AEROSOLS AND MICROBIOLOGY: CONNECTING DISCIPLINES IN THE POST-PANDEMIC ERA

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INVITED AND OFFERED TALKS

#MicroAerosols24

Mercure Bristol Grand Hotel
Broad St, Bristol BS1 2EL

MICROBIOLOGY SOCIETY

Invited talk: Innovations for detecting and monitoring airborne plant pathogens

Jon West, Gail Canning, Kevin King

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Abstract

Plant diseases significantly reduce food supply despite control efforts. Some plant pathogens are adapted for long distance air dispersal, while others spread short distances in air due to rapid deposition or mortality by UV light, desiccation, etc. Successful dispersal depends on the size of spore sources and environmental conditions affecting release, dispersal and deposition processes. Dilution of spores in air as they disperse means we can infer whether the source is close-by or distant by comparing the concentration of spores sampled at two heights. If the concentrations are similar, the spores come from a distant source but if there is a much greater concentration of spores close to the ground, it means the source is relatively close. We find that the species diversity at 10m height is about double that of 1m due to greater turbulence mixing air at 10m.

A wide range of air sampling devices are available to study aerobiology, the aerobiome and to detect airborne plant pathogens. These sample different volumes of air with different sampling efficiencies, which can bias samples by omitting collection of smaller spores. The number of samplers needed per farm or region varies depending on the pathogen but networks of samplers are increasingly being used to monitor disease risk. There is a trend for these to be automated using optical methods or rapid immunological or DNA-based diagnostics. Air sampling is also a good way to monitor an entire population of a pathogen for changes in genetic traits such as fungicide sensitivity or pathotype.

Invited talk: The Role of Aerosol Measurements in Understanding and Mitigating Disease Transmission

Joshua Santarpia

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Abstract

The study of infectious diseases includes both the progression of the disease in its host and how it transmits between hosts. Understanding disease transmission is important for recommending effective interventions, protecting healthcare workers, and informing an effective public health response. Sampling the environment for infectious diseases is critical to public health since it can provide an understanding of the mechanisms of transmission, characterization of contamination in hospitals and other public areas, and the spread of a disease within a community. Insights gained from environmental sampling, and the challenges presented by the COVID-19 pandemic, have highlighted the need for novel methods for understanding pathogen stability as an aerosol and novel methods for rapidly measuring airborne pathogens. In this talk, I will overview various techniques for studying various infectious aerosol transmission in both the field and laboratory and how those studies fit into the overall challenge of understanding infectious disease.

Biological mechanism governing bioaerosol survival: A future for preventing airborne pathogens?

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Abstract

In the indoor environment, reduction of airborne pathogen transmission can be achieved by different means: 1) engineer the indoor environmental conditions to minimise the survival of airborne pathogens and 2) adopt disinfection technologies to inactivate airborne pathogens.

To understand how one can engineer the indoor environment to reduce pathogen transmission, it is necessary to study the effects of different environmental conditions on the survival of airborne microbes. By carrying out bioaerosol research in a fabricated aerosol chamber, it was found that the survival of airborne *Klebsiella pneumoniae* was significantly lower at 30 ºC than that at 20 ºC. Moreover, although a lower relative humidity (RH) at 20 ºC (50% compared to 80%) appeared to reduce the airborne survival of *K. pneumoniae*, the effect of temperature tended to be stronger in airborne *K. pneumoniae* inactivation. The subsequent RNA-seq analysis further revealed three important stress response pathways in the airborne survival of *K. pneumoniae*, namely starvation, osmotic and oxidative stress responses.

For disinfection, synergistic effect by simultaneous operation of 222-nm and negative air ions were investigated by establishing a novel bioaerosol disinfection system. Results show that the combined operation rendered a synergistic inactivation of airborne *Escherichia coli* and *Staphylococcus epidermidis*, but not airborne viruses P22 and Phi 6, in which only additivity was observed. Further studies into the mechanism regulating the synergy stemming from the simultaneous operation of 222-nm UV and negative air ions are underway.

Gas-Phase Composition and Humidity Affect the Stability of Enveloped Viruses

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Abstract

The stability of respiratory viruses in aerosols and droplets can be influenced by many factors, including temperature, relative humidity (RH), physico-chemical properties of droplets, and virus strain, but the mechanism of inactivation of viruses remains elusive. One hypothesis is that a change in pH in the droplets, caused by changes in the concentration of CO_2 or other trace gases as droplets transition from the respiratory tract into ambient air, damages the virus. Another is that reactive oxygen species in the droplets cause damage. The objective of this study was to determine the effect of different atmospheres (ambient air vs. pure nitrogen vs. high carbon dioxide) and RH (27, 55, and 82%) on the stability of two enveloped viruses: bacteriophage Phi6 and influenza A virus in 1 µL droplets over 4 hours. For Phi6 suspended in culture medium, there was no difference in decay at low RH, but at mid and high RHs, decay was significantly greater at certain time points (2,4 and 4 hours, respectively) in ambient air vs. nitrogen. For influenza virus suspended in human saliva, decay was significantly greater at earlier time points (0.5, 1 and 0.5 hours) in ambient air vs. nitrogen and ambient air vs. carbon dioxide, respectively, at mid RH. This experiment has been extended to low and high RHs. Overall, this study suggests that the composition of the surrounding gas affects inactivation of viruses in droplets. Understanding the drivers of virus inactivation is important for developing interventions for reducing transmission of respiratory viruses.

Characterising Viable Virus from Air Exhaled by SARS-CoV-2 Infected Hamsters

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Abstract

Introduction

The transmissibility of SARS-CoV-2 depends on its ability to efficiently replicate and be released from an infected host, retain viability as it passes through the environment, and then initiate infection in the next host. Therefore, directly assessing viable virus emitted into the air from infected hosts provides insights to understand transmission of SARS-CoV-2.

Methods

Airborne transmission of SARS-CoV-2 was assessed using the hamster model in a custom-made ISOcage. A novel apparatus was developed to characterise infectious viruses from droplets emitted by infected hamsters.

Result

Infectious virus is predominantly released early after infection of hamsters with SARS-CoV-2. This correlates with different rates of transmission 100%, 50%, and 0% following exposure of infected hamsters to naïve sentinels on days 1, 2, or 5, respectively. Viral load in nasal wash samples partially correlated with emitted viable virus ($R^2 = 0.48$, $p = 0.036$). Additionally, although most variants (wildtype, Delta, Omicron BA.1, BA.5, and EG.5.1) showed a strong correlation between viral loads in nasal wash samples and viable virus from droplets, the Alpha variant did not, suggesting that variants have different abilities to be emitted from the upper respiratory tract into the air.

Conclusion

The hamster animal model is useful for the risk assessment of airborne transmission of SARS-CoV-2. Directly measuring viable virus emitted into the air can improve our understanding of the determinants and mechanisms of SARS-CoV-2 transmissibility and could ultimately be applied to studies of airborne viruses exhaled from infected people.

Air-Seq: Using DNA sequencing to detect airborne strawberry pathogens

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Abstract

Air-Seq is an airborne plant pathogen identification method which is faster and less-biased than prior microscopy and culturing techniques. Air-Seq combines high throughput air sampling with whole genome sequencing allowing for rapid detection of microbes, including those which do not culture. In this study Air-Seq was applied to a commercial farm setting.

A Coriolis liquid cyclone sampler was used for monthly airborne collections at three locations within a Strawberry farm. DNA was extracted from the samples, sequenced using Oxford Nanopore Technologies, and taxonomically identified. Plant disease scores for Botrytis spec., Phytopthora spec. and Podosphaera spec, temperature and humidity data was integrated into the analysis.

Air-Seq accurately identifies pathogen type and abundance as the number of sequenced reads varies with the disease score. Our findings indicate a complex relationship between airborne pathogen abundance and the future measured disease scores. Often there is a peak in measured airborne inoculum before the disease is seen on the plants suggesting it is possible to detect fungal pathogens before they damage crops using Air-Seq. Additionally, we observed fungicide application did not reduce the prevalence of fungi in the air or on the crops suggesting the application was not sufficient or the pathogens had developed resistance which has been documented in the monitored species (Marin et al., 2021; Palmer and Holmes, 2021; Weber and Hahn, 2019).

These results highlight Air-Seq as a valuable tool for airborne pathogen identification and with increased frequency of collections, fungicide application could be targeted to improve plant disease control.

Assessing population exposure to fungal spores in the UK using high-throughput sequencing methods over one year

Samuel Anees-Hill[1,2], Philippa Douglas[1,3,4], Sameirah Macchiarulo[5], Anna Hansell[1,2], Emma Marczylo[1,5]

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Abstract

Every breath we take contains fungal spores. Inhalation of fungal spores can trigger allergy and infection. While microscopy helps identify some fungi, high-throughput sequencing (HTS) offers a deeper, faster analysis. This study aims to further understanding of airborne fungal composition in two UK regions by using HTS.

120 daily samples per site taken in Leicestershire (lat: 52.623, long: -1.123) and Oxfordshire (lat: 51.575, long: -1.318) in 2021at 7-8m above ground level were processed using HTS. ITS2 and the D1/D2 LSU rDNA regions were selected for amplification. Sequencing was performed on Ion 520TM chips with an Ion S5TM instrument with controls. Bioinformatics was used to assign fungal taxonomic identities. Fungal diversity and composition were analysed with respect to location, meteorological variables and season.

The most abundant genera at both locations were *Cladosporium, Alternaria, Aureobasidium*, and *Epicoccum.* Abundance of different taxa gradually increased across all taxonomic levels until August, decreasing over the colder months to the end of the year. There were no significant diversity differences across seasonality or location. Air temperature was a statistically significant driver of changes in abundance at both locations (Oxfordshire: $p = 0.005$, Leicestershire: $p = 0.02$). Wind speed was also a significant driver of composition in Oxfordshire ($p = 0.04$).

This work illustrates the use of HTS as a tool to assess fungal diversity and composition, identifying many taxa that cannot be detected with microscopy alone, and may have associations with health outcomes.

Identification, mapping and engagement of stakeholdesr for bioaerosols

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Abstract

Bioaerosols are airborne particles from biological origin that can have diverse and serious effects on public health. Bioaerosols is an area overlapping multiple disciplines (aerosol and exposure science, epidemiology, clinical medicine, toxicology and public health). High quality research in this area requires multi-disciplinary collaborations. Stakeholder mapping provides essential information on the main players and who is affected within a certain project or field. Stakeholders can have diverse perspectives and priorities, some complementary and others conflicting, and it is important to identify effective engagement strategies to facilitate progress. The aim was to bring together partners with an interest in bioaerosols and create a stakeholder map of current and future stakeholders.

Stakeholders were identified and characterised using an MS Forms questionnaire distributed via personal networks, Health Protection Research Units, and BioAirNet (a Clean Air Network focussed on bioaerosols). The questionnaire incorporated stakeholder interests and sector, engagement type, level of importance and influence, as well as the frequency of engagement to allow creation of a stakeholder map. Two stakeholder workshops were held in October 2023 to discuss results and how to utilise the map, and foster collaborations for further work. Over 20 stakeholders attended the workshops.

The mapping exercise identified 129 different stakeholder organisations and 93 individuals. Interest and influence were scored. Available and desired expertise were also identified.

The workshop feedback showed this information is invaluable for the facilitation of collaborative multi-disciplinary bioaerosol research projects, risk communication and dissemination of research findings.

A Novel Approach to Understanding Bioaerosol Stability

Elizabeth Klug[1], Danielle Rivera[1], Don Collins[2], Ningjin Xu[2], Sean Kinahan[1,3], Vicki Herrera[1], Ashley Ravnholdt[1], Daniel Ackerman[1,3], Joshua Santarpia[1,3]

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Abstract

Viral disease poses a significant risk to public health. This is especially true of viruses that transmit as aerosols due to the potential for widespread disease transmission. These viral diseases may be transmitted from person-to-person, such as SARS-CoV-2, or as environmentally generated aerosols, like hantaviruses.

The threat a virus may pose as an aerosol depends on several factors, including its stability as an aerosol. Bioaerosol stability is generally studied as the decay of aerosol properties (viability/infectivity, detectability, etc.) in response to environmental conditions (sunlight, relative humidity, etc.). However, there is little understanding of how intrinsic viral properties drive this stability. We have developed a novel system and approach to investigate and determine the aerosol and viral properties that drive the stability of viruses that infect humans as aerosols. The Biological Aerosol Reaction Chamber (Bio-ARC) is a flow-through system designed to rapidly expose biological aerosols to environmental conditions (ozone, simulated sunlight (UV), temperature, and humidity) and determine the sensitivity of those particles to simulated ambient conditions. Using this system, we examined the stability of a well-understood viral model, MS2 bacteriophage. Furthermore, we have examined the aerostability of SARS-CoV-2, the causative agent of COVID-19. In future work, we aim to better understand the key factors that determine viral stability and to better predict the stability of emerging viral diseases that pose a threat as an aerosol.

Computational and Molecular Dynamics Modeling to Reduce Environmental Effects on Bioaerosols and Mitigate Disease Transmission

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Abstract

Viral infectivity and antimicrobial resistance (AMR) of bacteria pose increasing threat to global public health, requiring effective treatment strategies. Even though antibiotics, metals, and other pollutants are the main drivers for AMR development, growing evidence suggests that other environmental stressors such as air conditioning airflow contribute to triggering resistance in aerosolized pathogens. Due to mechanical and molecular forces, deposition, resuspension, attachment, and detachment from surfaces may change the behavior of bioaerosols in ventilation airflow. Our study using biolayer interferometry and molecular dynamics simulation demonstrates that different environmental conditions affect the binding kinetics of SARS-CoV-2 proteins to different surfaces. Experimental and modeling results with bacteria show that aerosolization not only exerts mechanical stress on bacteria, but also affects their cell membrane, indicating correlation with the airflow triggering resistance to antibiotics that inhibit cell wall synthesis. Although bacteria are robust creatures, environmental conditions, including airflow parameters can be manipulated to disrupt their proliferation. The layout of a facility will directly affect whether the rooms are more likely to become contaminated with aerosolized pathogens or not. Of greatest concern in food processing facilities and healthcare settings is the possibility of generating bioaerosols containing resistant pathogens. Therefore, it is critical to optimize the airflow patterns to minimize the entrainment of pathogens and the residence time of contaminated air. Computational airflow models developed in this study show that facility layout strongly affects the transport and behavior of bioaerosols, opening new avenues for engineering to reduce adverse responses triggered by environmental effects and combat their spread.

Remote aerosol SARS-CoV-2 transmission from COVID patients to sentinel animals

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Abstract

We performed studies investigating the feasibility of human to animal (H2A) model system to test whether patient generated respiratory bioaerosols hold infective capacity when traversing long distance airborne transport within the built environment. Clinically confirmed South African COVID-19 patients were housed in a clinical ward with a ventilation system continuously channeling exhaust airflow to animal caging units located proximal but segregated from clinic space. Seven (7) COVID-19 patients spent a cumulative 400+ in-residence hours in the ward during 17-day period. Pair-housed naïve golden Syrian hamsters (n=216) received continuous exposure to clinical ward ventilatory exhaust during this time. Analysis of available animal serum indicated anti-SARS-CoV-2 IgG in over half (61%) of all animals exposed to clinic exhaust. Viral lineages from donor patients identified and matched in sentinel animal lung suggests differential aerosol efficiency SARS-CoV-2 strain predominance during transmission. Collectively, these results support the concept of long-distance transport of infectious viral bioaerosols generated from patients remaining infective as evidenced by anti-SARS-CoV-2 IgG in rodent sentinels. Findings promote the posit that remotely generated bioaerosols manifest as stochastically dictated disease induction rather than a deterministic threshold effect. Further confirmatory studies are necessary to broaden our understanding of infection patterns in this idealized H2A transmission model system.

Assessing exposure to fungal bioaerosols in transport environments: Analysing fungal composition of passive dust samples collected in UK railway stations

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Abstract

Bioaerosols are complex mixtures of airborne microorganisms including fungi, bacteria, pollen, viruses and their by-products. While exposure to diverse microorganisms is essential for normal immune system development, bioaerosol inhalation (particularly of fungal spores) has been associated with respiratory allergy and inflammation. Bioaerosol are ubiquitous, yet their composition within different environments is not well understood. Here we focus on the fungal composition of transport environments, namely railway stations (RSs), which pose a potential source of occupational and community exposure.

Over 250 passive dust samples collected from 9 RSs across the UK between 03/2014-05/2015 were analysed by high throughput sequencing (HTS) with a metabarcoding approach targeting the ITS2 region. Such HTS techniques provide an opportunity to measure a wider range of microorganisms than traditional culture or microscopy techniques. RS characteristics were varied and included enclosed, open-ended and outdoor layouts. Annual footfall ranged from 10-50 million. Fungal composition of the settled dusts was dominated by yeasts, in keeping with other indoor environments. The most abundant fungal taxa included those known to cause opportunistic infections (Papiliotrema, Rhodotorula, Debaryomyces, Sporobolomyces & Filobasidium) or act as aeroallergens to those with allergic airway disease (Aureobasidium, Vishniacozyma). There were significant differences in both diversity and composition between stations and seasons. Differences between RS may be driven by station location and layout, while differences across seasons were likely to be driven by meteorological factors.

Such work is key to better understanding fungal exposures within public spaces and assessing their potential associated health impacts.

Comparison of aerosol and surface survival of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae* and vancomycin-resistant enterococci

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Abstract

Background

Transmission of *S. pyogenes* is thought to occur primarily through respiratory droplets and contaminated surfaces. A recent contact tracing study of scarlet fever cases in UK schools showed heavy asymptomatic shedding; *S. pyogenes* was detected in classroom air while surface recovery was very rare. To our knowledge there has not been a direct comparison of survival of clinically relevant pathogens in aerosols and on surfaces. The use of different methodologies, environmental conditions, and species have limited such comparisons.

Aims and Methods

This study aimed to compare the ability of *S. pyogenes* and six other clinical species to survive in aerosols and on surfaces. A simple bioaerosol generating system was validated using an aerodynamic particle sizer. Surface survival was assessed by drying bacterial suspensions on plastic and recovering viable cells at set timepoints up to a month. Three clinical isolates of each *S. pyogenes*, *S. pneumoniae*, *S. aureus*, VRE, *P. aeruginosa, E. coli* and *K. pneumoniae* were tested.

Results

Detected aerosol particles measured up to 14 µm. *S. pyogenes* and *S. aureus* demonstrated a 1-2 log-fold survival advantage compared to other species in aerosol. In contrast, *S. pyogenes* and *S. pneumoniae* failed to survive on plastic over the course of the experiment compared with other species. VRE survived exceptionally well on surfaces but poorly in aerosols.

These results highlight that aerosol transmission of *S. pyogenes* should be considered in future infection control guidelines

Airborne Antimicrobial Resistance: comparing the respiratory microbiome of hospital workers with the surrounding airborne resistome

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Abstract

To tackle the global health threat of antimicrobial resistance (AMR), greater knowledge of how AMR spreads in the environment and healthcare settings is needed. Despite increased research into antibiotic resistant genes (ARGs) in the aquatic environment and soil, research into the airborne resistome is lacking.

To investigate if the air in clinical environments represents a significant source of AMR, the airborne resistome of four hospital wards was compared to the respiratory microbiome of hospital staff. Across five weeks, nasal swabs from hospital staff were collected alongside sampling of airborne microorganisms with electrostatic dust cloths. The abundance of key AMR bacteria was quantified with culture and culture-independent methods. Whole-genome sequencing of isolates and metagenomics sequencing was used to identify key ARGs and determine the evolutionary relationship between the airborne and respiratory microbiomes to establish the degree to which the air is a source of clinically significant AMR infection.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from multiple staff, ranging from 10 to 4020 colony forming units (CFU) per swab. Extended beta-lactamase (ESBL) producing *E. coli* and KESC group bacteria (resistant to antibiotics such as penicillin) and *E. coli* resistant to drugs of last resort (such as carbapenems) were also detected.

Crucially, this research investigates the transfer of clinically important AMR microorganisms between the air and the human respiratory microbiome. Such knowledge is essential for understanding the transmission of AMR pathogens between the environment, healthcare and human microbiome and will enable regulators to prioritise effective interventions to slow the spread of AMR.

Air-seq: Measuring air metagenomic diversity in an agricultural ecosystem

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Abstract

Plant agricultural ecosystems are essential for human nutrition, but modern industrial farming typically uses large monocultures, in which diseases can easily spread and have devastating effects on yield and food security. Thus disease monitoring, chemical treatments, breeding of resistant cultivars etc. are essential for maintaining food security. Importantly disease propagation and outbreaks are not solely based on short distance transmission to neighbouring plants, but also on long distance aerial dispersal of spores over longer ranges.

Here we show that the low concentration of airborne DNA from microbes can be recovered, whole genome sequenced and taxonomically classified, including down to the species level. Data from wind tunnel microbial release experiments shows the method is reproducible, and that classified reads increase as the released spores levels increase. Monitoring a field growing key crops, we show that Air-seq can identify the presence of agriculturally significant pathogens and quantify their changing abundance over a period of 1.5 months, which often correlates with weather conditions. We add to the evidence that aerial environmental DNA can be used as a source for biomonitoring terrestrial ecosystems, finally we show that genotype data can be recovered from airborne microbes and matched to the closest known pathotypes.

Infectivity of exhaled SARS-CoV-2 aerosols is sufficient to transmit covid-19 within minutes

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Abstract

Background

Exhaled SARS-CoV-2-containing aerosols contributed significantly to the rapid and vast spread of covid-19. However, quantitative experimental data on the infectivity of such aerosols is missing. Knowing the emission rates of infectious viruses from normal respiratory activities enables more accurate modelling of disease transmission in indoor environments.

Method

We collected the exhaled aerosols from breathing, talking and singing, respectively, from 38 individuals with covid-19 using a BioSpot (Aerosol Devices), and cultured the aerosol samples that contained detectable levels of SARS-CoV-2 RNA. In another setting we collected exhaled aerosols from one individual with covid-19 using a cascade impactor to determine the size distribution of SARS-CoV-2 RNA in aerosol. Then, we used the size distribution and the emission rates in an indoor air inhalation model to calculate the time needed to inhale one infectious dose.

Results

50% of the 38 individuals had detectable levels of SARS-CoV-2 RNA in the exhaled aerosol samples. From three individuals, six aerosol samples were culturable, of which five were successfully quantified using $TCID_{50}$. The source strength of the three individuals was highest during singing, when they exhaled 4, 36, or 127 $TCID_{50}$/s, respectively. Calculations with an indoor air transmission model showed that if an infected individual with this emission rate entered a room, a susceptible person would inhale an infectious dose within 6 to 37 min in a room with normal ventilation.

Conclusion

Our data show that exhaled aerosols from a single person can transmit covid-19 to others within minutes at normal indoor conditions.

Antimicrobial Resistance in Bioaerosols: Characterising the Airborne Resistome in the Classroom and Beyond.

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Abstract

Antimicrobial resistance (AMR) is an emerging public health threat. Deaths caused by resistant pathogens are estimated to exceed 10 million annually by 2050. Despite this, the presence and dispersal of AMR in bioaerosols has been overlooked. The airborne resistome constitutes a major gap in our knowledge, particularly in the context of the One-Health approach to combating the AMR crisis. Our ongoing study aims to characterise the airborne resistome in environments occupied by children who, due to their developing immune systems, are a key at-risk group. Our initial results reveal that extended-spectrum beta-lactamase (ESBL) producing bacteria are common in classrooms and outdoor air. However, the diversity of these bacteria differs between indoor and outdoor air. ESBL-producing *Escherichia coli* were found exclusively in classrooms and not in outdoor air. While ESBL-producing *Klebsiella* spp. were found at similar concentrations in both. Lastly, concentrations of resistant fungi were significantly greater in outdoor air. These observations suggest that human occupancy drives the diversity and distribution of resistant airborne bacteria in indoor air, as it differs significantly in composition to outdoor background levels and to those we found at other outdoor sources (composting and wastewater treatment facilities). Our results demonstrate that AMR in bioaerosols is widespread in environments occupied by children, potentially acting as a source of infection, evolution, and dispersal of resistance. Further attention is therefore needed to determine the health risks associated with resistant bioaerosols across the indoor-outdoor air continuum and their role within the One-Health concept.

Assessing the risk of airborne transmission in commercial poultry production settings during clade 2.3.4.4b H5N1 high pathogenicity avian influenza virus (HPAIV) epizootics.

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Abstract

Since October 2021, Europe has experienced the largest high pathogenicity avian influenza virus (HPAIV) epizootic ever. Many poultry infected premises (IPs) have clustered geographically, raising questions around potential transmission by airborne particles.

To explore the risk of specific transmission pathways, we conducted extensive environmental sampling from IPs where clade 2.3.4.4b H5N1 HPAIV was confirmed, each representing a major poultry species (ducks, turkeys, chickens and pheasants). A range of environmental samples were collected from inside and outside poultry houses, including airborne particles, deposited dust, feathers, and other potential fomites. Viral RNA (vRNA) and infectious virus were detected in air samples collected from inside and outside, but in close proximity, of infected houses, with vRNA alone being detected within 10m of the infected shed. Dust samples collected within a very short distance (<2m) outside of the affected houses occasionally contained infectious virus, while feathers from the affected houses, located up to 60m away only contained vRNA. Low viral RNA levels were also detected in air samples from the environment of chickens and ducks experimentally infected with different H5Nx HPAIVs.

The distances infectious virus can travel from infected poultry houses might contribute to between house transmission on the same premises but are not likely to contribute to spread to neighbouring premises as supported by the low level of lateral spread found in genetic assessments. Consequently, other factors, including indirect contact with wild birds, fomite spread and the strict implementation of biosecure practises represent greater importance in preventing disease dissemination.

Modelling the generation of bioaerosols during SARS-CoV-2 infection in ferrets: Implications for virus transmission

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Abstract

The generation of infectious bioaerosols from individuals infected with SARS-CoV-2 has been widely studied during the COVID-19 pandemic. Ferrets are an established model (human/companion animal) for asymptomatic/mild SARS-CoV-2 infection and can be used to investigate bioaerosol generation and virus transmission. Bioaerosol generation following direct inoculation of ferrets with SARS-CoV-2 Delta and Omicron variants were characterised. Liquid- and gelatin filter-based air samplers were affixed inside ferret cages and positioned approximately 0.5m away from the ferret cages. Air samples were collected for up to seven hours per day for 25 days. The contribution of viral genome in 'naturally' generated bioaerosols from SARS-CoV-2 infection in ferrets was compared to upper respiratory tract (URT) viral shedding. SARS-CoV-2 Delta variant was capable of establishing robust infection in ferrets and transmitted efficiently to naive ferrets co-housed within the same cage. SARS-CoV-2 Delta viral RNA was detected in air samples collected from within the ferret cages and the room that ferrets were housed, albeit at lower levels. Genetic polymorphisms identified from air samples collected from within the cages were also detected in the ferret URT samples. Infection was established in ferrets following inoculation with Omicron variant however limited viral RNA was detected in the air samples. Collecting air samples during *in vivo* experiments enabled a comparison of viruses with particular genetic signatures that may allow dissemination of SARS-CoV-2 through bioaerosols, identifying the potential to cause environmental contamination beyond the immediate area within which the infected individual is residing, thus increasing the risk of virus transmission.

Use of microthreads for studying foot-and-mouth disease virus aerosols

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Abstract

Foot-and-mouth disease virus (FMDV) affects cloven hooved livestock species and is endemic across much of Asia and Africa. Losses in productivity in infected animals and reduced access to trade markets cause significant economic impact. Transmission occurs through direct contact between infected and susceptible individuals, but long-distance transmission can be also facilitated through survival of the virus on fomites or in aerosols. Investigating the ability of FMDV to survive in aerosols is critical for understanding control and surveillance of outbreaks.

Studying the behaviour of pathogens in aerosols is a complex task, particularly when containment conditions need to be considered in experimental work. Here, we describe the use of microthreads to capture and study foot-and-mouth disease virus (FMDV) aerosols within a high containment laboratory environment. Using the microthread methodology, spider's silk is wound around frames and then exposed to aerosolised FMDV, allowing virus decay rates to be determined and the effects of environmental conditions measured.

Experimental data from this methodology provides valuable information on the conditions in which aerosol transmission is likely to occur, based on the effects of environmental conditions and differences between virus strains. The microthread methodology can also be applied to other pathogens in order to investigate the role aerosol transmission plays in the spread of disease.

Facemask sampling and the exhaled microbiome.

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Abstract

It is now clear that the lower respiratory tract has a resident microbiome and that, along with invading pathogens, some of its microbes are expelled during tidal breathing without coughing or sneezing. The pattern and significance of these expulsions and the underlying mechanisms are not established.

Facemask sampling (FMS) is a newly established method enabling assessment of microbes exhaled during natural or directed activities. Although primarily developed to quantify infectiousness, the approach enables study of the abundance and identity of the microbes we naturally breathe out. Here, we present our investigations into the exhaled microbiota obtained by FMS from volunteers participating in clinical and non-clinical studies. Using sampling times from 15 minutes up to 8 hours with masks presenting either gelatine or polyvinyl alcohol sampling matrices and subsequent analysis by PCR, we show that FMS detects exhalation of at least 10^3 bacterial cells per minute from tidal breathing in most individuals. The effects of sampling time and respiratory efforts including vocalising, coughing and breath holding on exhalation to enhance bronchial fluid film burst, will be presented. Using assays of surfactant protein A and enzymes (e.g. amylase, lysozyme), we attempt interpretation of the abundance, phylogenetic distribution and sites of origin of the bacterial signals detected. In addition, we have used exhaled particle detection, analysis of spatial distributions of signals and size fractionating FMS to explore the droplet sizes carrying the microbial signals we detect.

FMS opens up our opportunities to study the nature and significance of the human exhaled microbiome.

Spatiotemporal stability of the air microbiome: Implications for simulation of bioaerosol backgrounds in aerosol test chambers

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Abstract

Efficient detection and identification of airborne biological threats is critical for timely response and public safety. However, assessing effectiveness of monitoring equipment in real-life scenarios is challenging. This study aims to develop a biological air background simulation enabling evaluation of equipment in aerosol test chambers with a microbial composition representative across spatiotemporal variation.

Air samples were collected over 8-hour periods in Kjeller (suburban/rural), Elverum (battlefield), and Svalbard (Arctic), Norway. Samples were shotgun sequenced and classified to identify the distribution of fungal and bacterial sequences over time and space. The top 20 species were used to decide the species temporal stability (year, season, day, within-day) and was also compared to published data to decide their spatial resolution.

Fungal sequences predominated (60%), although temporal and spatial variations were observed. Estimates based on the number of reads, average genome sizes, and bioaerosol particle number, indicated concentrations of 1×10^4 fungi/m^3 and 7×10^4 bacteria/m^3 which was consistent with literature. Five bacterial and two fungal species displayed high temporal stability across consecutive years, seasons, days, and within-days. Additionally, several species exhibited stability across seasons, days, and within-days. Several species/genera appeared in diverse global environments when compared to published data.

Shotgun sequencing of air samples revealed bacterial and fungal species with notable temporal stability and spatial resolution, suggesting their utility in simulated aerosol backgrounds as representative bioaerosol species. Simulated real-world backgrounds can be used, in conjunction with live agent, in aerosol test chambers for enhanced test and evaluation of biological detection, identification, and monitoring systems.

Real-time detection and characterisation of biological particles in aerosols

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Abstract

Characterisation of biological particles in aerosols (bioaerosols) and the interaction between their abiotic and biological components remains understudied. This significantly limits understanding of the role and impact of bioaerosols in the context of public health (allergenicity, toxicity, infectivity), climate (absorbing/scattering light) and ecosystems (nutrient transport/ dispersal of reproductive units). The existing evidence base on the detection and characterisation of bioaerosols stems from disconnected scientific disciplines each with its own perspectives and methods. Whilst these analytical approaches have advanced specific knowledge of the biological particles in aerosols, they have limitations in advancing understanding of the spatio-temporal characteristics of bioaerosols as well as the transformation and the governing influences on viability, toxicity and infectivity during airborne transport in a complex physico-chemical and biological matrix of aerosols in diverse environments. Step-change in the understanding of the bioaerosols can be achieved through the characterisation of single particles in real-time. Of the many methods developed to rapidly characterise airborne biological particles, fluorescence spectroscopy has shown promise to broadly classify organic compounds including those relevant to biological particles in aerosols in real-time. This presentation will offer an overview of the capabilities of single particle ultraviolet light-induced fluorescence detection systems combined with optical particle measurements to provide real-time quantification and temporal characterisation of bioaerosols in diverse environments and how this can provide a step change in understanding of bioaerosols in public and planetary health.

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Using saliva as a research material to measure the airborne viability of bacteria and viruses

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Abstract

Understanding the drivers of microbial decay in the aerosol phase is important for understanding how infectious respiratory diseases propagate between individuals. However, replicating respiratory aerosol is challenging due to the respiratory fluid composition being heterogenous and highly specific from person to person, and over time. The project looks to compare the airborne viability of bacteria and virus in surrogates of respiratory fluid and compare those with real respiratory fluid samples to ascertain a robust model of airborne disease transmission. Viability measurements are made using Controlled Electrodynamic Levitation and Extraction of Bioaerosol onto Substrate (CELEBS) in tandem with comparative kinetics electrodynamic balance (CK-EDB) measurements and allow for direct comparison between viability and evaporation kinetics of the aerosol as a function of time.

This research confirms previous findings, where physicochemical processes unique to evaporating respiratory aerosol drive the gradual loss of viability. Explicitly, water and solvent evaporation from the aerosol alongside carbon dioxide (CO_2) flux resulting in non-physiological conditions inside the respiratory aerosol. By building complexity into respiratory fluid surrogate formulations, through the introduction of clinically relevant concentrations of mucin, we report an increased airborne viability of coronavirus. However, an increased aerostability was not evident with *E coli* implying different mechanisms for biological decay between virus and bacteria in the aerosol phase. Advancing this technique further, from the initial model organisms, we describe the airborne viability of bacteria species more relevant to endemic respiratory diseases in our society currently.

Airborne SARS-CoV-2 in healthcare settings – when, where and how to prevent it?

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Abstract

Background

In order to improve infection control guidelines for healthcare workers, we investigated SARS-CoV-2 presence and risk factors for airborne transmission in healthcare settings during the covid-19 pandemic.

Methods

Briefly, a liquid cyclone was used for air sample collection close to patients at several wards, and an 8-stage NGI impactor (ranging from 0.1 to >8.1µm) was used for a longer measurement campaign in corridors of infectious disease wards. RT-qPCR was used for SARS-CoV-2 detection in collected air samples.

Results

During 2020 and 2021, we collected a unique material of 1100 air samples from several hospital environments, as summarized in Table 1. In patient rooms, across all collected samples, 10.6% were positive for SARS-CoV-2 by RT-qPCR. Shorter distance to the patient, higher patient viral load, and fewer days since symptom onset increased the risk of obtaining a positive air sample, while increased ventilation lowered the risk. So called aerosol-generating procedures (AGPs), which were of initial concern, were less significant.

In corridors, the positivity rate was only 2.7%. The large number of samples from corridors is attributed to the size-fractionated collection method, where 8 size fractions were collected every week. Positive samples were found in particle sizes ranging from 0.1-8.1 µm, with the majority of positive samples in size fractions below 1.7 µm.

Conclusion

Airborne SARS-CoV-2 RNA was mainly found close to patients in healthcare settings. Patient characteristics and ventilation rates significantly influenced the risk of a positive air sample, indicating that infection control measures should be aimed at controlling these factors.

Far-UVC (222 nm) efficiently inactivates microorganisms in the air and can produce measurable Ozone in a single-bed-sized chamber.

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Abstract

Far-UVC (222 nm) radiation can inactivate various microorganisms, including SARS-CoV-2. However, the inactivation efficiency of different microorganisms in the air and on surfaces at various ventilation rates is still unknown. Also not well defined is the potential ozone generation at 222 nm. This study has carried out experimental measurements in a room-scale chamber (32 m^3) to evaluate the performance of filtered Krypton-Chloride (KrCl) lamps in reducing the concentration of microorganisms in the air and on surfaces and has measured the production of ozone in indoor settings.

Aerosolised *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (NCIMB 10848) were released into the chamber using a 6-jet Collison nebuliser at four ventilation rates (1.5, 3, 6, and 9 air-changes-per-hour (ACH)) under steady-state conditions. The air sampling was collected using an Anderson air sampler.

Results show that the microorganisms' inactivation in the air is effective across a range of ventilation rates. At 3 ACH, the reduction reached 95.5% when using 1 lamp and 97.8% when using 5 lamps. Ozone levels measured inside the chamber increased with increasing number of Far-UVC lamps with an average room irradiance of 2.54 µW cm^{-2}.

The findings indicate that Far-UVC is a viable technology that operates at safe exposure and that can effectively enhance airborne infection control. These findings pave the way for future work to explore its efficacy in real-world scenarios as well as studies to quantify usability and acceptability.

Transmission of tuberculosis: *Mycobacterium tuberculosis* adaptation for entry into and within aerosol droplets

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Abstract

Tuberculosis (TB) is the single greatest cause of death from any bacterial disease. Globally, more than one-quarter of the population harbour the agent of TB, *Mycobacterium tuberculosis* (Mtb) in their lungs, testament to its transmission success. Mtb transmits between individuals via aerosol droplets expelled from the lungs of those infected. Surprisingly, little is known of the properties of Mtb that promote this. Understanding factors that impact Mtb entry into aerosol droplets, the physiological state of Mtb as it does so, and how it behaves and responds therein to changing physicochemical conditions following release (e.g. changes in temperature or osmolarity) will identify new opportunities to control TB. We have sampled Mtb aerosols and sputum samples expelled by TB patients and examined bacterial transcriptome within. Preliminary results indicate that aerosols contain bacterial populations enriched in Mtb and have properties not recognised in previous studies. In order to investigate how these properties may change in response to changes in environment on entry into, and residence within in aerosol droplets, we first investigated the survival of Mtb in aerosols. We maintained the bacterial aerosol in a Goldberg Drum and found that both the Mtb strain, H37Rv, and related vaccine strain, BCG, survived well, with a decline in culturability of 50% after 2 hours. We sampled Mtb H37Rv to measure changes in gene expression before entry into, and within aerosol for up to 2h. We identify distinct gene expression profiles, with evidence that Mtb responds to both the nebulisation process and aerosol residence.

Characterization of a Tower System for Respiratory Delivery of Medical Countermeasures

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Abstract

We have recently acquired a Buxco nose-only 'SmartStudy' inhalation tower system to evaluate whether respiratory delivery of an antiviral to mice and ferrets would be efficacious for treatment of viral encephalitis caused by alphaviruses. The SmartStudy system allows for precision controlled respiratory delivery using accumulated tidal volume-based exposures. Prior to initiating animal studies, we have performed initial characterization of the system using bovine serum albumin (BSA) as a surrogate for protein drug delivery. Higher levels of relative humidity (>40% RH) in the system had a negative impact on the ability of the photometer and filter samplers to reliably determine the aerosol concentration of BSA. Higher nebulizer flow rates increased RH in the tower unless an extended inline dryer was added to the system. Increasing the number of chambers attached to the tower also increased the time required to achieve the desired dose which could be overcome by increasing nebulizer flow rate. Using simulated breathing, delivering 200 ug of aerosolized BSA to four mice simultaneously would require approximately 10 minutes at a nebulizer flow rate to 0.05 liters per minute. With the use of the extended inline dryer, this did not increase RH above 40%. We are moving forward with rodent studies to assess lung deposition and efficacy against encephalitic alphaviruses.

Aerosol sample collection based detection of Foot and Mouth Disease Virus (FMDV) 48H prior to clinical signs.

Stanislaw Koper

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Abstract

Background:

Viral aerosols present an escalating risk to agriculture, animal farming, and public health. Intensified farming practices and densely populated locales heighten the vulnerability to infectious diseases. Early pathogen detection is paramount for mitigating loss of life and curtailing economic impacts.

Methods:

In this study, three specialised aerosol collection devices, comprising two filter-based samplers and one electrostatic precipitator (ESP), were evaluated alongside three established commercial samplers: Coriolis μ, Coriolis Compact, and NIOSH BC251. An infectious Foot-and-Mouth Disease (FMDV) challenge was executed in swine at The Pirbright Institute. Aerosol monitoring was performed throughout the study to ascertain efficacy of each device in detection of viral RNA in aerosols.

Results:

The findings indicate successful detection of FMDV RNA in aerosols 48 hours prior to the observation of clinical signs across all commercial & specialised but one specialised filter-based sampler did not. The ESP demonstrated superior performance, achieving viral detection at concentrations two orders of magnitude higher than those recorded by the commercial systems. Simplicity of the specialised EPS collector onto a microscope slide allows rapid processing and concentration of sample. A 15-minute sampling interval proved sufficient for positive detection across all devices. It is important to nite that the samplers positioning and pig activity influenced the measurable viral concentration.

Conclusions:

This investigation confirms the feasibility of detecting FMDV aerosols from infected pigs up to two days before clinical symptoms were observed. Such pre-emptive detection reinforces the viability of aerosol surveillance in extensive farming operations and has potential applications for monitoring multiple airborne pathogens.

Characterisation of aerosol recovery from hydrophobic collection surfaces using a mechanised droplet actuation system

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Abstract

Background: Electrostatic precipitators (ESP) are compact aerosol collectors, however, efficient reliable recovery of aerosol from the collector surface is critical for their wider adoption. This research characterizes the recovery of collected aerosol particles from hydrophobic surfaces using low-volume droplets.

Methods: Fluorescent particles of 1 µm to 4.8 µm were nebulised over a wide range of concentrations. An ESP was used to collect the aerosols onto a hydrophobic coated microscope slide. The recovery of the solids was achieved using 9 ul droplets of DI water containing 0.01 % Tween-80. An automated device was developed to move the droplets across the microscope slide in a repeatable and precise manner. The recovery efficiency was determined by microscopy particle counts before and after recovery.

Results: A recovery efficiency of 97 % was achieved for a particle concentration on a plate of 100 count/mm^2. The recovery efficiency remained high up to a plate concentration of 723 count/mm^2. However, concentrations of particles where it nearly covered the entire plate resulted in the inability to actuate the droplet. The difficulty was due to the change in surface characteristics since a pre-loaded droplet of particles (1.2×10^8 count/ml) showed no change in contact angle and its actuation across the plate resulted in no loss of particles. The recovery efficiency remained high at all tested particle sizes.

Conclusion: Droplet-based recovery of aerosol particles demonstrated high efficiency across all tested particle concentrations and sizes, suggesting that this may be a useful technique for a wide range of aerosol monitoring applications.

Investigating the Dynamics of Respiratory Droplet and Aerosol Transmission: Insights from the IMADGENN Study

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Abstract

Understanding respiratory aerosols in the context of pathogen transmission presents a complex challenge for public health. This study examined the generation and spread of respiratory droplets and aerosols, utilizing commensal upper respiratory tract bacteria as surrogates for respiratory pathogens. Utilizing a purpose-built Isolator to Measure Aerosol and Droplet GENeratioN (IMADGENN), our study investigated how individuals differ in terms of the aerosols and droplets produced during various respiratory activities including loud speech and the effectiveness of face coverings in reducing dispersal. We employed both passive and active air sampling to capture respiratory particles generated by healthy volunteers (n=31). Analysis was carried out using microbiological and molecular techniques with the data collected also being used to optimize methods used for detecting airborne respiratory organisms.

Our results revealed substantial inter-individual variability in aerosol and droplet production with a small number of 'super-spreaders' being responsible for the majority of bacterial dispersal. Face coverings significantly curtailed aerosol and droplet emission, especially when used by the high emitters. For all participants, activities that involved elevated vocal effort increased aerosol generation with each decibel increase in speech volume equating to a 34% increase in the likelihood of detecting higher levels of airborne bacteria.

This research demonstrates how the dispersal of respiratory droplets and aerosols differs with individual and highlights the potential role of 'super-spreaders' in transmission of infection. Activities increasing droplet and aerosol production are more significant amongst these individuals. Face coverings notably reduce emissions, and have highest impact when worn by 'super-spreaders'.

Modelling multiple routes of transmission in complex environments – using quantitative microbial risk assessment approaches with detailed human behaviour simulation

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Abstract

Modelling can act as a tool to synthesise knowledge on disease transmission and improve understanding of the importance of disease transmission routes in different scenarios. Quantitative mechanistic models can also provide information on the relative benefits of mitigation measures in reducing the likelihood of disease transmission and support better decision making. During the COVID-19 pandemic we developed quantitative microbial risk assessment models for transmission in transport and workplace settings. These models included small aerosol, large droplet and surface mediated transmission routes. To generate these models we used detailed descriptions of occupant behaviour, including movement, activity and touch as well as probabilistic descriptions of key parameters, such as viral load, that determine infectivity. The models were used to study a number of scenarios and mitigation options. They were run repeatedly with variation to build up a picture of the range of outcomes for each case. Under the assumptions used, the results suggested clear differences between the dose received via different transmission routes. The variability via each route also differed considerably, highlighting the importance of considering a large number of cases and individuals when assessing scenarios. The impact of different occupant behaviours on exposure was striking. For example, for workplaces, minimising interactions between offices, avoiding the use of shared spaces and removing contact with frequently touched surfaces greatly reduced exposure and transfer between occupants in different offices.Mechanistic models provide rich information on transmission and the representation of detailed human behaviour is critical to accurate predictions as well as good quality microbiological data.

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