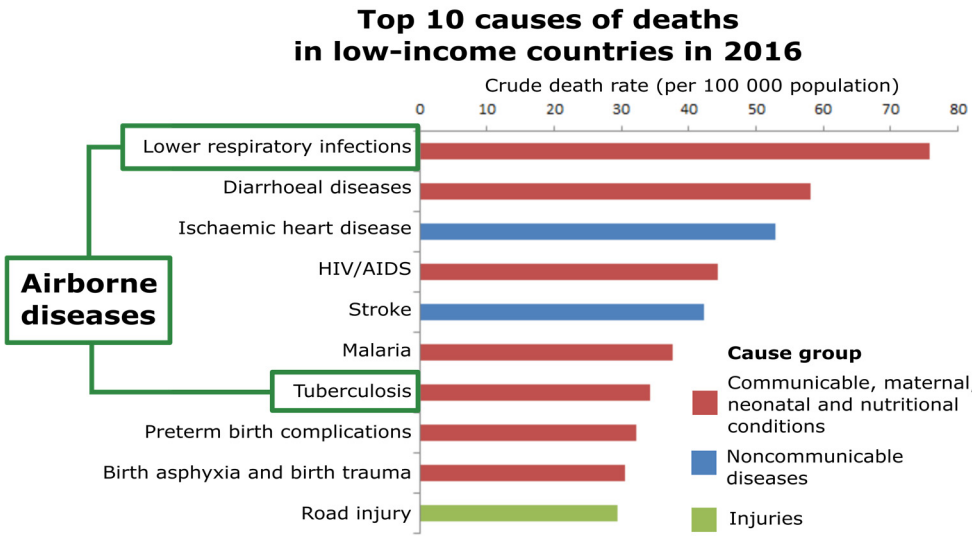


Figure 1. World Health Organization top 10 causes of deaths in low-income countries in 2016.
In low-income countries, infectious diseases constitute five of the ten most common causes of death (lower respiratory infections, diarrheal diseases, HIV/AIDS, malaria and tuberculosis), of which two are airborne diseases. In middle-income countries lower respiratory infections, tuberculosis and diarrheal diseases are on the top-ten list, and in high-income countries, lower respiratory infections are the only communicable disease on the list. Modified from [17].

1.2 Aim and objectives

The overall aim of this thesis was to increase the knowledge about sources and the airborne transport of infectious bioaerosols in order to prevent diseases from spreading via air (Figure 2). This was achieved by field measurements in hospital environments and by laboratory experiments on model bacteria and viruses.

The specific objectives of this thesis were to:

1. Identify possible sources of infectious bioaerosols through field measurements in hospital environments (Papers I and IV).
2. Compare bioaerosol viability or infectivity after aerosolization, transport and collection in controlled laboratory experiments. Investigate the effect of aerosolization processes and relative humidity during airborne transport (Papers II, III, IV and V).
3. Evaluate ventilation techniques and rapid bioaerosol detection techniques as prevention strategies in hospitals to avoid nosocomial infections, especially from surgery (Papers I and VI).

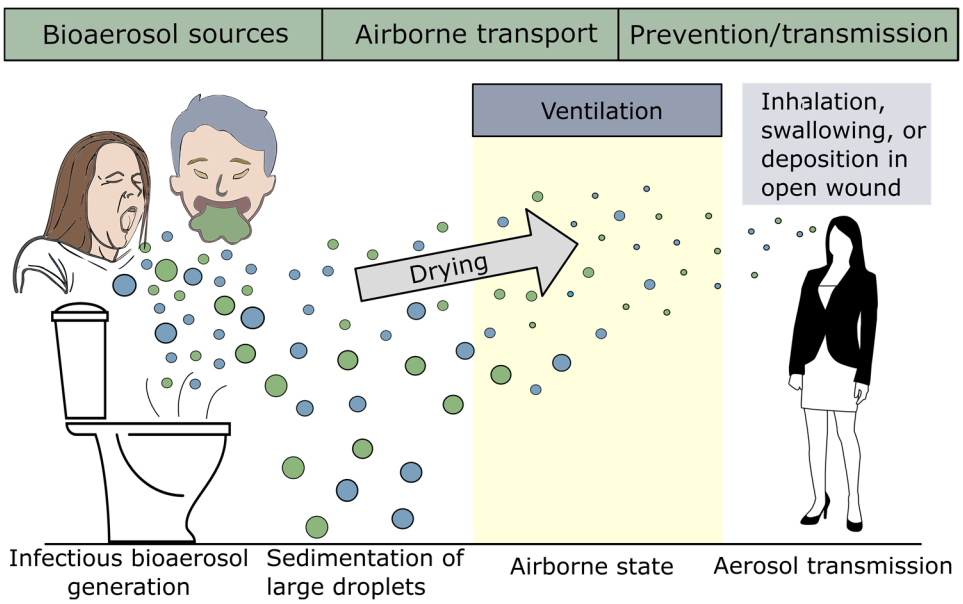


Figure 2. Bioaerosol sources, airborne transport and transmission.
Bioaerosols are for example genreated from sneezes, vomiting and toilet flushing (and many other ways). Large droplets (>100 µm) sediment to the ground after a short while. Smaller droplets dry out and remain airborne for longer times (minutes to hours). These dried particles can be removed by efficient ventilation or other prevention techniques. Particles that reach a susceptible host in a high enough dose via inhalation, swallowing, or depositioning in an open wound can reasult in an infection via aerosol transmission.

2 Background about bioaerosols

2.1 Bioaerosols in outdoor and indoor air

Bioaerosols can be of great diversity: bacteria, pollen, viruses, fungal spores, allergens or fragments of biological materials. They span a large size range: viruses can be 0.02-0.3 µm, bacteria 0.5-2 µm, fungal spores 2-30 µm and pollen 10-100 µm (Figure 3) [21]. Thus, the term "bioaerosols" encompasses a diverse range of aerosols, both in terms of origin, content and size. Although aerosol transmission in medicine often is defined as airborne particles <5 µm, here, I include particles <100 µm according to the conventional definition of aerosols [22].

In nature, microorganisms are dispersed into the atmosphere from essentially all surfaces. From water surfaces, vegetation and soil, an estimated number of 10^{24} bacteria or by weight around 50-100 Tg biological particles are dispersed into the atmosphere every year [21, 23]. Humans and their activities, such as agriculture, waste and wastewater treatment, animal farming and bioengineering industry are also contributors to bioaerosols in the atmosphere.

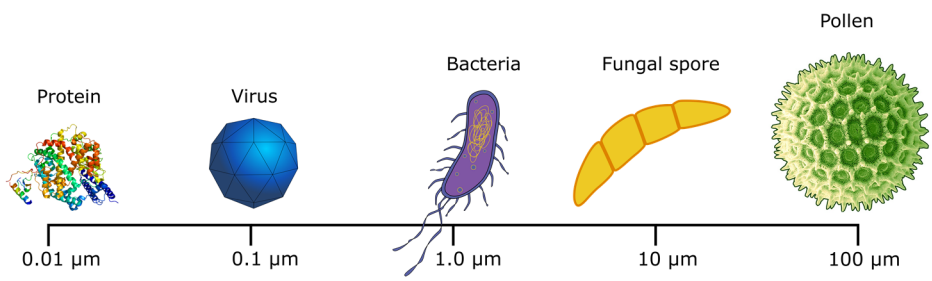


Figure 3. Scale of common bioaerosol components and their sizes.
A protein ~0.01 µm, a virus ~0.1 µm, a rod-shaped bacterium ~1 µm, a fungal spore ~10 µm, and a pollen particle ~100 µm.

In outdoor air, the total concentration of (non-bio-) aerosol particles are often on the order of 10^{9}-10^{11} m^{-3}, while the concentration of bioaerosols are several orders of magnitude lower, ranging from 10^{2}-10^{4} m^{-3} [23]. However, among coarse aerosol particles, >1 µm, about 30% are bioaerosols, considering both particle mass and number concentrations [24]. Generally, the atmosphere is a hostile environment for microbes. They are exposed to rapid changes in temperature and humidity, UV

irradiance, and limited nutrition [21]. Many species have developed protection mechanisms against these stress factors, such as dormant stages (*e.g.*, spores), anti-freeze proteins and pigmentation [21].

In indoor environments, humans and pets are major contributors to bioaerosol concentrations [25] through shedding of the trillions of human and bacterial cells that compose our individual ecosystems [26]. For example, a single human sheds about a million particles every hour from the skin and hair [27]. The indoor environment is more constant in terms of temperature and ventilation than the ambient atmosphere and it is protected from UV radiation, which all favor survival of microorganisms. Considering that people in developed countries spend more than 90% of their time indoors all year round [28], the air quality of the indoor environment is crucial for human well-being. Thus, the effect of indoor environments on viral and bacterial survival is important to investigate. Since the consequences of exposure strongly depend on what types of bioaerosols are present, there are no general guidelines on bioaerosol concentrations [29]. There are countries and organizations that have set their own guidelines for bioaerosol concentrations, where some levels are conditional to, for example, specific allergenic spores [29].

2.2 Bioaerosol sources

2.2.1 Natural bioaerosol sources

In nature, bioaerosols are generated by either wet or dry aerosolization processes (Figure 4). Dry aerosolization occurs when wind or mechanical forces resuspend microorganisms from soil or surfaces into the atmosphere [30, 31]. The number of microorganisms living on vegetation, on animals and in soil is vast, and dry aerosolization is estimated to contribute the majority of atmospheric bioaerosols [24]. Although bacteria aerosolized through dry processes constitute the major part of the total airborne bacteria, higher cultivability ratios (number of cultivable bacteria/total number of bacteria) have been found in airborne bacteria generated from wet aerosolization [24, 32].

Wet aerosolization occurs when droplets containing microorganisms are formed. Droplets are generated from films that break in splashing waters, from bubbles that burst, or as spume drops sheared off from waves by the wind (Figure 4). Bubble bursting creates two types of droplets: film drops and jet drops. Film drops are produced when the bubble cap film breaks, often generating high droplet numbers (up to 1000 droplets per bubble) with small droplet sizes (typically <1 μm) [33]. Jet drops are formed from the breakup of the liquid jet that is formed when the bubble

cavity collapses, creating fewer (~10 droplets per bubble) but larger droplets (typically >10 µm) per bubble than the film drop mechanism [33].

The number of film droplets produced from a bursting bubble, and the ejection velocity of these droplets, depend on surfactants and other compounds present in the water [34, 35]. Bacteria, viruses and algae are present in enriched concentrations (up to 10^{9}-10^{12} L^{-1} seawater) at the sea surface microlayer (the top 1-1000 µm of the seawater). Some microorganisms produce surfactants that are excreted to the surrounding water [36] where they are involved in regulating the bioaerosol generation. Aerosolized surfactants can act as cloud condensation nuclei (CCN). CCN and ice nucleation particles (INP) are prerequisites for cloud formation, and some bacterial species are known to be efficient INPs at higher sub-zero temperatures than non-biological particles [37]. As 70% of the earth's surface is covered by water, it is important to understand the extent to which marine bioaerosols contribute to atmospheric processes, so that climate models can be verified. At present, there is large uncertainty about the climate forcing of marine bioaerosols [36].

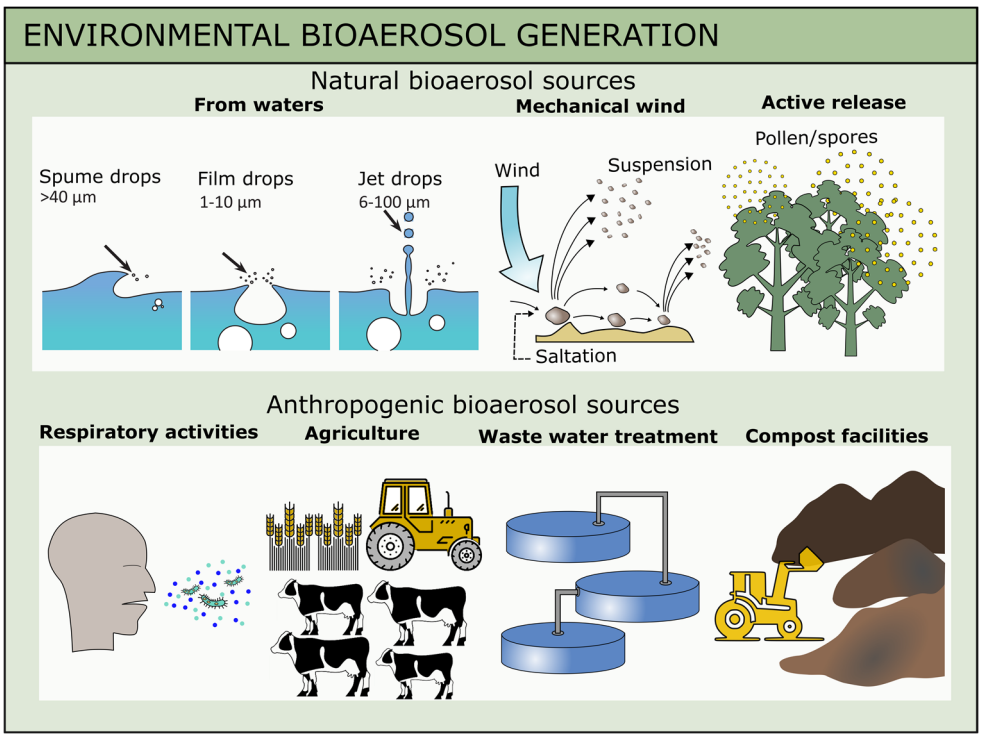


Figure 4. Natural and anthropogenic bioaerosol sources.
Illustrations of natural bioaerosol generation from waters (spume drops, film drops and jet drops), by wind and by active release (upper panel). The lower panel illustrates bioaerosol generation from some anthropogenic sources: human respiratory activities, agriculture, waste water treatment and compost facilities. Reprinted from [38].

2.2.2 Anthropogenic bioaerosol sources

Anthropogenic activities contribute locally with high concentrations of bioaerosols. For example, bioaerosols are generated during mechanic turning of waste in waste treatment facilities, during aeration of wastewater, in agriculture and animal farming, and from urban environments. Bioaerosols from anthropogenic sources can often reach high concentrations of certain species. Exposure to these, especially in working environments, are known to give rise to allergic responses or disease: mycotoxins and endotoxins at waste treatment facilities and agriculture; *methicillin resistant Staphylococcus aureus* (MRSA) in swine production facilities [1]; and viruses in wastewater treatment plants [39]. Our built environments can sometimes act as bioaerosol sources, for example, cooling towers from where *Legionella* species bacteria have been spread [1], toilet flushing after patients with diarrhea [40], or from mold growth in damp buildings [41, 42].

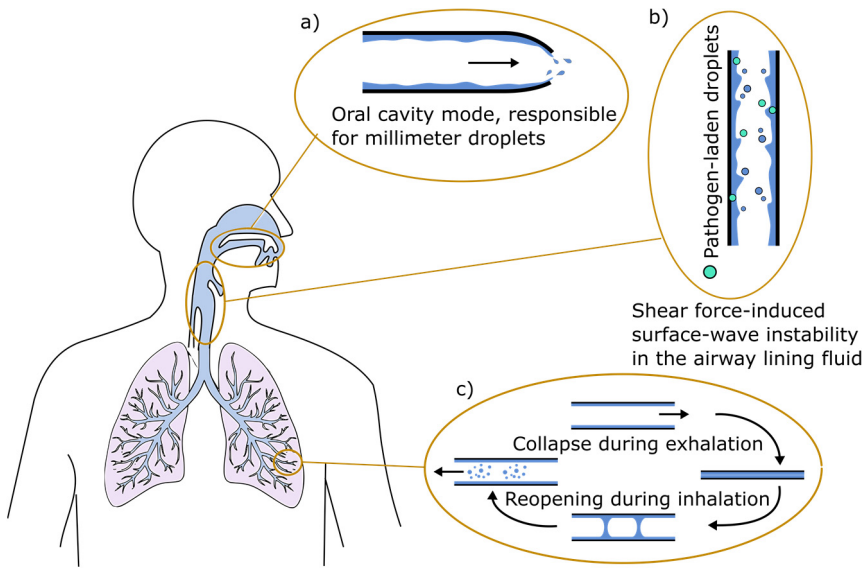


Figure 5. Droplet generation from human respiratory tract.
Schematic illustration of three droplet generating processes in the human respiratory tract. (A) Large, millimteter sized droplet generated from shear forces in the oral cavity. (B) Shear-force induced droplet generation from the airway lining fluid in the upper respiratory tract. (C) Small, sub-micrometer sized droplets generated from film rupture in the lower respiratory tract. Figure adapted from [43].

Potentially infectious bioaerosols in indoor environments are to a high degree generated through the symptoms of disease: sneezing, coughing, vomiting or skin rash. One sneeze can generate 40 000 droplets, and one cough about 3 000. Substantial amounts of these droplets are <100 µm and thereby likely to dry to droplet nuclei and remain airborne and inhalable [43]. Although sneezing and coughing generate high droplet numbers at high airflow speeds, these are low-

frequent events compared to breathing and talking. During two hours of breathing and talking, one order of magnitude larger volume of bioaerosol particles is ejected than from 100 coughs [44]. Droplets are generated in three ways in our respiratory tract during breathing (Figure 5): droplets are sheared off from saliva in the oral cavity (~100 μm); droplets are sheared off from the airway lining fluid in the upper airways (> ~1 μm); and droplets that form when liquid films in the smaller airways burst (< ~1 μm) [43].

2.3 Infectious diseases transmittable via air

In ancient Europe and China, "corrupt air" was thought of as the causative agent of diseases such as cholera and the Black Death [45]. Later on, during the 17th century, the term "miasma" was introduced, which means pollution in Greek. The miasmic theory was popular throughout the Middle Ages and until the 19th century. It implied that foul air and poor hygienic conditions would make a place polluted with corrupt air or miasma that would cause sickness. The good thing about the theory was that hygienic improvements were made regarding waste and sewage systems, for instance, promoted by Edwin Chadwick's report on the poor conditions in London [46]. However, it also delayed the recognition of the germ theory of disease until 1854, when John Snow identified a pump-well contaminated with cholera in London [47].

Infectious diseases can be transmitted by three routes: contact spread, droplet spread or airborne spread. Most diseases can be transmitted by contact spread [48], by either direct contact with the infected person or indirect contact via contaminated matter (*e.g.*, a door handle). Droplet spread is when millimeter-sized droplets are generated from, for example, sneezing or toilet flushing. These relatively large droplets fall to the ground within a short distance, typically 1-2 m, and thereby have a limited reach. Smaller droplets, <100 μm, often dry out within seconds before they reach the ground, and as they dry they shrink in size [49]. These dry particles, often defined as <5 μm or <10 μm, are small enough to remain airborne for longer periods of time, to transport longer distances, and to be inhaled and deposited in the respiratory system [48].

Typical airborne diseases are lung tuberculosis and measles. Tuberculosis is caused by the bacteria *Mycobacterium Tuberculosis* and can be designated as an obligate airborne disease [50]. Measles, caused by the Measles morbillivirus, may spread by contact but is a preferential airborne disease. Many other diseases that are mainly spread by contact can be opportunistic airborne diseases, meaning that under some circumstances they spread through air, and then often cause large outbreaks [50]. Opportunistic airborne diseases are, for example, influenza, norovirus, SARS, covid-19 or smallpox.

For a disease to spread via air, the infectious microorganism needs to be aerosolized from a source, transported by air and remain viable/infectious while airborne, and finally, needs to reach the susceptible cells of the new host and in high enough numbers to cause an infection. Most infectious agents are present in low number concentrations in air. Due to the low concentrations, most airborne infectious agents either need to be very contagious, or the host needs to be extra susceptible to acquire an infection. In accordance, noroviruses (NoV) are extremely contagious and survive up to weeks in the environment [51], which makes it reasonable to believe that they may transmit via air. Though it is contrariwise that inhaled airborne NoV would cause infections in the GI tract, a possible, yet not confirmed explanation for NoV infections, is by mucociliary clearance: particles deposited in the upper respiratory tract are transported by mucociliary clearance to the trachea where they are swallowed. Extra susceptible patients are, for example, those who undergo joint surgery, where commensal skin bacteria such as *Staphylococcus aureus* or *Staphylococcus epidermis* may cause surgical site infections [52].

2.4 Airborne infectious disease prevention strategies

Strategies to prevent the spread of infectious bioaerosols have been developed for different settings: hospital wards, operating rooms, isolation units, spacecrafts, aircrafts, etc. The main technique is ventilation and air filtration. Particulate filters are used to remove airborne contaminants and to ensure the introduction of clean air to the room. Other techniques are: positive air pressure in the room (ensuring no airflow into the room); negative air pressure (ensuring no airflow out from the room); anterooms between the corridor and the patient room; patient isolation in single rooms; access to the patient room from outdoors; separate transport flows for highly contagious patients; and personal protection equipment (an example of extensive personal protection equipment shown in Figure 6) including a respiratory mask, eye protection, apron and gloves [50, 53].

Figure 6. Infection prevention equipment during the covid-19 pandemic.
Although SARS-COV-2 was considered to spread primarily by contact and large respiratory droplets, infection prevention guidelines toward airborne spread were applied in some places. Photo by Tedward Quinn on Unsplash.

2.4.1 Operating room ventilation systems

The term "operating theater" comes from the way surgery was performed during the mid-19th century – built as amphitheaters with plenty of space for spectators and students. After Joseph Lister in 1867 published his results on antiseptic surgical work and the clear reduction in surgical site infections, the way of working changed with the aim to eliminate infections [54]. Improvements were made concerning sterilization of instruments, and exhaust fan ventilation was introduced to remove the steam from autoclavation. However, the exhaust fans pulled in air from the corridor outside the operating rooms, which contained a lot of bacteria [55]. The significance of clean air lowering the SSI rate, was shown by Shooter et al. in 1956 [55] who took in outdoor air instead of air from the hospital ward, and then by sir John Charnley in the 1960s who introduced ventilation with high air exchange rates [15].

Since then, several ventilation techniques have been developed, and the most frequently implemented and well-studied techniques are turbulent mixing airflow (TMA) and laminar airflow (LAF). Filtration systems that efficiently collect particles down to sub-micrometer sizes that operate at high airflow rates and that temperate the air require high energy consumption. Ventilation and filtration systems are voluminous and expensive both to purchase and to maintain, which is an important reason for the ongoing studies on their efficiency and discussions on their necessity in operating rooms [56-58]. In addition to ventilation systems, special

surgical clothing is used to minimize particle emissions from the staff (Figure 7) [59], and behavioral interventions are used to improve hygiene routines [60].

Figure 7. Me in an operating room.
Me, dressed in surgical clothes (not correct surgical hood) in one of the operating rooms where we measuremed colony forming units (CFU) concentrations in the air during ongoing surgeries (Paper I). Photo: Helena Bohm-Nilsson.

2.5 Bioaerosol sampling and detection

Sampling of bioaerosols is challenging due to their often low concentrations in air and due to the difficulty to capture and preserve sensitive biological structures that need to remain intact for the analysis. The number concentrations of bioaerosol particles are often one millionth of the total number of aerosol particles, which requires high sampling airflows and/or long sampling times. Long sampling times can lead to further disruption of sensitive biological structures due to extensive drying. Many airborne microorganisms cannot be detected by cultivation in the laboratory, as techniques to cultivate them are not yet known or because they are in a state called “viable but non-cultivable” (VBNC) [32, 61].

Many bioaerosol sources are local and originate from short-time events (*i.e.*, sneezing), which makes the timing and positioning of sampling devices crucial. This is often the case when it comes to infectious aerosols, as most infectious diseases spread during short periods. The spatio-temporal heterogeneity in the distribution of active sources of infectious bioaerosols imposes further difficulties on bioaerosol sampling and detection.

2.5.1 Bioaerosol sampling techniques

Bioaerosol samples are collected either on filters, into liquids or on solid substrates (*e.g.*, cultivation plates) (Figure 8) [62]. Filter sampling has the advantages that it is simple and that it has high collection efficiency for a wide range of particle sizes [22]. However, the viability of microorganisms may be lost due to extensive drying during sampling [63]. To avoid drying, gelatine filters can be used, but short sampling times are still recommended [64]. The collected bioaerosol may also stick to some filter materials making it difficult to extract for analysis [65, 66].

Sampling by impaction implies acceleration of an airflow towards a collection substrate. Particles with too high inertia (*i.e.,* mass and velocity) will impact on the substrate, while the air and particles with low inertia will flow around the substrate [22]. Sampling by impaction is used in several types of collectors and the substrate can be varied. Impaction directly on cultivation plates has traditionally been one of the most common sampling techniques, and is still frequently used in, for example, hospital hygiene measurements [67]. It is a simple method, but one has to be aware that the type of growth media and the incubation conditions select what microorganisms grow on the cultivation plates as colony forming units (CFU) [68]. The impaction force at high airflows can also damage sensitive structures. In addition to impaction on cultivation plates, impaction can be done on filter or metal surfaces, which then requires similar extraction processes as filter sampling.

Impaction into liquids is called impingement. The sample airflow is pulled through a critical orifice that accelerates the airflow to high velocities into a container filled with liquid, and the particles impact on the liquid surface. Some collection by diffusion may also take place as the air bubbles through the liquid. Due to extensive splashing inside the impinger, re-aerosolization of the collected material occurs to some extent [69] and liquid is lost due to evaporation. Evaporation is an issue in all liquid collectors that necessitates either short sampling times, refilling, or using large liquid volumes [63]. The main advantages of sampling with impingers is that: 1) viability is preserved to a higher degree when sampling into liquids [70], 2) many downstream analyses are based on liquid samples, and 3) the liquid can be varied. As with impaction, impingers are most efficient for collection of particles >1 µm.

Liquid cyclones are cone-shaped containers containing liquid where the sample airflow enters in a tangential direction at the upper rim. The airflow spins around inside the cone and accelerates toward the narrowing bottom, applying a centrifugal force to the particles. As the air swirls around, so does the collection liquid, causing particles deposit in the liquid. The cyclone collection mechanism is also based on inertia, and is therefore most efficient for particle sizes >1 µm. The advantage of using a liquid cyclone sampler is that high airflows of 100-1000 L min^{-1} can be used without extensive pressure drops, which means that less powerful and less noisy pumps can be used [63].

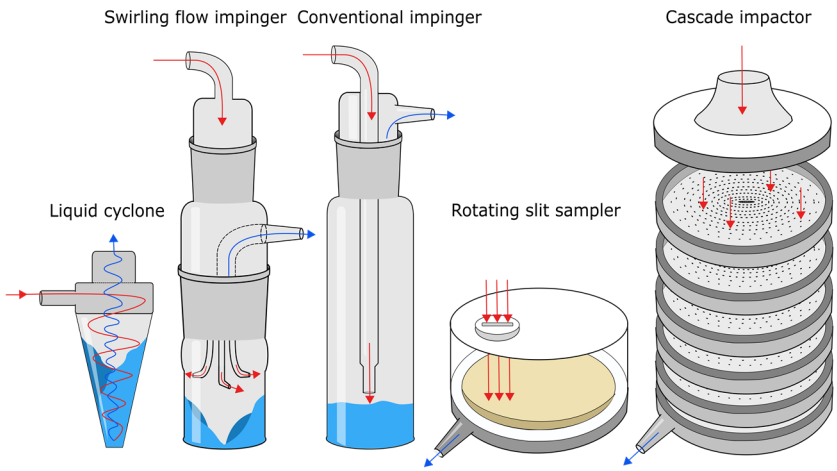


Figure 8. Common bioaerosol samplers
Schematic figures of some common bioaerosol samplers. Incoming air is indicated by red arrows and flow lines; outgoing air after the collection is indicated in blue. The liquid cyclone, the swirling flow impinger (BioSampler), and the conventional impinger sample into liquid. The slit sampler (an impactor), and the cascade impactor can be operated to sample directly on agar plates, or on the metal surface. Figure adapted from [62].

Electrostatic precipitation (ESP) is a sampling technique that can be used to sample either on solid substrates or in liquid. It is based on the charging of particles in the sample airflow that then are collected by electrostatic forces. ESP samplers that collect into liquid have been shown to reach high sample concentrations because the liquid volume can be kept very small [71]. One should be aware of the fact, though, that high voltage corona chargers are likely to produce ozone, which may influence the collected material. In addition, it may be unhealthy for operators to remain in close proximity to an ESP for long periods in small and poorly ventilated rooms.

The choice of sampling technique and collection media is important and depends on the downstream analysis and the microorganisms one expects to collect. There is also an option to add stabilizing agents to the collection liquid that enhance preservation of the collected material during sampling and until analysis is performed [72].

2.5.2 Sample analysis

The majority of bioaerosol analyses are performed offline using diverse microbiological, optical and molecular biology methods on the collected sample material. As mentioned in the previous section, traditionally counting CFUs on cultivation plates has been the most prevalent technique [62]. With the development in molecular biology and single-cell detection techniques, non-cultivation based techniques are being utilized more because only a small fraction (~1%) of environmental bacteria are cultivable in standard laboratory settings [73].

Fluorescent dyes can be used to stain certain molecules, commonly nucleic acids, in order to quantify, for example, cells with intact membrane integrity, membrane potential or active metabolism. The staining results can be evaluated by either fluorescence microscopy or flow cytometry. Electron microscopy can also be used to observe cells and their physiological conditions.

To determine which species are present in air, nucleic acid analysis by polymerase chain reaction (PCR) techniques can be used. Quantification of a specific bioaerosol type can be done with quantitative PCR (qPCR), where a standard curve from serial dilutions of a sample with a known concentration in included. Sequencing of suitable pathogen genes can identify specific agents and Next Generation Sequencing techniques allow identification of the entire total microbial diversity in a sample, generating information on what organisms that are present.

2.5.3 Online detection techniques

Recently, online detection techniques have been developed to give real-time information about the concentration of airborne microorganisms. One such technique is the use of laser-induced fluorescence (LIF) to discriminate between bioaerosols and non-biological aerosols.

LIF detection is based on auto-fluorescence from the tryptophan, riboflavin and nicotinamide adenine dinucleotide phosphate (NADH) biomolecules, which are present in most living materials. UV lights of wavelengths in the range of 270-405 nm are used for excitation of aerosols and fluorescence emission is subsequently detected at one or several wavelengths [24]. The fluorescence spectrometer technique can be used primarily to determine the total concentration of bioaerosols, and to some extent to classify bioaerosol particles into larger groups such as pollen, fungal spores and bacteria [74]. LIF-based techniques have great potential to identify rapid changes in bioaerosol concentrations, and could therefore be applied to the pharmaceutical industry, military defence or in hospitals, for example.

Another is matrix-assisted laser desorption/ionization (MALDI) aerosol time-of-flight (TOF) mass spectrometry (MS), which can be used to more specifically identify bacterial species in biological particles if a reference MALID-TOF-MS spectra from isolated cultures is available [75]. In laboratory experiments where the type of generated bioaerosol is known, optical aerosol particle counters are often used for size and concentration measurements.

2.6 The importance of bioaerosol particle size

The size of a bioaerosol particle determines: 1) how long the particle can stay airborne before it deposits on a surface, 2) where in the respiratory tract it will deposit if it is inhaled, and 3) how much infectious material it may contain [48]. The size of the bioaerosol particle is governed partly by the microorganism(s) that are contained in/on the particle, and partly by the bioaerosol source and aerosolization mechanism [76]. Microorganisms emitted from wet sources will constitute a particle together with other material in the droplet water when it dries to a smaller particle (Figure 9) [62]. Thus, there may be several viruses or bacteria in one droplet, and they may be surrounded by salts and organic material from the liquid source. Microorganisms aerosolized from dry sources can constitute a particle together with, for example, the dust particle or the skin flake particle that it is attached to. Conversely, bioaerosol particles can also be smaller than the microorganism, as for instance pollen and fungal spores that are fragmentized by environmental factors such as humidity [77]. The particle size fractions that contain pathogens or allergens can thus give an idea of where the bioaerosol comes from.

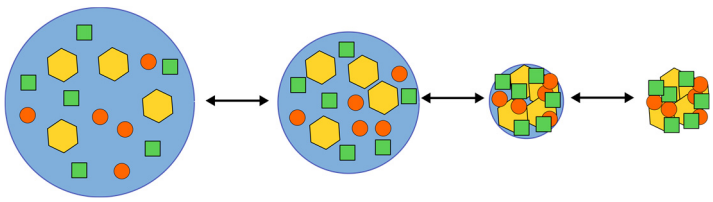


Figure 9. Droplet drying to dropelt nuclei.
A droplet containing viruses (yellow) and other organic and inorganic material (red and green) that is concentrated during water evaporation and finally a dry droplet nuclei (left to right). Rewetting of dry particles may also occur; hence, the double direction on the arrows.

For the purpose of transmission of infectious bioaerosols, both large and small particles may be advantageous for the ability of microorganisms to infect a new host. For example, a 10-µm particle has a thousand times larger volume than a 1-µm particle and is therefore likely to contain more pathogens. The pathogens are also more protected from environmental stress in large particles, as the surface-to-volume ratio is lower than in small particles [76]. However, the larger particles (>10 µm) are more likely to deposit on surfaces and sediment to the ground (within minutes), and in so doing, spread shorter distances from the sources compared to smaller particles. Smaller particles (<1 µm) that contain infectious agents have a negligible sedimentation velocity and will consequently follow the air currents (for hours). Sub-micrometer particles are more likely to be inhaled and deposited in the lower respiratory tract and for influenza, that often leads to more severe symptoms than if deposited in the upper airways [48].

2.7 Environmental factors affecting bioaerosols

There is an ongoing discussion on the reason(s) behind the distinct seasonal pattern of increased upper respiratory viral infections, gastroenteritis and other infectious diseases during winter. Historically, exposure to cold weather was thought to be the exclusive explanation for these epidemics because the start of respiratory viral infections strongly correlates with decreased outdoor air temperature [7, 78]. The exposure to cold as being the only explanation, though, was declared as insufficient in the early 20th century, and instead, crowding in indoor environments was thought to be the reason. However, in the urban lifestyles of the 20th and 21st centuries, the amount of crowding at workplaces and in transportation systems is the same all year around [78]. Nobody has so far been able to explain the seasonality of these infectious diseases that peak during the winter in temperate regions. However, several environmental, as well as human health related, aspects are plausible contributing factors to the increased infection rates:

- Lower temperatures outdoors lead to heating in indoor environments that makes the air dryer. Dry air dries our respiratory mucous membranes in the respiratory tract, damaging the epithelial cells, and by that, increasing our susceptibility to infections [79].
- Cooler air has a lower ability to contain water vapor. This results in the absolute humidity being lower than in warm air. Indoor heating of cool outdoor air decreases the relative humidity (RH) by increasing the temperature (although the total water content is the same) and we thus have generally dryer indoor air during the winter. Low RH increases the evaporation rates of aerosol droplets, which has been associated with low inactivation rates of airborne viruses [10, 12, 13] and longer residence times. At low RH, an increase of infectious airborne particles is relevant for large droplets ~100-300 μm [49]. Dryer air increases the number of large droplet nuclei; however, because they are large, they can only remain airborne for seconds. For smaller droplets, <100 μm, all will dry out to droplet nuclei before they sediment to the floor at regular RH, <70%.
- The effect of temperature on airborne infectious viruses has been investigated in numerous studies, which indicates that lower temperatures increase their infectivity [80, 81]. One possible explanation is that lower temperatures stabilize cell membranes [82], leading to more intact pathogens that may reach a new host; however, not all viruses have envelopes.
- Cool air can induce vasoconstriction in the nose and upper respiratory tract blood vessels. This constriction diminishes the ability to humidify the

inhaled air [83], decreasing its humidity and in turn, the mucociliary clearance function and phagocytic activity [78].

- Cool air may lower the temperature of the epithelial cells in the upper respiratory tract. This may prolong viral reproduction periods, extending the time when there is a risk for transmission (observed for rhinovirus and influenza A virus in animals) [79, 84].
- Little sunlight in the winter months results in lower vitamin D levels. Vitamin D has been proven to boost the innate immunity by activating antimicrobial peptides, and protecting against respiratory infections [85, 86].

3 Methodology

In this thesis, experimental and observational research was conducted in the field and in laboratory settings to gain knowledge on sources and concentrations of bioaerosols, and what transport conditions affect bioaerosol viability. Moreover, this research includes an evaluation of ventilation techniques in operating rooms and of rapid bioaerosol detection techniques that can be used for the prevention of infection from bioaerosols, especially surgical site infections. The field work included fast identification of cases that met the study criteria, preparation of sampling instruments that were brought to the hospitals, collection of air samples, and collection of information related to the samples according to a defined protocol. Analyses of field samples were performed by the Department of Clinical Microbiology, Lund University. The laboratory work comprised preparation of the organism in the aerosolization solution, aerosolization and collection while monitoring the aerosol concentration and size distribution, and the analysis by viability, fluorescence and molecular assays.

3.1 Study designs

Paper I

Airborne bacteria that give rise to surgical site infections are highly unwanted, and because of this, high airflow ventilations are used in operating rooms. Our study evaluated three ventilation systems for operating rooms with regard to airborne bacterial concentrations, airflow efficiency and working environment comfort. A newly developed ventilation technique using temperature-controlled airflow (T_CAF) was compared to the more conventional laminar airflow (LAF) and turbulent mixed airflow (TMA) (Figure 10).

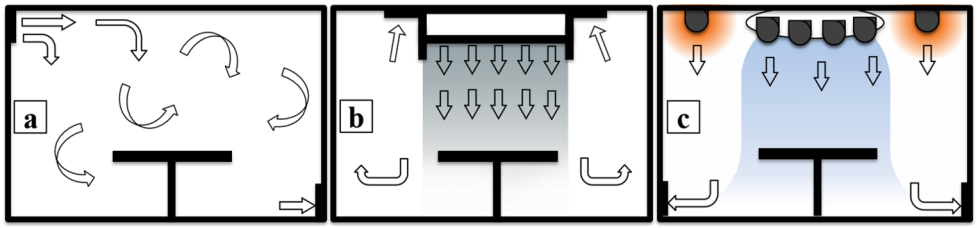


Figure 10. Operating room ventilation.
Schematic illustration of the three ventilation techniques for operating rooms: a) turbulent mixed airflow (TMA), b) laminar airflow (LAF), and c) temperature-controlled airflow (T_CAF). Arrows indicate airflow directions in the room with the operating table in the center [87].

Paper II

Noroviruses (NoV) is the main cause of acute gastroenteritis in the world. Although there are case studies of disease transmission that cannot be explained by its nominal transmission routes (contact, food or splashing droplets), transmission via air has not been confirmed. The aim of the study was to collect air samples in hospital wards where there was an outbreak of NoV infection and to correlate NoV positive samples to vomiting and diarrhea events.

Air samples were taken in the patient room and in the patient room toilet of NoV symptomatic patients, as well as in the corridor outside the room, using a liquid cyclone (Figure 11a). Both outbreaks (defined as more than one NoV infected patient in a hospital ward) and sporadic cases (defined as a single NoV infected patients in a hospital ward) were included in the study. In addition, air sampling with a cascade impactor and online detection with a laser-induced fluorescence instrument was performed in the hospital ward (Figure 11b). Air samples were analyzed by reverse transcription (RT) qPCR for NoV genomes, and correlated to the patient's recent symptomatic episodes.

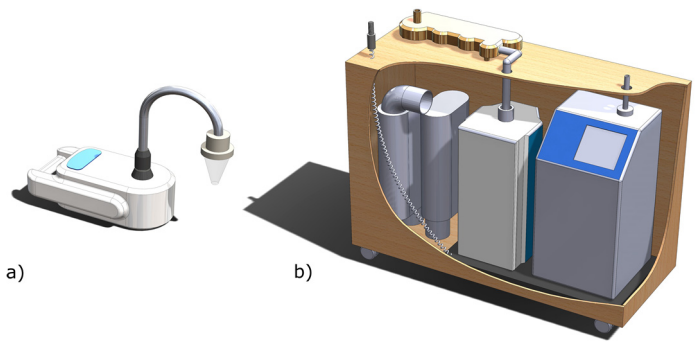


Figure 11: The liquid cyclone and the impactor box.
a) The liquid cyclone that was used to sample air close to patients with norovirus infection. b) The wooden box that was brought to hospital wards with norovirus outbreaks containing a pump connected to the cascade impactor placed on top, and the BioTrak.

Paper III

Bacteria are ubiquitous in both outdoor and indoor air, but only a small fraction are viable and thus prone to cultivate in new environments or cause infections. A comparison was made of the survival of the ice-nucleation active bacterial species *Pseudomonas syringae* (and model for the pathogenic *Pseudomonas aeruginosa*) after aerosolization from a liquid environment, and after surface drying in order to assess how the environmental source (dry or wet) affects bacterial dispersal (Figure 12). In addition, the effects of environmental factors, such as relative humidity (RH) and salt concentration in liquid suspension, on bacterial viability were investigated.

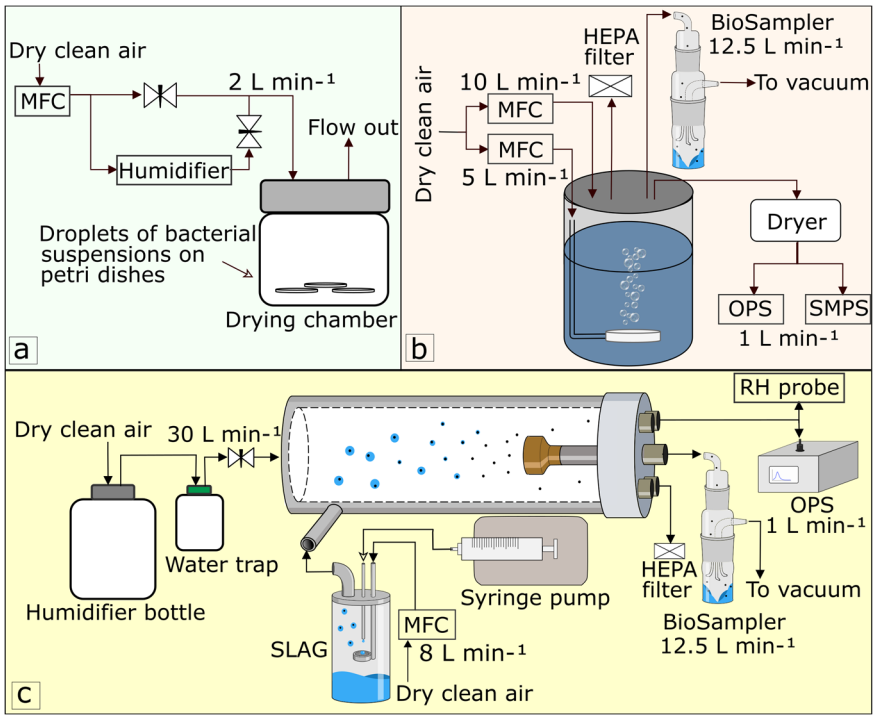


Figure 12: Experimental setups for evaluation of bacteiral viability from dry and wet sources.
a) Drying of bacteria on a dry surface in air with varying relative humidity and in salt solution with varying salinity. b) Drying of bacteria aerosolized by bubble bursting using a bubble tank. c) Drying of bacteria aerosolized by bubble bursting using the SLAG in air with varying relative humidity [88]. MFC: mass flow controller, HEPA: high efficiency partilculate arresting, OPS: optical particle sizer, SMPS: scanning mobility particle sizer, SLAG: sparging liquid aerosol generator.

P. syringae was aerosolized with a sparging liquid aerosol generator (SLAG) into a flow tube where the RH in the air was varied (10, 30, 60 or 90% RH), and the aerosol was then collected into an impinger. A bubble tank was used as another simulation of wet-environment aerosolization. Dry environments were simulated by drying droplets of bacterial suspensions on a surface in air with different RH and in

suspensions with different salt concentrations. Survival was evaluated quantitatively by live/dead fluorescent staining followed by flow cytometry analysis, and qualitatively by transmission electron microscopy imaging.

Paper IV

The presence of airborne NoV in hospitals in Paper III was detected by RT-qPCR, but those results did not indicate if the airborne viruses were infectious. As there is no robust cultivation assay for human NoV *in vitro*, the cultivable (and to human non-pathogenic) murine norovirus (MNV) is frequently used as a model virus to simulate NoV. In this study, we developed an experimental setup for studies on the infectivity of aerosolized MNV (Figure 13). Two aerosol generation principles were evaluated: bubble bursting (using the SLAG), a common natural aerosolization mechanism, and nebulization (using the Atomzier), a common aerosolization technique in laboratory studies. The aerosolization setup was characterized by physical and viral dilution factors, the generated aerosol particle size distributions, and the viral infectivity after aerosolization.

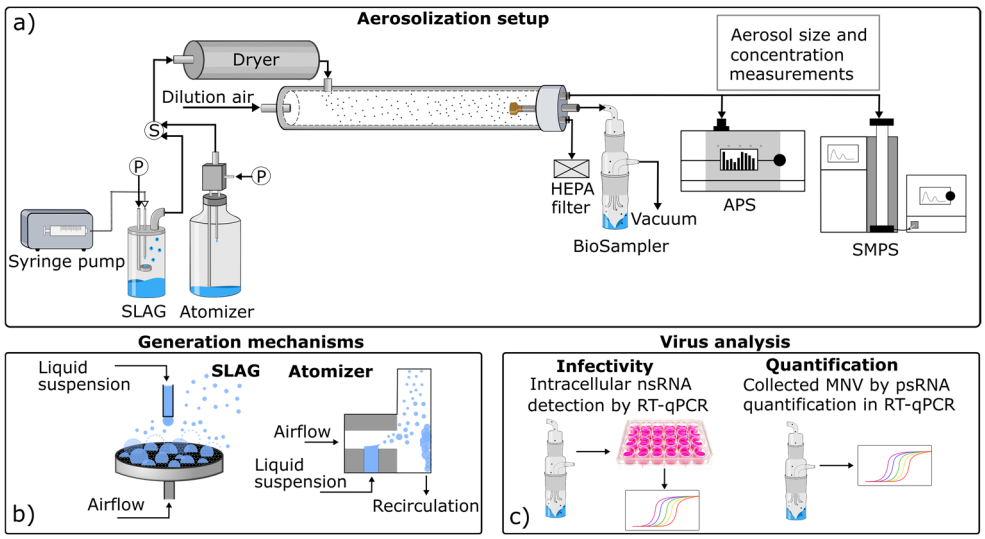


Figure 13: Experimental setup for aerosolization and ananlysis of murine norovirus.
a) The experimental setup for aerosolization of MNV using either the SLAG or the atomizer. b) Illustration of the two aerosolization mechanisms: bubble bursting (SLAG) and atomization (atomizer). c) Analysis workflow: the collection liquid in the BioSampler was used to infect cells, and RT-qPCR was used to confirm infection by detection of negative sense RNA. RT-qPCR targeting the positive sense RNA was used to quantify the amount of MNV in the collection liquid. APS: aerodynamic partilce sizer, nsRNA: negative sense RNA, psRNA: positive sense RNA, RT-qPCR: reverse transcription quantiative polymerase chain reaction.

Paper V

Attendants of The International Aerosol Conference in Saint Louis, Missouri in 2019, initiated a special issue of review articles on the topic of bioaerosols. I was included in a group of specialists in the field invited to contribute to a review article on bioaerosol generation in nature and in laboratory experiments that was managed by Richard J. Thomas at the Defence Science and Technology Laboratory, Porton Down, Salisbury, London.

Paper VI

Bioaerosol collection in exhaled breath can be employed to identify sources of infectious bioaerosols and allow fast isolation of infected patients. A recently developed bioaerosol sampling device was therefore evaluated, with focus on the designs of the liquid collectors inside the sampler (Figure 14). The particle collection efficiency was evaluated by three methods: polydisperse wet droplet collection, polydisperse dry droplet nuclei collection, and monodisperse microsphere particle collection in the size range 0.5-3.0 µm.

A bioaerosol nebulizing generator (BANG) was used to generate dye aerosol droplets, radioactive droplet nuclei particles, and aerosolized fluorescent microsphere particles. Aerosol was collected at stepwise increasing needle voltages, from 0 to -10 kV. The material collected in the liquid was quantified using light absorption, gamma ray spectroscopy, and flow cytometry for the three aerosol types, respectively. For comparison, radioactive and microsphere aerosols were also collected with the BioSampler, and the resulting sample concentrations were compared.

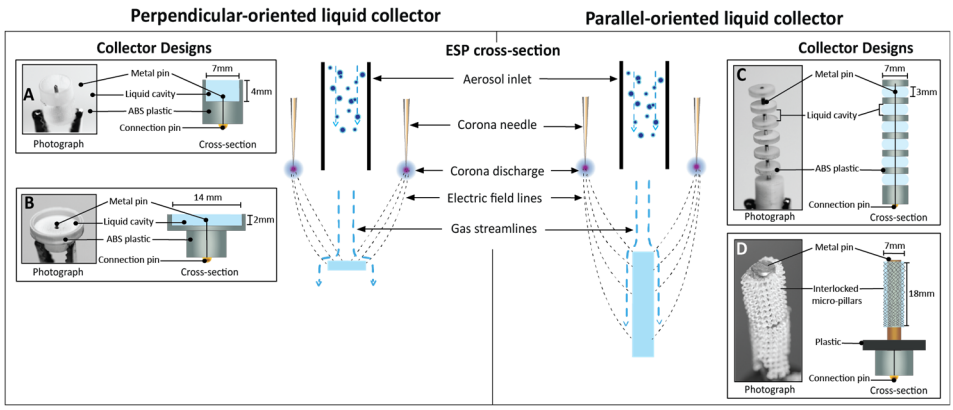


Figure 14: Electrostatic precipitator with four liquid collector designs.
Left) Collectors A and B with the liquid surface oriented perpendicular to the aerosol flow. Right) Collectors C and D with the liquid surface oriented parallel to the aerosol flow.

3.2 Bioaerosol sampling in field studies

Filter sampling in the operating room

In the study reported in Paper I, sampling of airborne bacteria was performed close to the open surgical wound (within 40 cm) using a filter sampler (MD8 airscan, Sartorius GmbH, Germany) operated at an airflow rate of 100 L min^{-1} for 10 min. Gelatine filters were used to prevent the bacteria from drying. The filter was oriented vertically during sampling. After the sampling time, the gelatine filter was placed directly on an agar plate (horse blood agar) where it dissolved. The agar plate was then incubated at 35 °C for 48 h, and analyzed as described in section 3.5.

Slit samplers in the operating room

Slit samplers were used for bacterial sampling by impaction onto agar plates (horse blood agar) in the operating rooms next to the instrument table and in the periphery of the rooms (Paper I). The two slit samplers (Impactor FH5, Klotz GmbH, Germany) operated at an airflow rate of 100 L min^{-1} for 10 min, and had a cutoff diameter (d_{50}) of 3 µm. The impactor plates were oriented horizontally during sampling. After the sampling time, the agar plates were incubated at 35 °C for 48 h and analyzed as described in section 3.5.

Liquid cyclone

A liquid cyclone (Coriolis µ, Bertin Technologies, France), operating at an airflow rate of 200 L min^{-1} for 10 min, was used to sample NoV from air in patient rooms, patient toilets and hospital ward corridors (Paper II). In the collection cone, 15 mL of phosphate buffer saline (PBS) was used as the collection liquid. Typically 1-3 mL of collection liquid evaporated during a 10 min sampling. Since only a small volume was needed for the RNA extraction protocol, the collection liquid was concentrated to 200 µL using centrifugal filter units (Amicon Ultra-15, 50 kDa, Merck Millipore). The Coriolis µ has a cutoff particle diameter of ~1 µm [89].

Cascade impactor

A cascade impactor (Next Generation Impactor, Copley Scientific, U.K.) was used to sample particles into eight size fractions: 0.14-0.34 µm, 0.34-0.55 µm, 0.55-0.94 µm, 0.94-1.7 µm, 1.7-2.8 µm, 2.8-4,5 µm, 4.5-8.0 µm and >8 µm. It was operated at an airflow rate of 60 L min^{-1} for 17-120 h in hospital ward corridors during outbreaks of NoV. After collection, each collection cup was swabbed with a nylon swab (Copan Scientific, U.K.) wetted in universal transport media (UTM), and then the swab was placed in 1 mL UTM and vortexed for 15 s. The samples were analyzed by RT-qPCR.

3.3 Bioaerosol sampling in laboratory studies

Swirling flow impinger

The swirling flow impinger, the so called BioSampler® (SKC Inc., USA), has three impinger heads (unlike conventional impingers that have one) oriented at an angle that makes the collection liquid swirl in the collection jar. The swirling causes the liquid to rise on the sides of the container, flushing over the spots where the impinger heads are directed (*i.e.*, where the impaction takes place). It was operated at an airflow rate of 12.5 L min^{-1} according to the manufacturer using an external vacuum pump and with a liquid collection volume of 20 mL.

The BioSampler was designed to preserve biological particles and to collect sub-micrometer particles more efficiently than earlier impinger designs [62]. Initially in the thesis research, a conventional impinger was used but the collection efficiency was too low for successful analysis by flow cytometry and cultivation; thus the method was changed to use the BioSampler instead. Earlier characterizations of the collection efficiency of the BioSampler show that it is most efficient for micrometer-sized particles and less efficient for sub-micrometer particles [70, 90]. Nevertheless, there is a lack of better techniques for efficient collection of bioaerosols and preservation of viability of the microorganisms [91]. Hence, the BioSampler has become the standard/reference sampler in many laboratory studies on bioaerosols. The BioSampler was used for sampling bacteria (Paper III) and MNV (Paper IV). It was also used as a reference in the collection efficiency evaluation of an electrostatic precipitator (Paper VI).

Electrostatic precipitator

Recently, more attention has been given to the advantages of sampling bioaerosols into liquids using electrostatic precipitation: low pressure-drop in the device allows simpler and less noisy sampling equipment; its simplicity make it portable and useful for point-of-care collection; low collection volume results in high sample concentrations [71]. The electrostatic precipitator that was evaluated in Paper VI, was operated at an airflow of 3 L min^{-1} using an external vacuum, and with a liquid collection volume of ~300 µL. The applied voltage was varied between 0 and -10 kV in increments of -2 kV.

Aerodynamic particle sizer

The number concentration and size distribution of aerosol particles were measured with an aerodynamic particle sizer (APS, Model 3321, TSI Inc., USA) in the Paper IV study. The instrument principle is based on the time of flight (TOF) of a particle. The resulting measure – aerodynamic particle size – is governed by the size and shape of the particle, as well as its density. The APS measures particles in the size range 0.5-20 µm; however, as the accuracy is low for the smallest particles [92],

only data from 0.8-20 µm were included in Paper IV. The APS was also used in the Paper VI study to measure the concentration of microsphere particles in the air upstream the ESP collector.

Scanning mobility particle sizer

A scanning mobility particle sizer (SMPS, consisting of a DMA, model 3080, TSI Inc. and a CPC, model 3775, TSI Inc.) system was used for measuring particle number size distributions in the size range 15-500 nm in the Paper IV study. The SMPS consists of a differential mobility analyzer (DMA) and a condensational particle counter (CPC). The polydisperse aerosol goes into the DMA where it is charged and particles of the selected electrical mobility (*i.e.*, size) exit as a monodisperse aerosol that goes into the CPC. In the CPC, liquid condenses on the small particles, increasing their size to what is detectable with light optics, and finally, counted.

Optical particle sizer

An optical particle sizer (OPS, model 3330, TSI Inc., USA) was used for measuring particle size and concentration in the Paper III study on aerosolized bacteria. The OPS detects scattered light from aerosol particles to classify their particle size into 16 size bins in the size range 0.3-10 µm.

3.4 Choices of bioaerosol sampling methodologies

The sampling methodologies used in the experimental studies are summarized in Table 1 for the comparison of collection characteristics and their advantages and disadvantages.

Table 1. Bioaerosol samplers used in laboratory experiments and field sampling.
The bioaerosol samplers used in the thesis research are summarized in the table to provide an overview of the choices of methodology in the different settings and the advantages and disadvantages of each of them.

Aerosol source	Bioaerosol sampler	Particle collection size range (µm)	Sampler airflow (L min^{-1})	Collection media	Advantages	Disadvantages	Sample analysis	Paper no.
Bacteria in operating rooms	Filter sampler (Sartorius Airscan)	All	100	Gelatine filter	High physical sampling efficiency	Drying on filter during sampling	CFU counts	I
Bacteria in operating rooms	Slit sampler impactor (Klotz)	>3	100	Horseblood agar plate	Deposition directly on nutrition media	High cutoff particle size	CFU counts	I
Bacteria in operating room	BioTrak fluorescence detection	1.0 – 25	28.3	-	Rapid detection in real time	Highly unspecific of what is "viable"	Optical: single particle fluorescence	I
Human norovirus in hospital wards during outbreaks	Liquid cyclone (Coriolis µ)	>1	200-300	15 mL PBS	High sampler airflow. Small and convenient for field sampling	Short sampling time due to liquid evaporation	RT-qPCR targeting the viral genome	II
Human norovirus in hospital wards during outbreaks	Cascade impactor (Next generation impactor)	0.14 – 20	60	Metal collection cups	Collects in 8 size fractions and low particle cutoff size	Requires a powerful and loud pump	Swabbing collection cups + RT-qPCR	II
P. syringae generated by SLAG and bubble tank	BioSampler impinger	>0.5	12.5	20 mL PBS	High biological collection efficiency	Bacteria stick to collection cup wall at impingement point	Live/dead staining + flow cytometry	III
Murine norovirus generated by SLAG and atomizer	BioSampler impinger	>0.5	12.5	20 mL PBS	High biological collection efficiency	Low collection efficiency of sub-micrometer particles	Quantification by psRNA RT-qPCR and infectivity by cell culture infections + nsRNA RT-qPCR	IV
Fluorescent microspheres generated by BANG	Electrostatic precipitator	>0.5	3	0.3 mL PBS	Low liquid collection volume and conventient for field sampling	Not commercialized and low sampler airflow	Flow cytometry	VI

3.5 Bioaerosol detection techniques

Colony forming units

Counting colony forming units (CFU) was the major analysis method in the Paper I study. Sampled agar plates were incubated at 35 °C for 48 h and then the number of bacterial colonies were counted (CFU) and classified into major bacterial genera, mainly: *staphylococcus, micrococcus*, and *bacillus*. In the Paper III study, CFU counts were used as a qualitative measure to verify the results obtained by live and dead staining and flow cytometry analysis. In this case, the bacteria collected into liquid were plated on agar plates in serial dilutions and triplicates, incubated at room temperature for 2-3 days and then counted.

Real-time bioaerosol detection by laser-induced fluorescence

An instrument based on laser-induced fluorescence (LIF) detection (BioTrak Model 9300, TSI Inc., USA) was employed for measuring aerosol concentrations in clean environments with low aerosol concentrations. The BioTrak has a higher airflow rate (28.3 L min^{-1}) than other LIF instruments (usually 1 L min^{-1}). It consists of: 1) a conventional optical particle sizer (OPS) that detects all aerosol particles due to them scattering light, 2) a virtual impactor that concentrates the aerosol flow, and 3) a fluorescence spectrometer, where light λ=405 nm is used to excite auto-fluorescent molecules. It has two fluorescent light detectors for the wavelength intervals 405-500 nm and 500-650 nm, respectively. The instrument uses an algorithm that, based on the fluorescence signals, determines if a particle is "viable" or not. All particles are classified into six size channels: 0.5-0.7 µm, 0.7-1.0 µm, 1.0-3.0 µm, 3.0-5.0 µm, 5.0-10 µm, 10-25 µm.

The BioTrak was used to measure the bioaerosol concentration in real time in the operating rooms in the Paper I study. Data from each 10 min of measurements were compared with the corresponding CFU counts measured on slit sampler agar plates. The BioTrak was also used in the Paper II study for measurements of bioaerosols in hospital ward corridors during NoV outbreaks (but not included in the article), in a field campaign on a (Danish) military patrol ship going north along the west coast of Greenland, and in a short study on sawdust generated in autopsies.

Flow cytometry with fluorescent staining

Fluorescent staining of live and dead bacteria was carried out using the BacLight™ kit (Life technologies, Thermo Fisher). The live stain SYTO9 is a small molecule that can diffuse across cell membranes and hence stains the nucleic acid molecules of all cells. SYTO9 fluoresces in green light (λ=530 nm). The dead stain propidium iodide (PI) is a larger and charged molecule that cannot diffuse across an intact membrane, and thus, stains the nucleic acid molecules only of cells with impaired

cell membranes (*i.e.*, dead cells). PI fluoresces in red light (λ=614 nm). The analysis by counting the stained cells was performed with a NovoCyte ACEA (Biosciences Inc., LOD: 0.2–50 µm cell size) flow cytometer with a λ=488 nm laser for excitation.

Virus infectivity in cells

As viruses cannot grow outside cells, the level of infectivity is analyzed by the concentration at which they infect and kill cells in cell cultures. In the Paper IV study, RAW 264.7 (mice macrophage cells) cell cultures grown in Dulbecco's minimum essential medium (DMEM, no pyruvate; catalogue no. FG 0435, Biochrom), supplemented with 10% low endotoxin fetal bovine serum (FBS, Nordic Biolabs), 1% non-essential amino acids (Life Technologies) and 5% penicillin/streptomycin (Life Technologies) were infected with MNV. Cell cultures were inoculated with MNV from the starting solution and the collection liquid after aerosolization in 10-fold dilution series. The 50% tissue culture infection dose ($TCID_{50}$) was determined either by observation of cytopathic effect (CPE) or by verification by detection of negative sense RNA (nsRNA) in cell lysates. The nsRNA is the complementary strain to the viral genome (positive sense RNA [psRNA]) and is only present during replication of new viruses; hence, it can be used as a proof of infection.

PCR and RT-qPCR in MNV experiments

Polymerase chain reaction (PCR) is a technique for amplification of DNA in cycles, doubling the number of amplicons per cycle. Quantification is done by detection of fluorescence from molecules that only fluoresce when attached to the DNA, and comparison with fluorescence from a sample of known nucleic acid quantity. When the target nucleic acid is an RNA strain, it is first transcribed to DNA by reverse transcription (RT) before quantification by qPCR.

In the Paper IV study, strand specific RT-qPCRs for psRNA and nsRNA detection were performed, as described by Vashist et al. [93]. The psRNA (the viral genome) was detected for quantification of MNV in the collected aerosol samples. Intracellular nsRNA was detected for verification of ongoing replication in the MNV infected cell cultures. RNA extractions from cells and from supernatant were done with the RNeasy mini kit (Qiagen, Germany) and the QIAamp viral RNA mini kit (Qiagen, Germany), respectively. Extracted RNA was treated with DNase (Thermo Scientific) for 30 min at 37 °C, according to the protocol of the manufacturer. Extracted RNA samples were stored at -80 °C until analyzed. Strand specific reverse transcription of RNA to cDNA was done using tagged primers and SuperScript IV in a thermal cycler (Applied Biosystems 2720) according to previously developed methodology [93]. qPCR was performed with a StepOnePlus Real-Time PCR system (Applied Biosystems) on sample reactions prepared using the SYBRgreen select master mix (Thermo Scientific).

Detection of human norovirus RNA in clinical samples

The samples collected by the liquid cyclone and the cascade impactor in the Paper II study were analyzed by RT-qPCR with a primer targeting human norovirus (NoV) genogroup I and II (as describe in Kageyama et al. [94]) at the Department of Clinical Microbiology, Lund University. In addition, positive samples were genotyped by semi-nested sequencing of the NoV GII ORF1/ORF2 junction [95].

Radioactive aerosol measurements

^{99m}Technetium is used in routine diagnostics of lung disease by scintigraphy. ^{99m}Tc has a half-life time of 6 h and emits gamma radiation with a photon energy of 140 keV. In the Paper IV study, ^{99m}Tc was added to the MNV solution that was aerosolized, in order to determine the physical dilution factor of the setup (concentration in collection liquid/starting solution) with high precision. The radioactive samples were analyzed by gamma-ray spectroscopy for 90 s each using a sodium iodine well count detector (1480 Wizard, Perkin Elmer).

3.6 Laboratory bioaerosol generation

Laboratory studies on bioaerosols are useful for carrying out controlled studies and evaluations of one parameter at a time since bioaerosols in the environment are complex to study. A detailed description of bioaerosol generation from natural sources and from experimental generation using laboratory techniques can be found in Paper V. There are several different aerosolization techniques, and the one that is chosen depends on the biological agent to be aerosolized and the natural aerosol source that is to be simulated. Most aerosol generators use pressurized air to spray a liquid containing the biological agent (Figure 15). Two of those techniques were used in the thesis research: bubble bursting and twin flow nebulization. There are several other nebulizers, of which many have been developed for the pharmaceutical industry and therefore produce higher numbers of micrometer-sized droplets.

Constant output atomizer

One of the most common nebulization techniques, atomization, relies on the Venturi effect and operates by a pressurized airflow that shears off droplets from the top of a liquid column (Figure 15, twin flow nebulization). The largest droplets impact into the wall opposite the inflowing air, while the small droplets follow the air stream up and out where the droplets evaporate to droplet nuclei. The constant output atomizer (model 3076, TSI Inc., USA) generates a polydisperse aerosol with the majority of droplet nuclei particles <200 nm in diameter. Atomization imposes high shear forces on the liquid, which presumably can disrupt bacterial membranes [96, 97]. The constant output atomizer is common and its performance is well validated in aerosol science, and was therefore used in the Paper IV study.

Bioaerosol nebulizing generator

In the Paper VI study, a bioaerosol nebulizing generator (BANG) was used. It is based on the same aerosolization principle as the constant output atomizer, and is said to minimize foaming in order to allow aerosolization from proteinaceous liquids [98].

Sparging liquid aerosol generator

The sparging liquid aerosol generator (SLAG, CH Technologies, USA) mimics the natural process of bubble bursting that occurs in water reservoirs, for instance, in the sea and when flushing a toilet (Figure 15, bubble bursting). The suspension to be aerosolized (containing the biological agent) is dropped onto a porous stainless steel sintered plate. At the same time, pressurized air is blown through the plate from below, bubbling through the suspension, thus generating droplets from bubble bursting. Large droplets drop down in the collection jar, while the smaller ones exit with the airflow.

The SLAG was chosen for studying bacterial cell survival from different aerosol sources in the environment (Paper III). There were two main reasons for that: it simulates bubble bursting but still generates a particle number concentration that is comparable to other nebulizers; and it has been shown to be less damaging to cells, due to lower shear forces than nebulizers working on pressurized air [96, 99]. The SLAG has so far been used in relatively few studies (and only one on virus), and was therefore compared with the more well characterized constant output atomizer in an experimental setup for aerosolization of MNV in the study presented in Paper IV.

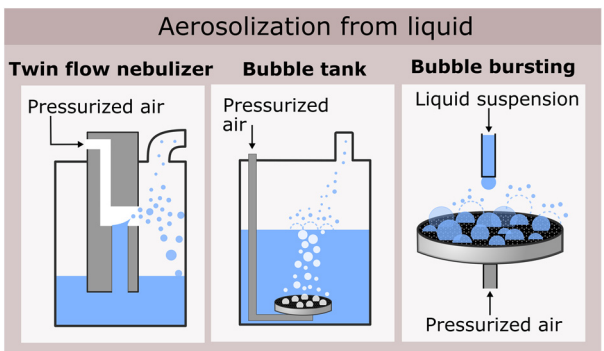


Figure 15. Aerosolization techniques from liquid suspensions.
Schematic illustration of aerosolization techniques for laboratory experiments: twin flow nebulization, bubble tank generation, and bubble bursting. In this thesis, twin flow nebulization was used for aerosol generation in Paper IV (atomizer) and Paper VI (BANG); bubble tank generation was used in Paper III; and bubble bursting with the SLAG was used in Papers III and IV. Modified from [38].

3.7 Bioaerosol transmission prevention techniques

Since aerosol particles are too small to be seen by the human eye, it is difficult to perceive when concentrations are high. Instead, we need to learn what procedures may generate infectious aerosols and how to minimize the spread from these. Minimizing the spread can be done by either technical measures or management and hygiene routine measures, targeting 1) the aerosol source, 2) the air, or 3) the susceptible patient. Measures targeting the aerosol source are mainly containment, for example, to close the toilet lid when flushing. Technical measures that target the air are ventilation, air filtration, disinfection by hydrogen peroxide vapor or disinfection by UV light. Management and hygiene routine measures include isolation of infected patients (preferably in single rooms), dividing staff (working with infected or non-infected), and positioning in relation to the ventilation airflow.

The main control measures preventing bioaerosols from spreading disease in hospitals are air filtration and ventilation. In infection departments, patient rooms often have high air exchange rates (AER, ~10 h^{-1}) [100] in comparison to other hospital departments (AER~2 h^{-1}, in residential homes ~0.5 AER h^{-1}). In addition, these patient rooms often have negative pressure (and anterooms) to avoid air from exiting into the corridor. In oncology and operation wards, patient rooms have positive pressure (and often anterooms) to avoid the corridor air from entering, since patients are extra sensitive to infections. In operating rooms, ultra clean air is required for sensitive surgeries, meaning bacterial concentrations <10 CFU m^3 [67]. Three types of ventilation systems for operating rooms were evaluated in the study presented in Paper I, and the airflow velocity pattern for each of them can be seen in Figure 16.

Turbulent mixing airflow ventilation

One of the two most common types of ventilation techniques in operating rooms is turbulent mixing airflow (TMA). TMA is based on the dilution principle: a high airflow rate introduces high efficiency particulate arresting (HEPA) filtered air that dilutes any existing concentration of airborne contamination. The TMA ventilation in our study (Paper I) had an airflow rate of 3200 m^3 h^{-1}. The ideal TMA ventilation would generate perfect mixing in the whole room; however, there is a risk that only parts of the room air is mixed and exchanged.

Laminar airflow ventilation

The other of the most common ventilation techniques is laminar airflow ventilation (LAF). In LAF ventilation, air is introduced from HEPA filters just above the operating table and directed downward at a vertical speed of 0.3-0.4 m s^{-1}. The LAF ventilation in this study operated at an airflow rate of 12 000 m^3 h^{-1}. The clean airflow directed towards the open wound often results in low bacterial concentrations at the operating table. However, the cleanliness in the other parts of

the room where there are no air inlets (*e.g.*, the instrument table) is a possible reason why no advantage of using LAF compared to TMA has been reported in large registry studies of SSI [57].

Temperature-controlled airflow ventilation

Another type of ventilation, based on temperature-controlled airflow (T_CAF), has sometimes been used in combination with other techniques or by itself. One type of T_CAF was investigated in the Paper I study, and compared to the more conventional LAF and TMA ventilations. In this version of T_CAF, air that is 1.5 °C cooler than the room air is introduced from eight air inlets above the operating table. The cooler air falls downwards due to its higher density than the surrounding air. A stable temperature gradient is maintained by having eight additional air diffusers in the room surroundings where warmer air is introduced. An advantage of this system is that clean air is dispersed both in the center and the surroundings of the room. The T_CAF ventilation in this study operated at an airflow rate of 5 600 m^3 h^{-1}.

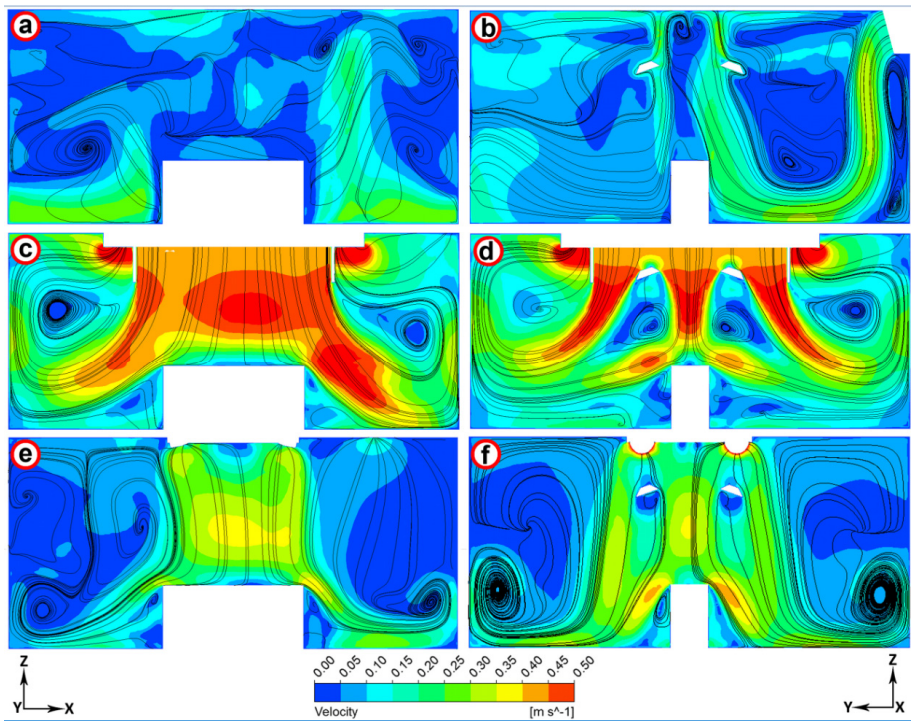


Figure 16: Airflow velocity simulation in OR ventilation using computational fluid dynamics
Cross-sectional figure of the operating room along the long side of the operating table (white) in a) ,c) and e), and cross-sectional along the short side of the operating table in b), d) and f). High airflow velocity (0.5 m/s) is represented by red, medium airflow velocity is represented by green and yellow (0.3-0.4), and blue represents low airflow velocity (<0.2 m/s). a-b) turbulent mixed airflow (TMA), c-d) laminar airflow (LAF), and e-f) temperature-controlled airflow (T_CAF). Reprinted from [87].

3.8 Statistics and analysis

The statistical tests used in this thesis research were mainly non-parametrical tests. In the Paper I study, the sign test was used to compare median values of bacterial concentrations with a specific value. Spearman's rho was used to evaluate correlations between bacterial concentrations and the factors suspected to give rise to increased concentrations. The Mann-Whitney-U test evaluated differences between concentrations of bacteria in the three operating rooms and at different sampling points. In the Paper III study, the Mann-Whitney U test was used to evaluate differences between the viability of bacterial cells dried under various condition, and Spearman's rho to evaluate correlations with varying RH in the air and ionic strength in the solution. Statistical tests were performed in SPSS Version 23-26. In the Paper II study, STATA was used to perform the chi-square test and Fisher's exact test for assessing crude differences between groups of patients and air samples. In addition, a random effects model was used to account for that some air samples were collected close to one patient (clustered analysis) in the regression analysis used to calculate odds ratios of finding positive air samples. In all studies, mean value and standard deviation (based on at least triplicate samples) were used to describe the data.

3.9 Ethical considerations

For the Paper I study, no ethical approval was needed, since the study did not involve sensitive personal data, nor biological patient samples or any physical or mental impact on patients.

The Regional Ethical Review board in Lund approved connecting personal patient data to air samples in the Paper II study (Dnr. 2015/51 and 2016/961). Informed consent was not necessary, but if patients found the air sampling disturbing, they had the right to demand it to stop.

I took part in the work to implement a biosafety laboratory class 2 in our aerosol laboratory, in order to perform the laboratory studies included in this thesis in a safe manner.

4 Results and discussion

Both airborne bacteria and viruses were detected in the research presented in this thesis. As described in Paper I, we measured airborne bacteria in operating rooms with the median concentrations in the range 0-22 CFU m^{-3}, but we found no correlation with potential sources or contributing factors. The Paper II study shows that the norovirus (NoV) RNA can be present in the air in hospital wards, and that the NoV positive samples correlated with short time after vomiting during outbreaks, suggesting that vomiting was the source. The aerosol particle size fractions that contained NoV were >4.5 µm and 0.14-0.94 µm. The latter size range represents small particles that are likely to remain airborne for long times and can thus, spread to other rooms in hospital environments. Paper III and IV describe laboratory studies on model organisms. In Paper III, we concluded that *P. syringae* viability was high when dried rapidly, that is, dried in small droplets at low RH and at high salinity. The Paper IV study investigated aerosolized murine norovirus (MNV), and aerosol droplet drying in a low solute solution was suggested to be the main factor leading to loss of infectivity, though variations in RH were not investigated. Paper V reviews bioaerosol generation processes in nature and in the laboratory.

Ventilation is an important strategy for preventing transmission of infectious bioaerosols in hospitals. We found that the type of ventilation technique that is used in operating rooms was important for maintaining low bioaerosol concentrations at critical locations (Paper I). To enable more convenient methods for air sample collection in for example hospitals and indoor environments, we evaluated the particle collection efficiency of a novel bioaerosol sampler that can for example be used for collection of particles in the exhaled breath of infected patients (Paper IV).

4.1 Sources of infectious bioaerosols in the field

Sources of airborne bacteria in operating rooms were investigated in the Paper I study, and sources of airborne NoV were evaluated in Paper II. In both studies, we measured concentrations of airborne microorganisms and investigated correlations with potential aerosolization or source events.

4.1.1 Airborne bacteria in operating rooms

Air sampling in operating rooms during ongoing infection sensitive surgeries was carried out to evaluate the air cleanliness achieved by the three ventilation techniques: TMA, LAF and T_CAF (Figure 10). In total, we collected 750 air samples during 45 surgeries (15 in each operating room) at three sampling points: close to the wound, at the instrument table, and in the periphery of the room. In addition, we recorded the number of people in the room and the number of door openings.

Median airborne concentrations of bacteria were in the range 0-22 CFU m^{-3} depending on sampling point and ventilation type. As the incoming ventilation air is HEPA filtered, the bacteria detected in the room air were generated inside the room or entered from door openings. In our study, no significant correlations were found between the CFU concentrations and the number of people in the room or the number of door openings. A previous study, like ours, did not find a significant effect on airborne bacteria from the number of people in the room, and argued that the activity level of the personnel would be a stronger indicator [101]. However, it was clear that the incoming air from above the operating table in LAF and T_CAF kept the air close to the wound almost free from bacteria with median concentrations of 0 and 1 CFU m^{-3}, respectively.

Although no significant correlation with door openings was found in this study, it is still thought to be one of the main sources of airborne bacteria in operating rooms as shown in several earlier studies [101-105]. The insignificant correlation in our study can be explained by a low number of door openings to the corridor (median 3, range 0-11) and relatively clean air in the corridor outside the operating room with a median of 40 (range 18-66) CFU m^{-3}. There were also door openings to anterooms; however, these were well ventilated and therefore not included in the correlation analysis.

Though more people in the room generally would generate more airborne bacteria, the amount of particle shedding depends on individual factors, for example, hygiene routines and the activity level. Thus, people present with low activity, such as students standing by the wall for observation, are likely to contribute negligible amounts of bacteria that can reach the surgical wound. On the contrary, the skin condition of the surgeon, being close and with a higher activity level, could potentially be of more concern. Keeping a low number of people in the room is nevertheless favorable, to avoid disruption of the ventilation airflow pattern [106].

4.1.2 Airborne norovirus in hospital wards during outbreaks

Noroviruses are known to spread and cause outbreaks, especially in hospitals, and in some cases airborne transmission has been suspected to occur. Therefore, we collected air samples in patient rooms, patient toilets and in corridors (Paper II) as soon as possible after identification of suspected NoV cases or when patient samples

were confirmed NoV positive. In total, 86 air samples were collected with a liquid cyclone in hospital wards with NoV infected patients, and 21 (24%) contained detectable amounts of NoV RNA. All positive air samples were collected during ongoing outbreaks or preceding an outbreak; all air samples collected around sporadic (single) cases (n=34) tested negative for NoV.

By asking the patients and the staff, and collecting information from the patients' journals, we documented the times when patients had episodes of vomiting and diarrhea. We found the highest percentage of NoV positive air samples within 3 h from the last vomiting episode (64%, Figure 17), and analogously, an odds ratio of 8.1 (p=0.04) of finding a positive air sample within 3 h after the last vomiting episode compared to no vomiting the last 3 h (controlled for diarrhea within 3 h). The corresponding odds ratio for finding a positive air sample within 3 h since the last diarrhea episode was 2.2 and not significant (p=0.4).

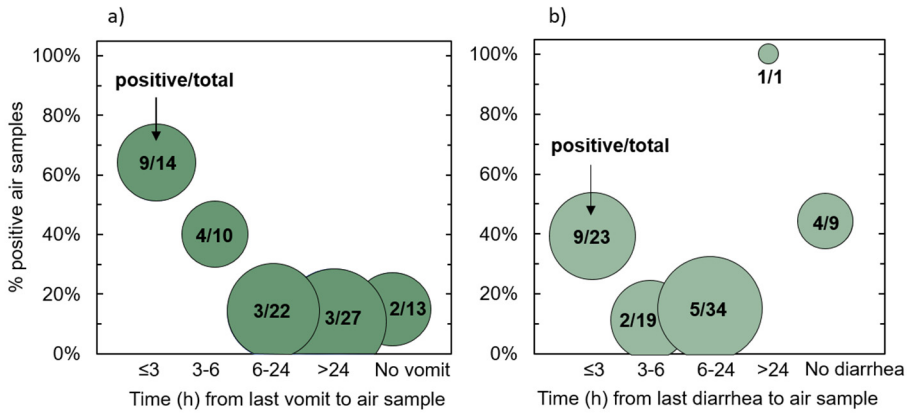


Figure 17. Percentage of NoV positive air samples in hopsital wards.
Percentage of NoV positive air samples after 3, 3-6. 6-24 and >24 h since a) last vomiting episode, and b) last diarrhea episode. The circle sizes are proportional to the number of samples. Modified from [107].

In previous epidemiological studies of NoV outbreaks, being present during a vomiting episode was linked to higher risk of infection [51, 108, 109]. The occurrence of vomiting from NoV patients in hospital wards has also been correlated with more outbreaks [110]. Vomiting has the potential to generate aerosol droplets because high forces are involved when the gastric content is expelled [111]. The emesis can be both large in volume (650-850 mL) and high in virus titers (about 5-6 $\log_{10}$ RNA copies mL^{-1}) [112]. Hence, an extensive viral shedding is plausible. There is unfortunately no study that has characterized the aerosol generated from vomiting [113]; however, infectious MS2 bacteriophages (a virus that infects *Escherichia Coli*) have been collected from air in a vomiting simulation study [111].

Many NoV infections start promptly with vomiting, which is later followed by abdominal cramps, nausea, fever and diarrhea [114]. The rapid symptom onset is a probable reason for extensive environmental contamination, especially for hospitalized elderly patients that are not able to go to the toilet themselves. The dominating NoV genotype since the mid-1990s, especially in nosocomial outbreaks, has been the GII.4 [114-116]. Genogroup GII infections have been reported to generate higher viral loads [117] and higher frequencies of vomiting in elder people than those from the GI genogroup [118]. We detected the NoV genotype GII.4 in clinical samples from 20 of 26 patients included in the Paper II study, and the two air samples that were high tittered enough to allow successful sequencing were also of the genotype GII.4. The incidence of GII.4 infections has been shown to have a stronger seasonal pattern in than GI and other GII genotypes [116]. Although the occurrence of NoV outbreaks has decreased in recent years – probably due to attained immunity in society and unsuccessful viral mutations – there are lessons to be learned that may decrease the future spread of similar viruses.

The high viral titers in feces (9.1 $\log_{10}$ RNA copies per g stool [119]) from NoV infectious patients are also a high risk for airborne transmission if aerosolized. A single toilet flush can generate 10^4-10^5 aerosol droplets (depending on flush mechanism) [6]; however, coverage with toilet paper and containment by closing the toilet lid may decrease the dispersal. Nevertheless, in the case of SARS-CoV-1, aerosolized feces inside the sewage system were put forward as the most likely explanation for the infection of 320 cases in an outbreak at an apartment complex in Hong Kong in 2003 [120]. Diarrhea was a common symptom of SARS-CoV-1, and the viral titers were higher in feces (7.0 $\log_{10}$ mL^{-1}) than in respiratory emissions (5.8 $\log_{10}$ mL^{-1}) [121].

4.1.3 Bioaerosol concentration in hospital wards during norovirus outbreaks

A laser-induced fluorescence (LIF) instrument was used to monitor the bioaerosol concentrations in hospital wards during six NoV outbreaks studied in Paper II. The measured concentrations of viable particles did not correlate significantly with the total particle concentrations during any of the investigated outbreaks. Instead, there was a significantly higher level of viable particles during daytime (07:00-19:00) than nighttime (19:00-07:00) (Student's t-test, $p<0.05$), suggesting daily activities as a strong contributor to the number of bioaerosols indoors. An interesting observation was frequent high peaks of viable particle concentrations at the department of gastrointestinal diseases (Figure 18a). The department of geriatric disease was located in the same building, with a comparable ward design (and the measurements were performed directly one after the other), but there, viable particle concentrations were generally low and with few high peaks (Figure 18b). As the

LIF instrument primarily detects bacteria (and fungal spores), this dataset suggests that symptoms of gastrointestinal diseases, such as vomiting and diarrhea, can generate high concentrations of airborne bacteria. Previous studies have, for example, found the *Clostridium difficile* bacteria in air samples collected in wards with infected patients [122]. *C difficile* most commonly spreads within hospitals and to patients with disrupted intestinal flora due to antibiotic treatment [123]. The bacterial spores can remain viable for months in the environment [124] and are therefore likely able to spread directly or indirectly (deposition on surfaces) via air. Toilets are known to spread bioaerosols and fomites in a bathroom [125] (which is one reason people put their toothbrushes in a cupboard); thus, there is reason for extra precaution when there are patients with diarrhea caused by infectious agents.

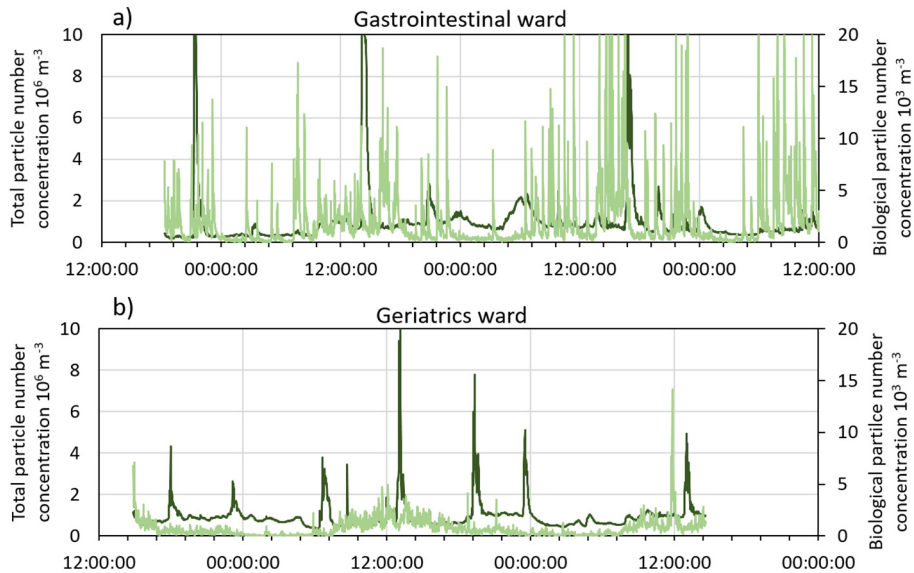


Figure 18. Viable and total particle concentrations in hospital wards during NoV outbreaks.
Total particle concentrations (dark green, left axis) and viable particle concentrations (light green, right axis) in the size range 0.5-25 µm, measured by optical scattering and laser-induced fluorescence, respectively, at a ward of: a) gastrointestinal diseases, and b) geriatric diseases. Measurements were performed in hospital ward corridors during norovirus outbreaks.

4.2 Bioaerosol generation and airborne transport in laboratory experiments

Many infectious bioaerosols are emitted through liquid aerosolization, creating aerosol droplets that evaporate to droplet nuclei in ambient air. The evaporation rate for aerosol droplets ≤ 20 µm in air with relative humidity <60% is within one

second, and for RH <90% within four seconds (Figure 19a); hence, a very rapid process. Drying of airborne droplets dramatically changes the particles' physicochemical composition by, for example, increased solute concentrations and altered pH. This may affect the bacteria or viruses in the droplet. Evaporation of water changes the droplet size, and thereby, how long it can remain airborne (Figure 19b). The effect of different drying rates (by varying the droplet size, RH in air and liquid solute concentration) on bacterial viability was investigated in the Paper III study. Aerosolization of MNV using two bioaerosol generators was performed in the Paper IV study and compared with results in the literature.

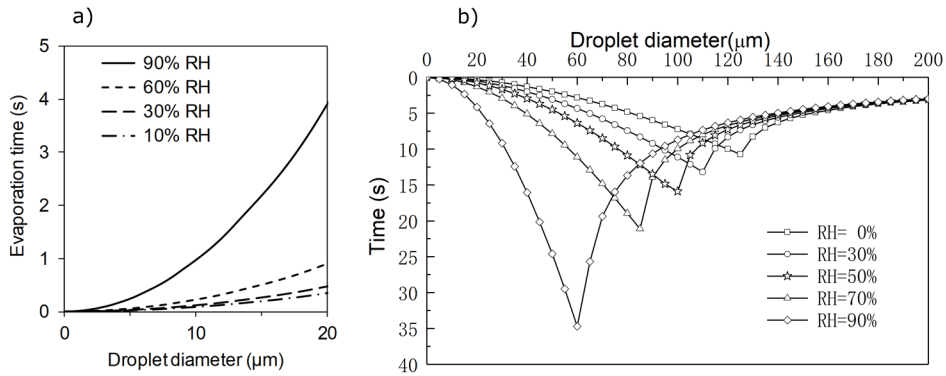


Figure 19. Calculated evaporation times for droplets.
a) Evaporation time for aerosol droplets with diameters in the range 0-20 μm at different levels of relative humidity (at room temperature) [88]. b) Prediction model by Xie et al. [49] of the droplet evaporation time (left curve) and the droplet sedimentation time from a height of 2 m (right curve) in air with varying relative humidity. The tip where the left and right curves meet represents the maximum droplet size that can evaporate to a droplet nuclei before it has sedimented to the ground.

4.2.1 Comparing bioaerosol generators

Bubble bursting is a natural aerosolization process that plays an important role for the generation of both atmospheric bioaerosols outdoors and infectious bioaerosols in indoor environments. The viability of bacteria aerosolized through bubble bursting using the SLAG and the bubble tank, respectively, were investigated in the Paper III study. Although the concentration of aerosolized bacteria in the SLAG setup was about 50 times higher than in the bubble tank setup, the aerosol particle size distributions were similar. We found no significant difference in bacterial viability, which was expected since the aerosolization mechanism was the same. Other studies have shown that bubble bursting is less damaging to bacteria than the more commonly used twin fluid nebulization technique, since the latter often recirculates the liquid and the bacteria are hence, repeatedly exposed to shear forces [97, 99, 126].

A comparison of these two aerosolization mechanisms was presented in Paper IV: twin fluid nebulization by a constant output atomizer, and bubble bursting by the SLAG, using MNV as the model organism. We found no significant difference in MNV infectivity due to the different aerosolization processes. Previous studies on the infectivity of aerosolized viruses also observed little or no effect from the aerosol generation process [127-129]. This can be explained by the small size of viruses compared to the large size of the generated droplets, resulting in a limited effect from the shear forces.

Nonetheless, an interesting observation was found in our comparison of the two bioaerosol generators as we looked into the physical and viral dilution factors for each of them. The physical and viral dilution factors were determined by the concentration ratios of radioactive tracers (physical dilution) and MNV genomes (viral dilution), respectively, in the start solution compared to the collection liquid:

$$Dilution\ ratio = \frac{C_{start\ solution}}{C_{collection\ liquid}}.$$

For the atomizer aerosol, the physical dilution ratio was three times lower than for the SLAG (Figure 20). Thus, more aerosol mass was collected when generated by the atomizer. However, the viral dilution factor was the same for both generators, indicating higher virus transfer efficiency in the experimental setup when using the SLAG. A possible explanation for this could be that MNV were distributed differently in the aerosol particles than were the radioactive tracers.

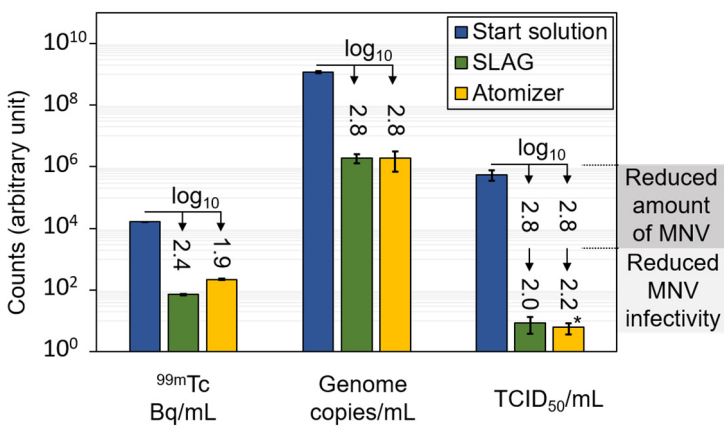


Figure 20. Physical and viral dilution factors of MNV aerosol.
Concentrations of radioactive tracers, MNV genomes and MNV infectivity ($TCID_{50}$) in the starting solution (blue bars) and the collected samples from aerosol generated by the SLAG (green bars) and the atomizer (yellow bars), respectively. The bars represent the mean of triplicate experiments and the error bars represent one standard deviation. *mean and standard deviation only based on duplicate samples.

One previous study investigated the distribution of bacteriophages in aerosol particles generated by twin-fluid nebulization (Collison nebulizer, Model CN25, BGI Inc.) and found a homogenous distribution [130]. Therefore, a heterogeneous virus distribution in aerosol could more likely have been generated by the SLAG. The physico-chemistry of the bubble bursting process was only recently evaluated in detail [34, 35], and needs to be further investigated to confirm or reject our hypothesis.

4.2.2 Drying of bacteria in air and on surfaces

Bacteria live in liquids or as colonies on surfaces, and they can become airborne by wet or dry aerosolization, respectively. In the Paper III study, we investigated the viability of the environmental bacteria *Pseudomonas syringae* after drying in microliter-volume droplets on surfaces and in femtoliter-volume droplets in the aerosol phase. In addition, the relative humidity of the drying air and the salinity of the bacterial solution were varied. We found that the viability of *P. syringae* was two orders of magnitude higher when dried in aerosol droplets than in surface droplets ($p<0.001$), regardless of relative humidity (Figure 21a). Since the surface dried bacteria were not aerosolized after drying, our results from surface drying represent the upper limit of viable bacteria of those that are aerosolized from a dry surface, as potentially destructive mechanical aerosolization forces were not applied. From studies on atmospheric bacteria, researchers have reported higher bacterial viability in air masses with aerosol originating from wet environments than in air with aerosol from dry environments [24], which supports our results.

In the same study (Paper III), we found a significant inverse correlation between bacterial viability and relative humidity after both drying in aerosol and in surface droplets (Spearman's rho= -0.56, $p<0.01$ for aerosolization and rho= -0.73, $p<0.01$ for surface drying) (Figure 21b-c). In addition, drying in 1 μL droplets on surfaces resulted in higher viability than in larger, 5-75 μm droplets ($p=0.004$). Taken together, we concluded that rapid drying in small droplets and low RH was less damaging than slow drying in large droplets and high RH.

Moreover, we evaluated the viability of *P. syringae* dried in surface droplets from liquids of varying salt compositions (NaCl and sea salt) and ionic strength: from 0 to 0.7 M. We saw a significant correlation between viability and increasing ionic strength (Spearman's rho=0.87, $p<0.001$). This could also be interpreted as a faster drying rate: as the salt concentration in the droplet increases with evaporation, less water is available for the bacterial cell, resulting in a high osmotic pressure on the bacterial cell. Increased osmolarity has been shown to decrease bacterial growth rate and metabolism [131-133]. Thus, higher salt concentrations during drying could speed up this downregulation of bacterial activity and act to preserve the cells. In addition, K^+ ions that were present in the sea salt but not in the NaCl suspensions can regulate internal osmoprotectants, which are contingent in the response to a

hypersaline environment [134]. It should be noted that the much longer timescale of drying in surface droplets allowed for a transcriptional response to osmotic stress, and not only the immediate response (within seconds) that is dominated by the downregulation of enzymatic activities [132].

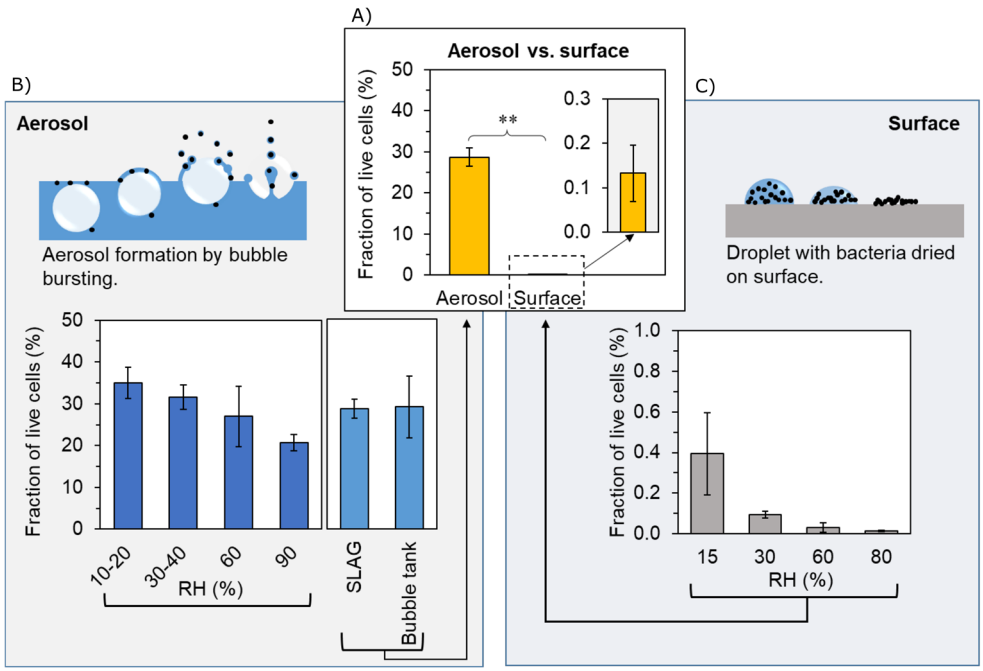


Figure 21. Viability of *Pseudomonas syringae* after drying in aerosol and in surface droplets.
a) Fraction of live cells after drying in aerosol (all samples included) and surface drying (all samples included). b) An illustration of the process of bubble bursting taking place in the SLAG and the bubble tank, and the fraction of live cells after aerosol drying in air with different relative humidity. c) Illustration of the surface drying in droplets, and the fraction of live cells after surface drying in air with different relative humidity. Reprinted from [88] .

Previous studies on bacterial survival in air employed different aerosolization methods, as well as different residence times in air, sampling methodology, bacterial species, environmental stress factors, etc. Aerosolization techniques are further discussed in Paper V, and some general recommendations are proposed regarding the preparation of the material, spray fluid composition, aerosol generation and aerosol particle characterization to allow for reproducibility and comparability between studies. A previous study on aerosolized *P. syringae* showed higher survival at a lower (12 °C) temperature than at a high one (22 °C), and since the water content in air was unchanged, the authors related low temperature to high RH. However, the aerosolized droplets were large (450 µm) and it is therefore likely that the droplets did not dry out completely at 12 °C (77% RH). Some studies that evaluated the survival of other bacterial species at varying RH also found, in line with our results, higher survival at low RH [135-137].

4.2.3 Drying of viruses in air and on surfaces

Unlike bacteria, viruses have no ability to respond to osmotic pressure during drying. Possibly due to this, the results on virus survival in aerosol particles exposed to varying RH is more conclusive with a rough consensus that enveloped viruses have lower inactivation rates at low RH and non-enveloped viruses have lower inactivation rates at high RH [137]. Many of the viral diseases that recur in wintertime are caused by enveloped viruses [7, 137], but the winter vomiting flu, caused by the non-enveloped NoV, also displays a significant pattern of seasonality [138]. In order to study the infectivity of airborne NoV we developed an experimental laboratory setup for the aerosolization of MNV (Paper IV). In our study, the infectivity per virus (the $TCID_{50}$/MNV genome ratio) decreased from 10^{-4} in the start solution to 10^{-6} after aerosolization and collection (Figure 20). Since we observed the same decrease in infectivity from bubble bursting and from atomization and due to similar infectivity reduction because of drying in a previous study [113], we hypothesized that the drying process instead of the aerosolization process caused the decreased infectivity.

The one study that previously evaluated infectivity of aerosolized MNV [128] found a decrease in infectivity that was less than one order of magnitude – distinct from our results. A possible reason is that Bonifait et al. [128] aerosolized MNV from the cell growth media (DMEM), containing a variety of amino acids and inorganic salts, while we diluted our viral stock solution (in DMEM with 10% FBS) 1:10 in phosphate buffered saline (PBS). Hence, the solute concentration in the aerosolized droplets in our study was lower and possibly less protective to the MNV. Human NoV has been shown to be infectious for long times in the environment, up to several days [108, 139]. Environmental contamination most likely occurs from an abrupt vomiting incidence, and the NoV would then be incorporated in the vomitus during drying – along with protective proteins, salts etc.

To date, nobody has studied the infectivity of airborne MNV at varying RH. Colas de la Noue et al. performed laboratory experiments on surface dried droplets containing MNV in varying RH and found that MNV infectivity was preserved to a higher degree when dried at low RH [11]. At intermediate RH, 55-85%, their results showed strongly reduced infectivity, while at high RH, >90%, infectivity remained high at least for shorter times (6 h). This V-shaped pattern of high infectivity at low and high levels of RH and low infectivity at intermediate RH has also been observed for Influenza A, and for other viruses as well [10, 13, 79, 140, 141] A recent study, though, found that the RH-dependent infectivity of aerosolized influenza virus disappeared when supplemented with extracellular proteinaceous liquid from human bronchial epithelial cells [142]. The effect on viruses of rapid versus slow drying in chemically complex droplets (containing concentrations of salts, proteins and surfactants representing natural body fluids) needs to be further studied to

conclude if the physicochemical effects seen in laboratory studies can explain the RH dependency of the influenza incidence.

4.2.4 Cultivability and infectivity of laboratory bioaerosols

The detection of NoV in hospital air was based on viral genome detection and the infectivity was hence not investigated. The infectious dose of NoV has been estimated to be as low as 28-2800 viral particles in human challenge studies [143-145], and the concentrations present in air could thus be enough for transmission of disease [128]. In addition, a recent study showed that NoV can be excreted in clusters in lipid cloaked vesicles, and that samples rich in these vesicles were more infectious than if the vesicles were depleted [146]. Thus, it is possible that the infectivity of NoV is altered with the amount of vesicle-transported NoV.

As many microorganisms are inactivated while being airborne, it is important to complement detection and quantification with infectivity or viability studies *in vitro*. For NoV, this was performed with the model virus MNV (Paper IV) as there is yet no robust *in vitro* cell assay for human NoV where low-titre samples (such as those from air) can be evaluated [147]. As seen in Figure 20, the infectivity of MNV was reduced more than the MNV copy number, indicating inactivation of virus.

In the Paper III study, where we examined aerosolized *P. syringae*, the main viability analysis was based on membrane integrity fluorescent staining measured by flow cytometry. We also cultivated the bacteria, but the results did not correlate significantly with the number of viable cells (flow cytometry). Furthermore, samples exclusively containing cells that were defined as “dead” and “damaged” by flow cytometry (not included in viable) were shown to contain cultivable cells. Thus, live and dead (and damaged) as defined by flow cytometry did not fully correspond to cultivable and non-cultivable.

4.3 Particle size of infectious bioaerosols

As described in the introduction to section 4.2, drying makes droplets shrink to smaller sizes, and shrinking results in a lowered sedimentation velocity and hence, a longer time airborne. Potentially infectious bioaerosols are then able to spread longer distances within indoor environments. The aerosol particle size also determines where in the respiratory tract they deposit if inhaled. The deposition site is important as the cell receptors in the mouth and nose, upper respiratory tract and lower respiratory tract have different susceptibility to various infectious agents [148]. A proposed theory for norovirus infections is by deposition in the mouth or upper respiratory tract (and transfer by mucociliary clearance to the pharynx)

followed by swallowing and subsequent infection of cells in the gastrointestinal tract [39, 128].

In order to gain more knowledge on the potential of NoV to spread via air, we collected aerosol particles in a cascade impactor in the hospital ward corridors during outbreaks of NoV (Paper II). From three of the four investigated outbreaks, NoV RNA was detected by qRT-PCR in one or two size fractions (Figure 22). In one case, NoV was detected in particles in the size range 4.5-20 µm. In the other two cases NoV was detected in the particle size ranges of 0.14-0.34 µm and 0.34-0.94 µm, respectively. Detection of NoV in both micrometer-sized particles, with the potential to contain more infectious material, and sub-micrometer particles with negligible settling velocity, confirms that the virus has the potential to spread within indoor environments. It should be taken into account that one 10 µm particle has the equivalent volume of a thousand 1 µm particles; thus it is remarkable that in outbreaks 2 and 3, only the small particle fractions were NoV positive.

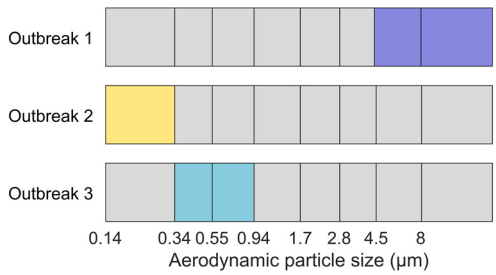


Figure 22. Norovirus positive particle size fractions in hospital corridor air during outbreaks.
NoV positive (colored) and negative (gray) particle size fractions from air sampling with a cascade impactor placed in the corridor during three of four investigated NoV outbreaks. In the fourth measured outbreak, no size fractions were positive.

We also measured the RH and temperature in the hospital wards during some NoV outbreaks (Figure 23). The duration of the measurement varied from 3 h (March) to 5 days (January) as indicated by the number of minute average points *n* in Figure 23. We can conclude that in general, RH was low, <30%, in indoor air during the winter, and higher in late spring (May). The indoor temperature varied little; all measurements had a mean temperature of 23-25 °C (with a standard deviation of 0.2-0.6). The RH measurement series that correspond to the ones with NoV positive impactor size fractions (Figure 22) were January (outbreak 3), May (outbreak 2) and December (outbreak 1). The reasons for not detecting NoV in the impactor during the February$_1$ measurement was likely due to starting collection late in the outbreak and positioning the instruments far away from NoV positive patients. In the February$_2$ and March measurement series, no impactor sampling was done. In essence, we saw that the indoor relative humidity was low during the NoV outbreaks we investigated.

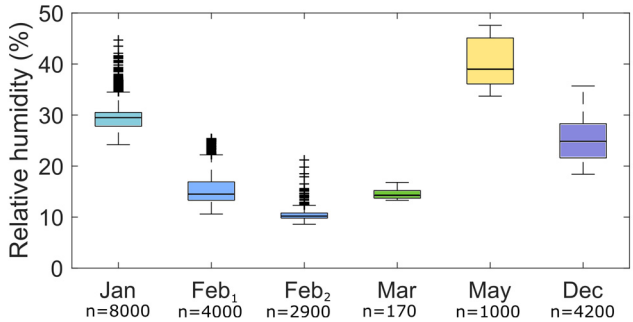


Figure 23. Relative humidity in hospital wards during NoV outbreaks.
The relative humidity was measured at different hospital wards during norovirus outbreaks. As the sampling times varied between outbreaks, *n* indicates the number of minute values of RH that were included in the box median, upper and lowe quartile values. The two mreasurement series in February took place in adjacent hospital wards on consecutive days. For comparison with Figure 22: Jan=outbreak 3, May=outbreak 2, Dec=outbreak 1, Feb_1=no positive size fractions, Feb_2 and Mar=no impactor samples collected.

The relationship between RH and the potential amount of infectious influenza in the air was investigated in more detail by Yang and Marr [149]. Apart from the change in particle size during drying, viral inactivation due to RH and air exchange rates in indoor environments were also included in the model. They concluded that low RH increased the amount of airborne infectious influenza virus within a shorter period due to larger dried-out particles and low inactivation. For times >30 min, large particles >10 µm settled to the ground regardless of RH. For the smaller particles <5 µm that remained airborne, it was concluded that ventilation was the most important removal factor [149]. Influenza A virus and several other respiratory viruses have, in concordance with our results on NoV, been detected in aerosol particle size fractions <5 µm [150-154]. Since these particle sizes have high deposition efficiency in the respiratory tract, efficient ventilation and source identification with subsequent containment is important to prevent the spread.

4.4 Prevention strategies in hospitals

In order to prevent the spread of infectious bioaerosols in hospitals, there are guidelines and practices for airborne diseases and aerosol generating procedures. However, infectious bioaerosols may be generated from diseases not considered to be airborne, in asymptomatic stages of disease, or from symptoms or treatments that are not considered to cause airborne spread. In these cases, the ventilation is of major importance. A situation when airborne bacteria can cause severe infections is during surgery [52], and there is an ongoing debate about the most appropriate ventilation types and protective clothing to use in operating rooms. In the Paper I study, we evaluated three ventilation systems for operating rooms according to their ability to

maintain clean air – known to prevent surgical site infections (SSI) – at critical locations during ongoing surgery. Moreover, a LIF instrument was brought to the operating rooms to investigate the correlation between viable particles and cultivable bacteria. In Paper VI, we presented the evaluation of a newly developed bioaerosol sampler that has the potential to be used for point-of-care collection of air samples, for example, exhaled bioaerosols.

4.4.1 Ventilation techniques for prevention of surgical site infections

Ventilation with a high airflow has been acknowledged to reduce the number of surgical site infections [15, 155, 156]. However, several studies question the benefit of laminar airflow ventilation systems [57, 157, 158]. We evaluated three types of ventilation systems for operating rooms (Paper I). We concluded that the airflow pattern played an important role for maintaining low bacterial concentrations at critical locations, that is, close to the wound. The airflow rates for the three ventilation systems were compared with the measured concentrations of airborne bacteria (Figure 24). Both LAF and T_CAF were more efficient than TMA (when assuming perfect mixing) in keeping clean air at the wound, and T_CAF was more efficient than both TMA and LAF in keeping clean air in the periphery of the room (Figure 24). Hence, the higher energy consumption related to the higher airflows in LAF and T_CAF was more efficiently used than what would be the case of higher airflow rates in TMA.

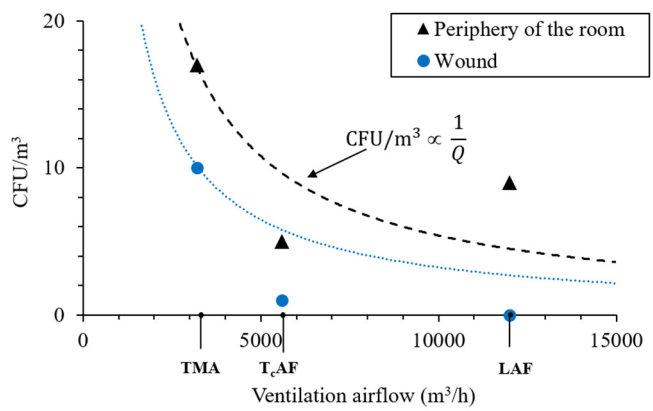


Figure 24. Operating room CFU concentraitons in relation to the ventilation airflow rate.
Median values of CFU concentrations at the wound (circles) and in the periphery of the room (triangles) in relation to the ventilation airflow rate. The dashed and dotted lines represent the assumption that the concentration of CFU m^{-3} is inversley proportional to the airflow rate Q m h^{-1}. The dotted and dashed lines are adjusted to the CFU concentrations for TMA at the wound and in the periphery of the room, respectively. Reprinted from [87].

The frequencies of SSIs from surgeries performed in operating rooms with LAF ventilation compared to those performed in operating rooms with TMA ventilation have been evaluated in retrospective registry studies [158, 159] and in a meta-analysis [57]. The conclusion in [57] is that LAF does not significantly prevent SSIs compared to TMA. In studies on airborne bacterial concentrations, the results are more consistent and in line with our results: lower CFU concentrations in LAF than in TMA [156, 160-162]. Besides direct contamination of the wound via airborne particles, bacterial contamination of the surgical tools [156] and hypothermia (cooled tissue) have been proposed to increase the risk for SSI [163]. However, the effect of hypothermia was recently evaluated in a meta-analysis and found to be non-significant [164].

Another parameter often included in the discussion is what surgical clothing material should be used to prevent particle emissions from the staff. In a study by Kasina et al. [59], three clothing materials were used during 37 surgeries in an operating room with TMA and an airflow rate of 2200 $m^3\ h^{-1}$. Although they found significantly lower CFU concentrations when using single use polypropylene clothing than with the traditional mixed materials clothing (69% cotton, 30% polyester and 1% carbon fiber), their median and average CFU concentrations were higher than those in our study (Paper I) where mixed material clothing was used. Thus, ventilation with higher air exchange rates likely contributes to lowering the CFU concentrations more efficiently than clothing materials. In addition, efficient ventilation is likely more tolerant to disturbances from, for instance, door openings that can increase CFU concentrations [102, 103, 105, 165]. Woven materials (like the mixed materials clothing and other reusable gowns) have been shown to be more comfortable than non-woven materials (single use materials) [166]. Thus, from a working environment perspective, woven material clothing is to be preferred.

Paper I reported on a questionnaire answered by the surgical staff. It indicated that working environment comfort was perceived as being better with T_CAF ventilation, because the temperature was comfortable and the noise level was low. The lower airflow rate in T_CAF compared to LAF was beneficial in terms of better working environment and 30% lower energy consumption, while keeping CFU concentrations below the recommended value of 10 CFU m^{-3} for infection sensitive surgeries [67].

It would be beneficial with a higher general knowledge among medical practitioners about how bioaerosols are generated, how they transport and deposit, and the way prevention techniques function. A study by Langvatn et al. showed that surgeons were mostly wrong about what ventilation technique was used in the operation room they worked in [167]. This could lead to behavioral mismanagement and errors in reporting statistics on SSIs. The latter is adverse, since this kind of data often lays the ground for decision-making.

4.4.2 Novel bioaerosol source identification techniques

Increasing our knowledge about bioaerosol sources and changes in bioaerosol concentrations would be useful for infection prevention work in general. In Paper I, a LIF-based instrument was brought into the operating rooms and a comparison was made between CFU concentrations and "viable particle count" concentrations during seven operations. No significant correlation was found and the range of viable particles detected by LIF (0-544 m^{-3}) was distinct from the range of CFU concentrations (0-40 CFU m^{-3}). No correlation with potential sources was investigated, but the LIF technique could potentially be useful to localize bioaerosol sources by detecting concentration changes.

Collection of air samples generally requires advanced equipment that is disturbing in indoor environments due to high noise levels and vibrations. In the Paper VI study, a device that could be used for point-of-care collection of air samples (*e.g.*, exhaled bioaerosols in exhaled breath) was evaluated for its particle collection efficiency. The sampler collected aerosol particles by electrostatic precipitation (ESP) into a small liquid volume. Having a low collection volume was found to be advantageous for the resulting ESP sample concentrations, which is in line with previous studies [71, 168]. For particles >1 µm and at collection voltages of 6-10 -kV, the sample concentrations of the ESP in our study were higher than those of the BioSampler (Figure 25), although the sampler airflow was four times lower than that of the BioSampler.

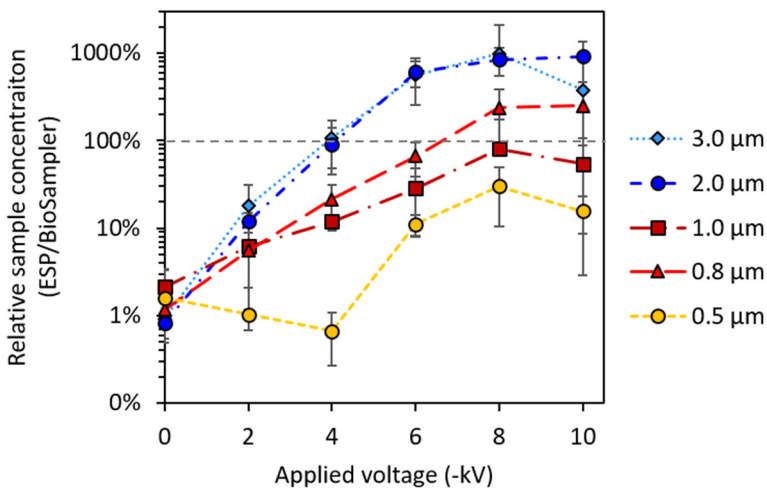


Figure 25. Particle collection efficicency of the ESP and the BioSampler.
The relative collection efficiency of the ESP compared to the BioSampler for each particle size at increasing applied collection voltage.

A point-of-care device, such as the ESP evaluated here, could be very useful in hospitals for screening of exhaled bioaerosols in the breath from patients with diffuse respiratory symptoms to prevent spread during ongoing outbreaks (or pandemics). Fast identification would enable rapid isolation of spreaders and avoid nosocomial infections. As for covid-19, there is evidence of patients testing negative by nasal swabs but positive by bronchiolavage fluid [169]; hence, exhaled air could be useful for the diagnosis of pneumonia.

4.5 Findings from side projects

During my thesis research, some side projects were carried out that touched upon the same topics as my main research area.

Airborne bone dust during sawing in autopsies

The LIF instrument was used to help evaluate prevention strategies for aerosolization of particles during autopsies of patients with suspected prion infections. During these kinds of autopsies, sawing in bone generates airborne bone dust particles that theoretically may be contaminated with prions from body liquids if the patient had prion disease. We evaluated the total concentration of airborne particles and the concentration of fluorescent particles in the room during two autopsies: one when sawing was performed inside a plastic bag that was draped around the body and not opened until after sawing was finished, and one when sawing was performed without the plastic bag as protection (Figure 26). Bone emits fluorescent light at wavelengths in the range 400-550 nm [170] that matches the detectors in the LIF instrument, resulting in the peak seen in Figure 26b.

Historically, pathologists working with autopsies have been at a high risk of getting infections from patients with infectious diseases, especially tuberculosis [171]. Nowadays, infection prevention has improved remarkably but some concerns remain considering brain biopsies from patients with suspected Jakob-Creutzfeldt disease, caused by prions. Prions are misfolded proteins that, when in contact with normal proteins of the same variant, may cause the latter to misfold as well. It may take years or decades to develop symptoms of the disease; however, from symptom onset, the condition usually has a fatal outcome within one year [172]. Thus, containment of sawing dust from possibly prion-infected patients is crucial, and our conclusion from the short pilot study was that sawing inside a plastic bag is to be recommended compared to sawing without the bag protection.

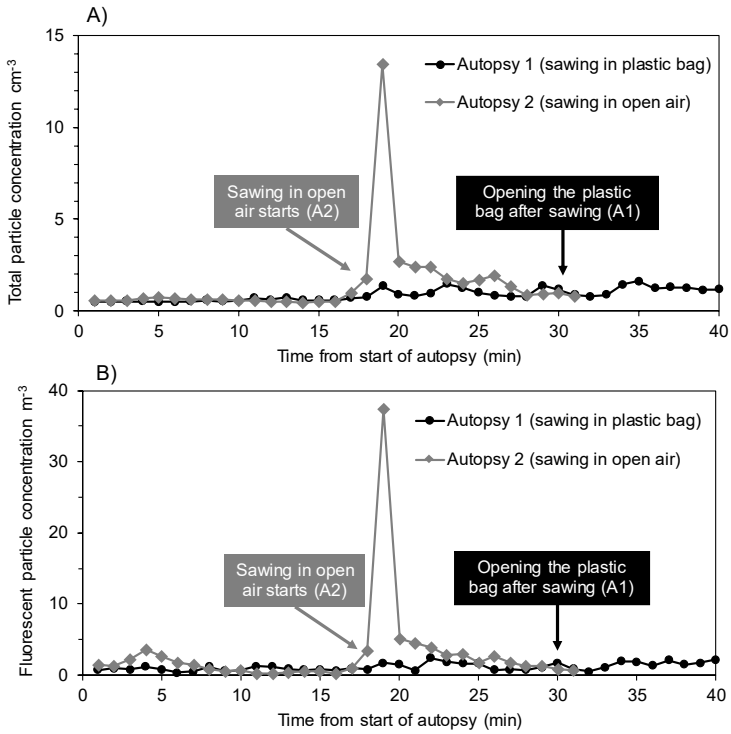


Figure 26. Total and viable particle concentrations during two autopsies.
a) Total particle concentration per cm^3 in the size range 0.5-25 µm measured during two autopsies, and b) fluorescent particle concentration per m^3 in the size range 1.0-25 µm during the same autopsies). In autopsy 1 (A1), sawing was performed inside a platic bag, and opened after the sawing was finished. In autopsy 2 (A2), sawing was performed without the plastic bag. Arrows indicate the opening of the plastic bag (A1) and the start of sawing (A2).

Airborne dog allergens

Allergens from pets are known to give rise to allergic responses in hypersensitive individuals. If inhaled and deposited in the bronchioles, they can also give rise to asthmatic responses. Therefore, we performed a study of airborne allergens from four dogs in a stainless steel chamber of 27 m^3. Each dog spent two hours in the chamber together with its owner, and air samples were meanwhile collected using a cascade impactor, a liquid cyclone and filters. Three different dog allergens were investigated: Can f 1, Can f 3 and Can f 4. Can f 1 was found in particles of sizes >2.8 µm, while Can f 4 was found in all size fractions from 0.14-0.34 to >8 µm (Figure 27). Can f 3 was not found in any air sample.

Our study on airborne dog allergens is the first to detect Can f 4 allergens in a wide distribution of particle sizes. This has only been done with Can f 1 previously [173]. The aerodynamic particle size is important because it determines where in the lungs the particles are likely to deposit, and may thereby be linked to symptom development in asthmatic individuals.

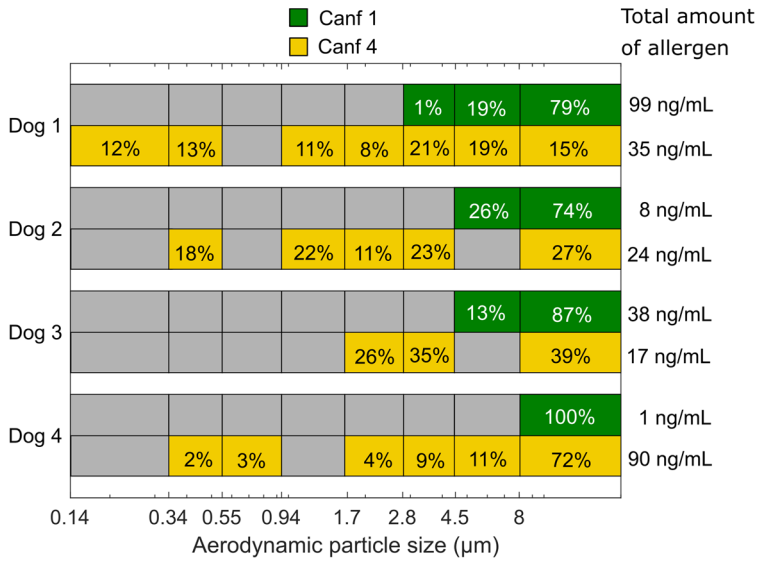


Figure 27. Dog allergens detected in cascade impactor size fractions.
Two dog allergens, Can f 1 and Can f 4 detected in eight respective size fractions in the next generation cascade imapactor. Allergens were detected by secondary antibody fluorescence in an inhibition ELISA assay.

Bacteria in the Arctic – a field campaign along the Greenland west coast

I was part of a field campaign on bacteria in the Arctic (see map in Figure 28a) that aimed to discover similarities and differences between bacterial species and concentrations in the sea, in the sea surface microlayer, and in the air. Air samples were collected from the front deck of a Danish military patroller ship, with two high flowrate impingers (about 2 m^3 min^{-1}) (Figure 28b). One of the impingers collected bioaerosols into PBS and the other into an RNA preservative that was to be used for sequencing analyses. In addition, real-time viable particle count measurements were performed by LIF.

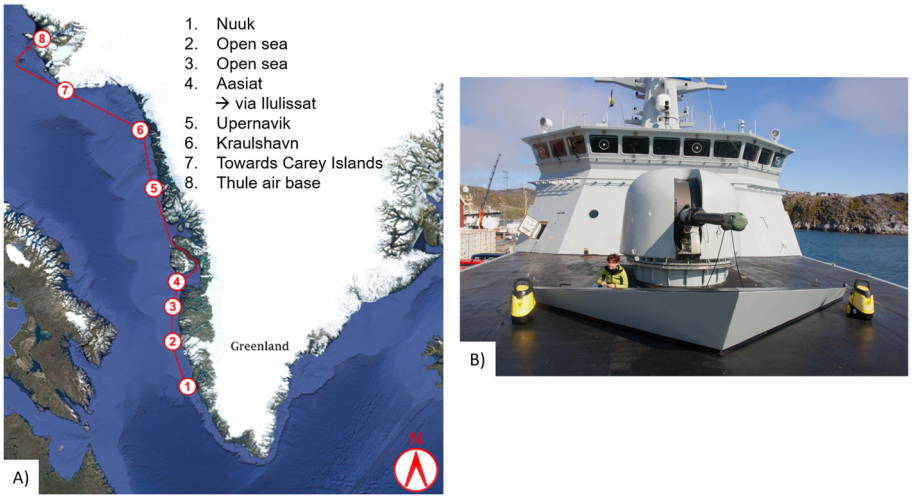


Figure 28. Greenland campaign map and ship front deck measurement setup with two impingers.
a) Map describing the ship route during the 10-day long field campaign. b) Photograph form the front deck of the ship where the two impingers and the BioTrak (not shown) were placed for air sampling and measurements, and the campaign project leader, Tina Šantl-Temkiv.

We observed higher total and viable particle concentrations close to towns (Nuuk, Aasiat, Upernavik, and Thule) and settlements (Kraulshavn) (Figure 29). Comparing the LIF measurement results in the outdoor air in the Arctic (~10^2 m^{-3}) with those from indoor hospital environments (~10^3 m^{-3}), shows about one order of magnitude lower concentrations of viable aerosol particles in the Arctic.

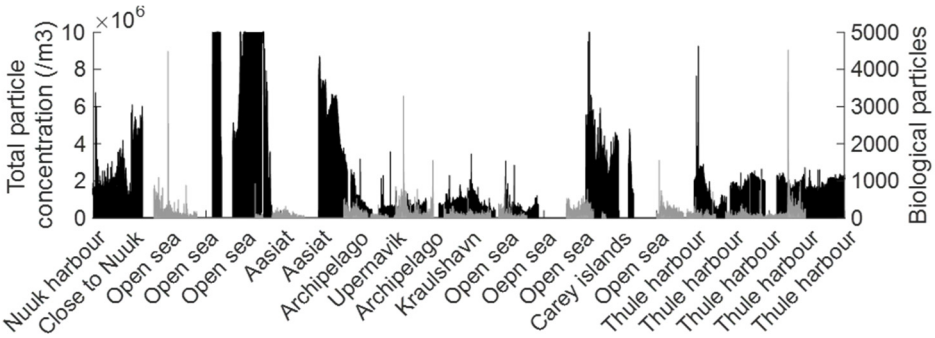


Figure 29. Total and viable aerosol particle concentrations along the Greenland west coast.
Total particle concentrations measured by an OPS (0.5-25 µm), black data series, left y-axis, and viable particle concentrations measured by LIF (1-25 µm), gray data series, right y-axis.

5 Conclusions

This thesis describes studies of sources, transport and some prevention strategies for infectious bioaerosols, primarily in hospitals. We identified vomiting as a probable source of norovirus-containing bioaerosols in hospitals. No significant correlation was found between airborne norovirus and toilet flushing after diarrhea; however, an association between airborne NoV and diarrhea could possibly had been found if the dataset was larger. Toilet flushing is a probable source of bioaerosols, as it is a common symptom during acute gastroenteritis and as numerous peaks of high bioaerosol concentrations were detected by laser-induced fluorescence at the gastrointestinal ward.

We developed an experimental laboratory setup to examine the airborne transport of two model infectious bioaerosols: murine norovirus and *Pseudomonas syringae* bacteria. Our findings showed that the infectivity of murine norovirus was reduced two orders of magnitude after aerosolization and collection, hypothetically due to extensive drying in air with little protection from the low-solute aerosolization liquid. The viability of bacteria was reduced to 20-35% when aerosolized by bubble bursting. Higher viability was observed when cells were aerosolized and dried in air with low relative humidity. Together with experiment where bacteria were dried in larger droplets on surfaces, these results suggest that rapid drying in small droplets, low relative humidity and high salinity favored survival of *P. syringae*. As the way bioaerosols are generated in laboratory studies seems to have a large impact on the outcome of viability studies, a literature review was conducted where general advice on how to perform such experiments was presented.

To minimize airborne transmission of bacteria and viruses, we need to know which prevention techniques are most efficient and when and where to use them. High airflow ventilation has been acknowledged to reduce the number of surgical site infections during open wound surgery by preventing high concentrations of airborne bacteria. Our evaluation of three types of ventilation systems for operating rooms showed that the introduction of clean air above the operating table, directed downwards, efficiently kept the air close to the wound clean, with low concentrations of airborne bacteria.

Simple methods for identification of bioaerosol sources are needed since early detection can lead to faster containment or isolation. We evaluated a new device for bioaerosol sampling and found that its sample concentrations were higher than those

of a commonly used impinger, owing to the low sample liquid volume. With a higher demand for mitigating transmission of airborne infectious agents, this device could be used for bioaerosol source identification in for example hospitals (*i.e.* patients or medical procedures).

Diseases that can spread via air are more likely to give rise to uncontrollable outbreaks and epidemics. As new infectious diseases emerge, the need for knowledge that can be generalized across organism types becomes highly valuable. Breathing fresh air was long seen as the ultimate cure, and conversely, all diseases were thought to arise from breathing corrupt air. After John Snow revealed that cholera was spread via contaminated water, the germ theory with focus on contact spread took over and airborne transmission of disease was to some extent forgotten and has since then been disregarded in many cases. However, in the last decades, the number of publications about airborne infectious viruses and bacteria has increased rapidly, and with the ongoing covid-19 pandemic the research topic is more relevant than ever. During the covid-19 pandemic, the lack of knowledge as well as the shortage of interdisciplinary studies providing knowledge by synergistically linking aerosol science, medicine and virology, has become apparent. Improved scientific collaborations between these fields of science are important to better understand when, where and why airborne transmission of infectious bacteria and viruses takes place.

If a bumblebee can fly,
probably, microorganisms would also qualify.
Yes, bioaerosols are ubiquitous in the air
and by breathing, we share the air.
But what airborne bacteria and viruses do,
is not for a single field of view.
Will they keep moist in winter?
Does long time sampling make their structures splinter?
Will they grow in your lab?
Or are they offended by your pipet stab?
Their genetic codes will make you sick.
And when you think they are dead they might just be playing a trick.
The questions are many and sometimes hard to seize,
though work involving a wide expertise,
could prevent from future infectious disease.

6 Outlook

In this thesis, I have presented my research about the possible sources of infectious bioaerosols in hospital environments, how aerosolization and airborne transport affect some model bacteria and viruses, and the types of ventilation that are preferential in operating rooms for prevention of surgical site infections. Many questions remain and some have arose during the research process.

In general, we know little about the contribution of airborne transmission to the spread of common diseases, such as influenza, SARS and covid-19 from coronaviruses and acute gastroenteritis caused by noroviruses, and the reason for their seasonal recurrence. Many theories have been proposed and one that has gained much attention is that dry air favors airborne transmission. However, the reason for the relationship between high viral survival at low relative humidity is unclear and partly in contrast with expectations since desiccation can be detrimental for functional proteins. It would be worthwhile to study aerosolization of viruses under conditions that are more similar to those of real life than has previously been done. For norovirus, this would include aerosolization by vomiting or toilet flushing of viruses in a gastric fluid-like liquid. At present, there is no characterization of the aerosol particle size distribution generated from vomiting. Moreover, if viability assays for human noroviruses are improved in terms of sensitivity, the infectivity of human noroviruses collected from air could be evaluated and used to confirm or reject the possibility of airborne transmission.

Since influenza and coronaviruses have been shown to spread during the incubation period, before symptomatic coughing and sneezing start, it would be relevant to investigate aerosolization during normal breathing and talking. The aerosolization liquid should resemble the airway lining fluid with potential modifications of protein and surfactant concentrations to match the changes in early onset of the immune response. Proteins and surfactants in the aerosolization liquid may significantly alter the aerosol droplet number and size, as well as droplet drying times and viral inactivation rates. During respiratory infections, the composition of the airway lining fluid may change, which should be included in further studies. For example, SARS-CoV-2 has been shown to infect cells rich in the ACE-2 receptor that is present in high density on alveolar type-2 cells, which are the cells that produce lung surfactants [174]. An improved understanding of the large variation between individuals in aerosol generation during normal respiratory activities, and

of the underlying principles of “superspreaders”, may be key points in preventing transmission of infectious bioaerosols.

During the covid-19 pandemic, questions regarding infectious bioaerosol generation from medical procedures, such as assisted ventilation techniques and intubation, have been frequent: What are droplet and aerosol generating procedures? What appropriate protection equipment does either require? The distinctive cut-off size at 5 µm for classifications of droplet and aerosol transmission should be reconsidered because it is sometimes misleading; aerosol particles up to 20 µm are inhalable. Further studies to evaluate the generation of infectious bioaerosols from sick patients under different respiratory treatments are also needed. Again, the element of studying real-life conditions is essential, although the data may be challenging to collect and even more so to interpret.

Infections from multi-drug resistant bacteria are constantly rising, primarily due to the redundant administration of antibiotic in human medicine and livestock. Antimicrobial resistance is referred to as a threat against modern medicine because antibiotics are key for numerous treatments and for prevention of nosocomial infections. Therefore, general prevention of airborne spread of bacterial infections, by efficient ventilation, for example, is important. On the contrary, bioaerosols could be used for the administration of therapeutics such as bacteriophages against antimicrobial resistant lung infections.

Technical advancements now allow gentle collection of sub-micrometer particles into liquid by condensational growth, which may show more accurate results for cultivable microorganisms in air samples. This improved sampling technique, together with single-cell analysis and molecular biology techniques constitute the prerequisites for advanced aero-microbial studies. Moreover, simple collection and detection techniques could be used to take air samples from breath as a part of routine diagnosis of lung infections.

Our societies continuously face the threat of emerging infectious diseases due to: close contact with animals in breeding and trading facilities, close contact between humans in urban environments such as transport systems and indoor environments, and frequent long-distance traveling. In order to be prepared for new contagious agents, we need to improve our understanding of the ones that already circulate in our societies. The path towards improved understandings of airborne transmission of infectious diseases is through interdisciplinary research that combines laboratory simulations and *in situ* studies.

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Field measurements of airborne microorganisms have been an important part of my thesis research. Here are two photographs from the fun in between air sample collections and lab work in Greenland and a hospital stairwell hall in Skåne.

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