Antimicrobial resistance in bioaerosols: towards a national surveillance strategy

Chief Scientist's Group report

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Dr Robert Bradburne
Chief Scientist

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Executive summary

Infections that do not respond to antimicrobial treatment are of increasing concern, representing a significant global threat that needs to be managed in order to protect human, animal and plant health. While antimicrobial resistance in clinical settings has received significant attention, comparably little attention has been given to the emergence and proliferation of antimicrobial resistant microorganisms in the environment. As well as land and water, air is increasingly also considered as an important environmental dissemination route for antimicrobial resistance, especially since airborne microorganisms can potentially travel great distances and affect vulnerable receptors far from the source.

This report gathered background information required to design a UK surveillance programme for airborne antimicrobial resistance. The approach consisted of:

- **An evaluation of different bioaerosol sampling methods**. This consisted of an expertise-informed evaluation of existing methods, followed by a practical evaluation via a field-based trials comparing potential methods for sampling source-associated and ambient air.
- **An assessment of the current scientific understanding of airborne antimicrobial resistance.** A rapid evidence assessment of the scientific literature relevant to the UK was performed to summarise the existing evidence on antimicrobial resistance targets and their prevalence in bioaerosols, methods for their detection and quantification, and factors influencing their dispersion. Additionally, evidence of the risk AMR in bioaerosols poses to human health was assessed.
- **A comparison of existing air quality surveillance operations in the UK** in order to identify cost-effective and pragmatic mechanisms for initiating a surveillance strategy for airborne antimicrobial resistance at a national scale.

We identified:

- **Effective methods for bioaerosol sampling** that permit flexibility in downstream analysis in both source-associated and ambient air.
- **Critical knowledge gaps** in the understanding of antimicrobial resistance in bioaerosols that need to be addressed in order to develop a fully optimised and operational surveillance programme.
- **Existing air quality surveillance operations** that could be leveraged to deploy early-stage monitoring rapidly and cheaply for antimicrobial resistance in bioaerosols in the UK.

Finally, steps are proposed for monitoring airborne antimicrobial resistance in both source and receptor environments in the UK. The approach consists of:

- **Establishing the prevalence, density and characteristics of AMR in bioaerosols at source and ambient environments.** Bioaerosol collection should include active sampling to enable a comparison of antimicrobial resistance in air across different locations. The AMR endpoints for analysis should include quantifying sentinel

antimicrobial resistant microorganisms densities using culture-based methods specifically for azole-resistant *Aspergillus fumigatus*, methicillin-resistant *Staphylococcus aureus*, and extended-spectrum beta-lactamase-producing *Escherichia coli.* Culture-independent techniques should also be used to characterise multiple surveillance targets in whole microbial communities simultaneously, in order to identify potential risks posed by antimicrobial resistance in other species and identify additional targets for future surveillance.

- **Source-focussed monitoring of AMR in bioaerosols based on the M9 framework to understand source apportionment.** AMR-focussed bioaerosol sampling could be deployed alongside samplers being used for statutory bioaerosol monitoring in order to combine and save resources.
- **Receptor-focussed monitoring of AMR in bioaerosols based on the Automatic Urban and Rural Network.** Deployment of samplers in the locations used by this existing air quality network would be an effective way to achieve large geographic coverage of air samples representing the quality of air to which the public are exposed. This will also link airborne AMR to other elements of air quality.

Developing a national surveillance strategy should proceed in stages, with each stage accruing evidence to better inform the next stage of surveillance strategy development and expanding to protect other critical receptors (such as animal and plant receptors). This will lead to a comprehensive, robust and informed strategy for monitoring AMR in bioaerosols in the UK.

Scope

- **Introduce** a source-receptor approach to surveillance of AMR
- **Review** existing literature to give an updated assessment of current state if scientific understanding of airborne AMR and its measurement in both sources and receptor environments
- **Pilot** methods for surveillance of airborne AMR in the field in both source and receptor environments
- **Review** existing air pollution surveillance models as models for deploying an airborne AMR surveillance strategy across
- **Recommend** an early-stage strategy for monitoring airborne AMR in source and receptor environments in the UK

Background

Infections that do not respond to antimicrobial treatment are of increasing concern. In 2019, there were an estimated 1.27 million human deaths directly attributable to antibiotic resistant infections (Murray *et al.*, 2022) - a number that is expected to rise and result in over 10 million deaths each year globally by 2050 (O'Neill, 2016). Resistant fungal disease is also of increasing concern, especially considering the comparably limited number of antifungal drugs available when compared to antibiotics (Antimicrobial Resistance Division, 2022; Fisher *et al.*, 2022). Antimicrobial resistance (AMR) therefore represents a significant global health threat that needs to be managed in order to protect human (as well as animal and plant) health for future generations.

Whilst the onus on managing the human health risk posed by AMR has conventionally fallen on those working in clinical and public health settings, a role for environmental surveillance in managing the AMR threat is also increasingly recognised (HM Government, 2019). Natural environments can act as direct reservoirs of the antimicrobial resistant pathogenic microorganisms commonly encountered in the clinic, which humans may acquire through interaction with the natural environment (e.g., via bathing in polluted water bodies). The environment is also a reservoir of AMR genes that predate the human use of antibiotics, or even the origins of humans themselves. Antimicrobial resistance genes emerge in clinical pathogens through complex processes that are poorly understood. Therefore, surveillance of antimicrobial resistant microorganisms, mobile genetic elements and resistance genes in the natural environment is increasingly being considered and put into practice by policymakers.

1. Current scientific understanding of airborne AMR

Antimicrobial resistance in the natural environment is diverse, complex, and multifaceted, making it challenging to monitor and regulate. There are many ways AMR enters the environment from other sources (Singer et al., 2016), although in practice, a few human activities that involve both high antimicrobial usage and high densities of microbial material are often the focus of researchers, regulators and policymakers. Such activities impose strong 'selective pressure', enriching or amplifying resistance and transmission within and between human, animal and environmental microbiomes. Particular activities of concern include:

- **Human and animal waste processing sites:** Human waste (e.g. municipal, hospital, industrial) and animal waste (e.g. livestock) can contain high levels of antimicrobial compounds and resistant microorganisms, which can then enter the environment through sewage treatment plants, agricultural runoff, or directly through poorly managed waste disposal.

- **Agricultural sites:** Antimicrobials, particularly antibiotics, are widely used in livestock farming to treat and prevent disease (and to promote growth in some countries), as well as in arable farming (particularly antifungals). This can lead to the spread of AMR through animal waste, soil, and water.

Pollution sites: Hotspots of pharmaceutical, industrial or municipal pollution can also contribute to the spread of AMR through the release of compounds with antimicrobial properties, along with resistant microorganisms, into the environment.

Assessing the current understanding of airborne AMR is a key step to informing the design of a potential UK surveillance strategy for airborne AMR. A rapid evidence assessment was conducted, summarising the relevant literature on airborne AMR, to identify the state of the evidence base upon which a sampling strategy may be developed and justified. A detailed summary of findings is presented in the full, appended report (Appendix A - Rapid Evidence Assessment for AMR in bioaerosols).

Briefly, the rapid evidence assessment on AMR in bioaerosols reviewed the available data on the detection and prevalence of AMR in bioaerosols across various source and receptor environments, including wastewater treatment plants, livestock farming (pigs, poultry, cattle), and ambient settings. It also considered the available evidence of factors that influence the variation and dispersion of AMR in bioaerosols, and the hazard AMR in bioaerosols poses to human health. The review focussed on studies conducted in high-income countries, similar to the UK, to generate insight into the types and prevalence of different AMR markers likely to be relevant in a UK context. This is because AMR in bioaerosols is expected to represent source material (George *et al.*, 2022), and resistomes appear to vary by geography (Hendriksen *et al.*, 2019). All studies indicate that various AMR microorganisms or resistance genes are present in bioaerosols collected from studied environments. Some of the data were over 15 years old, meaning that some AMR

prevalence reported in the literature may not reflect current levels. For example, environmental AMR levels may have changed over time, either due to rising prevalence, or to initiatives aimed at reducing antibiotic use in humans and animals.

AMR endpoints targeted

Diverse AMR markers have been quantified in bioaerosols using culture-independent and culture-dependent techniques and covering phenotypic resistance to a large range of antibiotics and antibiotic classes, as well as genotypic measures, such as the detection of antibiotic resistance genes and gene subtypes. Reports of detecting antifungal resistance markers in bioaerosols were limited to azole-resistant *Aspergillus fumigatus* and cycloheximide-resistant fungi using culture-dependent methods. Overall, AMR marker selection by researchers appears to have been biased toward pathogens and/or genes posing a direct public health risk. For example, in ambient air, investigators typically targeted diverse sources or ones associated with particular AMR threats that were of considered emerging epidemiological threats at the time of study (e.g., methicillin-resistant *Staphylococcus aureu*s). In source-associated environments, the AMR markers measured in bioaerosols included pathogens as well as resistance genes targets justified based on the antibiotics used and/or the material being aerosolised. The prevalence of each AMR target, when quantified, was reported in different ways, ranging from reporting percentage of total isolates that were phenotypically resistant to an antimicrobial, to the density of resistant isolates in a given volume of air, or to the relative abundance of anti-microbial resistant genes (ARGs), (e.g., ARGs per 16S rRNA copy number, or per ng DNA). This made drawing general conclusions about prevalence and density of resistance very difficult, though extracted information for specific studies and targets is provided in our full literature review (Appendix A – Rapid Evidence Assessment for AMR in bioaerosols).

Spatial patterns

Studies explored a few environmental variables likely to influence the dissemination of AMR via bioaerosols. A consistent observation of a distance-decay relationship was made, whereby the abundance of AMR markers decreases with increasing distance from the source. This is in line with the literature on dispersion of bioaerosols generally from specific emission sources. Furthermore, downwind samples tended to have higher relative abundance of AMR (gene copy numbers per ng DNA (Gaviria-Figueroa *et al.*, 2019) or percentage of viable resistant colonies recovered per m^3 air (Gibbs *et al.*, 2006) compared to upwind samples, with some studies indicating detection of AMR over 100 metres downwind from source or even many kilometres away. Wind speed and direction is expected to strongly influence the location and extent of AMR dispersal relative to the source. While not directly investigated by any of the included studies, topography in the local area was often discussed in relation to interpreting study findings: the natural and artificial features of the local landscape could serve to promote, or reduce, air circulation and thus AMR dispersal. There were no data on the effect of rainfall on AMR dispersion in the literature, as this affects the sampling equipment. In general, precipitation is expected to reduce AMR in bioaerosols as it promotes wet deposition, though moist conditions may prevent viable

airborne microorganisms from desiccation. Many species of bacteria and fungi create spores, which are more tolerant to environmental stresses (e.g., UV, temperature, moisture, nutrient availability etc.), and smaller than the microbial cells that produce them. Bacterial spores tend to be smaller (about 0.8µm to 1.2µm in length) than fungal spores (about 2µm to 100µm in length). Particle size is an important factor influencing microorganisms' dispersal by wind, with small particles becoming airborne more easily and remaining airborne for longer, and larger ones settling out more quickly. However, size appears not to be the only factors affecting dispersal: morphology, mass, and whether the particles clump easily may play important roles in the aerodynamics of microorganisms dispersal, as well as survival (Golan and Pringle, 2017).

Temporal patterns

Longitudinal study designs provided some data on how AMR prevalence varies over time. The frequency of sample collection varied across studies, with some papers reporting data on AMR variation over short time frames (days or weeks), whereas most performed sampling to capture and compare seasonal variations in AMR. Findings on seasonality were inconsistent across studies and may be due to the different methods used in each study, and the sources investigated.

Potential human health effects

The evidence indicates the atmospheric pathway to be a highly plausible but understudied transmission route for AMR. The evidence base on this topic is small, according to Stanton *et al.*, (2022)'s map of evidence on human exposure to, and transmission of antibiotic resistance from, natural environments. The reviewed studies focussed on human colonisation by a narrow selection of bacterial opportunistic pathogenic bacteria, in highly exposed study populations typically involving occupational exposures. The occurrence of azole-resistant respiratory infections caused by fungi (such as *Aspergillus fumigatus)* in patients without previous azole use indicates inhalation of AMR fungal spores, known to be present in the environment (Dauchy *et al.*, 2018; Jeanvoine *et al.*, 2020). Several studies in the review identified shared features of AMR across AMR source matrices, bioaerosols, and AMR analysed in human samples. In addition, genes conferring resistance to clinically important antibiotics recovered from bioaerosols samples were shown to be mobile, meaning that these genes could be acquired by human commensals or pathogens.

Key knowledge gaps

AMR in bioaerosols is an emerging area of science, and there are significant knowledge gaps. The lack of key information is a barrier to the immediate design of a surveillance strategy. Designing such a strategy is, therefore, likely to be an iterative process involving a number of stages of evidence gathering.

Some of the key knowledge gaps in AMR in bioaerosols that are pertinent to a sampling strategy include:

- **Limited UK-specific data across all key environments**. Current baseline data on the prevalence, temporal variation of AMR in bioaerosols across priority sources (e.g., WWTP, livestock, composting) and receptor environments (e.g., residential/urban locations) is needed. In particular, a baseline assessment of AMR in the atmosphere is required in order to shape specific surveillance objectives, and to inform answers to design questions such as spatial and temporal coverage needed to detect changes in ambient AMR with a given level of statistical confidence.
- **AMR transmission pathways**: The mechanisms and pathways by which AMR are transferred between sources and receptors found in bioaerosols are not well understood. Better understanding of these mechanisms is essential for developing effective control strategies.
- **Temporal Variation**: Lack of data on temporal variation of AMR within Bioaerosols.
- **Risk assessment**: There is a lack of standardised methods for assessing the risk of AMR transmission to human health via bioaerosols. Further research is needed to develop appropriate risk assessment tools and guidelines.
- **Detection methods**: Currently available methods for detecting AMR genes and bacteria/fungi in bioaerosols are not optimised for this purpose.
- **Exposure assessment**: The extent and frequency of human exposure to AMR via bioaerosols are not well understood. Further research is needed to quantify exposure levels and determine the associated health risks.
- **Control strategies**: Because sources and pathways to exposure are not well understood and quantified, there is a lack of effective control strategies for preventing the spread of AMR via bioaerosols. Further research is needed to develop and evaluate control strategies, including process and engineering controls, ventilation, and disinfection, as well as changes to mitigate drivers promoting the development of AMR in the organisms that become bioaerosol components.

Overall, the above knowledge gaps in AMR in bioaerosols highlight the need to understand the nature and extent of AMR in bioaerosols, to understand risks, transmission pathways, and control strategies related to AMR in bioaerosols. Further to this, given that such sources of AMR are often associated not just with high levels of antimicrobials, but also high levels of soil or water pollution, the few environmental AMR surveillance programmes that have been suggested/proposed to date have focussed on monitoring AMR in terrestrial and aquatic matrices. For example, the WHO's Tricycle protocol for One Health surveillance of extended spectrum beta-lactamase producing *E.- coli* includes sub-protocols for monitoring rivers and aquatic pollution sites.

Lessons Learnt

The lessons learned from such environmental AMR surveillance programmes on monitoring AMR in terrestrial and aquatic matrices can be applied to air sampling for AMR surveillance. For example, one important lesson is the need to identify clear objectives and endpoints for the air sampling programme. In terms of endpoints, the sampling programme should aim to detect the presence or absence of specific AMR genes or organisms in the air samples. This may involve using PCR or other molecular methods to identify the presence of resistance genes or sequencing methods to identify the microbial species and their resistance profiles.

Another lesson is the importance of selecting appropriate sampling methods and protocols for air sampling. The selection of appropriate methods will depend on the type of AMR being monitored and the specific locations to be sampled. For example, if the goal is to monitor AMR associated with animal agriculture, air sampling should be focused on livestock facilities or manure storage areas, using focussed high-flow rate sampling methods, as well. On the other hand, if the goal is to monitor AMR associated with hospital environments, air sampling would need to be focused on patient rooms or areas where antibiotic use is common using discrete unobtrusive filtration methods. Finally, it is important to consider the potential for environmental contamination to impact air sampling results. For example, high levels of soil or water pollution in the surrounding environment may impact the levels of AMR detected in the air samples. Therefore, it may be necessary to monitor these potential confounding factors and adjust sampling strategies accordingly. Addressing these knowledge gaps will be critical for developing effective strategies to monitor and mitigate the spread of AMR and protect public health.

2. Bioaerosol sampling methods for AMR detection in the atmosphere

Detecting AMR in different outdoor environments using bioaerosol sampling methods requires careful consideration of various factors such as the:

- specific objectives of surveillance,
- type of environment, and,
- potential sources of AMR.

Scientific expertise and existing literature can help in selecting appropriate sampling methods, as many sampling methods are available and standardised procedures have been established only for specific regulatory purposes. Other factors such as downstream analyses, sampler type, sampling frequency and duration, location, performance checks, and control parameters all contribute to the choice of sampling methods. However, comparing results from different studies can be challenging due to the use of different samplers that introduce biases and show high variability. In addition, there is no consensus on standardised metadata requirements, and sampling design can affect the representativeness of the bioaerosol sample. Where differing sampling methods are applied, this limits comparisons between studies due to different sampler variabilities and bias. These challenges make bioaerosol research and monitoring challenging for end-users, regulators, and health officials, particularly in terms of interpreting data in relation to health exposure (Environment Agency, 2022; Whitby *et al.*, 2022).

Active *versus* passive sampling approaches

There are two different sampling approaches as summarised in Figure 1. Active sampling methods involve physically drawing a known volume of air through or over a particle collection device, such as a liquid or solid media (Whitby *et al.*, 2022). The sampler inlet must be designed to maintain the airflow and accurately reflect the particle size of the sampled air. Results are typically expressed as colony forming units per cubic metre (CFU m^{-3}), but other metrics are also used (such as particle number m^{-3} or relative abundance). Active sampling is useful when low concentrations of microorganisms are expected, such as hospital operating theatres, and allows for quantification of bioaerosol concentration. Different types of devices, including air impactors, impingers, and filtration systems, can be used for active sampling (Environment Agency, 2022). In contrast, passive sampling methods do not involve the use of air pumps but instead rely on gravitational settling, electrostatic attraction, or a combination of both, to collect particles onto a collection surface such as a Petri dish with culture media (known as "settle plates"). Settle plates are exposed to the air for a specified time and then incubated (Whitby *et al.*, 2022). However, gravitational settling is affected by particle size, shape, and air motion, with larger particles preferentially captured. Smaller, lighter particles may remain suspended in the air for longer periods therefore passive methods using settling plates require careful decontamination because the collection surface is exposed to the ambient air, which may contain a variety of microorganisms, including potential pathogens and antibiotic-resistant bacteria (Whitby *et*

al., 2022). These microorganisms can settle onto the collection surface prior to sampling having commenced, potentially contaminating the sample, leading to false positive results. Rutgers' Electrostatic Passive Sampler (REPS) is a recent development that improves particle capture by using polyvinylidene fluoride film for both electrostatic and gravitational capture (Therkorn *et al.*, 2017).

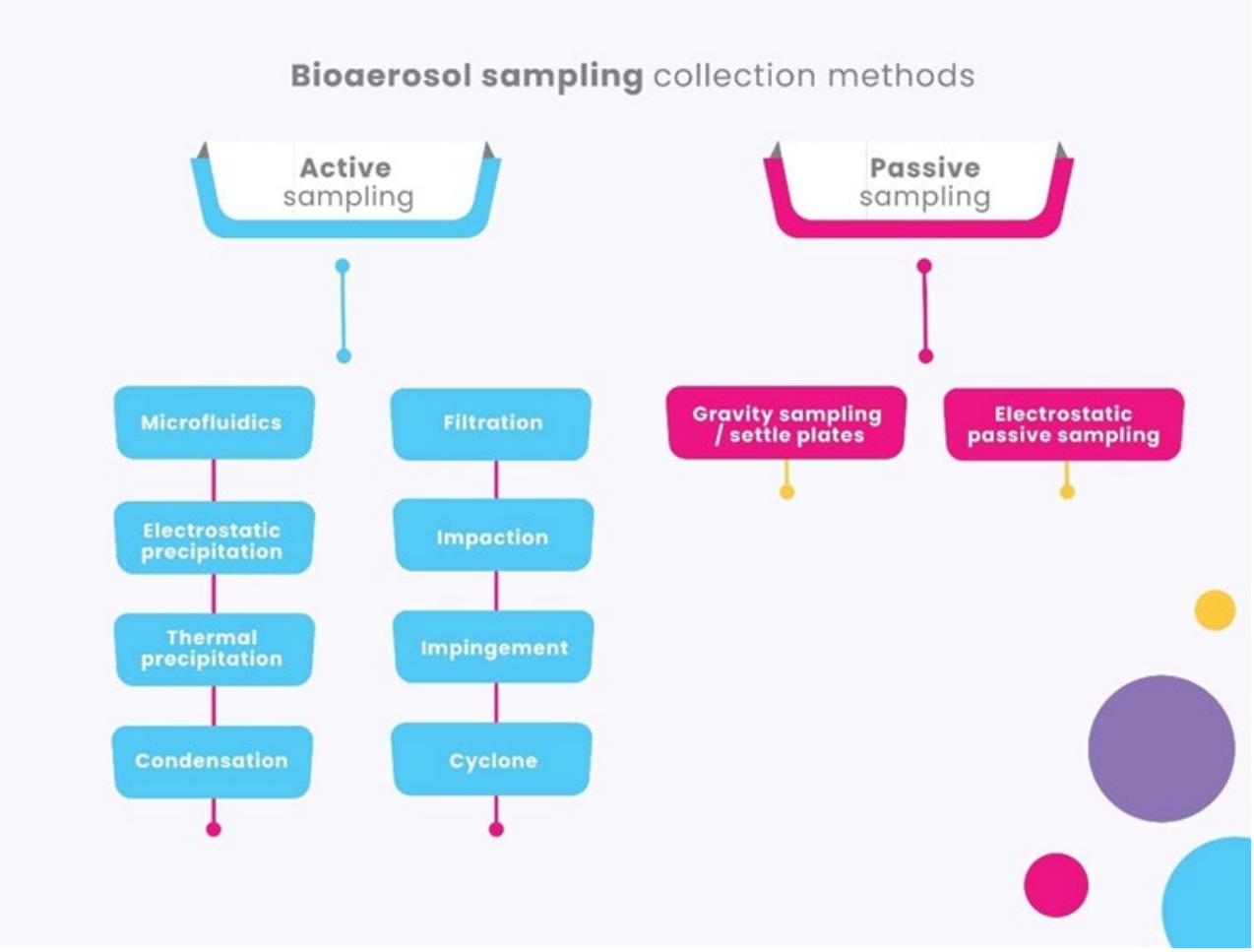

Figure 1. Flow chart summarising active and passive air sampling methods (reproduced from Whitby et al. 2022).

For further details on the list of typical samplers used for sampling bioaerosols along the advantages and disadvantages for each active and passive method, refer to Environment Agency (2022) and Whitby *et al.*, (2022).

Sample size, replication, and frequency considerations

The guidelines and recommendations on how to determine the optimal bioaerosol sample size to deploy and collection strategy for a site will vary depending on several factors, including the prevalence of AMR, the sensitivity and specificity of the detection method, and the desired level of confidence with respect to presence or absence of AMR. In addition, some general recommendations can be followed:

1. Determine the purpose of the sampling: The objective of the sampling will guide the number of samples and sampling strategy needed. For example, if the goal is to evaluate the general air quality of a specific site, fewer samples may be needed, but if the goal is to identify potential hazards for workers or risk to wider population, greater sample density may be required.
2. Use appropriate statistical methods: Statistical methods can help determine the optimal sample size and collection strategy. Consider factors such as the variability of the data, the desired level of confidence, and the level of precision required. Statistical methods such as power analysis can help determine the number of samples needed to achieve a specific level of precision. Selection and application of statistical methods for a surveillance network requires prior knowledge of the likely sources and distribution of AMR in the atmosphere and risk of emergence. Where this knowledge is unavailable, then a critical stage on the path to developing a network is to carry out work to plug knowledge gaps. The state of current knowledge is reviewed below.
3. Consider the size and layout of the area for sampling, and potential sources of AMR bioaerosols within it: The size and layout of monitoring at specific sites within the surveillance scheme will influence the number of samples and the sampling strategy needed for each type of site within a network. For example, sites known to be heterogeneous may require more samples, sampling locations should be representative of different areas of the site.
4. Existing protocols: Consider applicability of existing regulatory guidelines, such as the M9 guidance document published by the Environment Agency, which provides specific guidance on the monitoring of bioaerosols emitted from certain industrial operations, national and international guidance issued by standards bodies and occupational guidance used to sample workplaces and public spaces such as hospitals. Where these are appropriate to the objectives of surveillance for environmental AMR in bioaerosols, they offer the advantage of known performance characteristics and inter-compatibility of data with a wider base of evidence on bioaerosol source terms which may provide additional context when interpreting AMR measurements. Guidance typically recommends that sampling for exposure assessments should be conducted in the breathing zone of workers, where possible, and in areas with potential emission sources. Also consider the applicability of existing sampling protocols.
5. Sampling frequency: The sampling frequency should be determined by the level of exposure and the characteristics of emission. Periodic sampling may be sufficient where the risk of AMR emergence is low, while continuous or frequent sampling may be indicated for situations where the risk of emergence, and the likely consequences of emergence, are high. The number of samples should be sufficient to provide a statistically representative sample of the area or activity being monitored.

3. Field-based Trials of Bioaerosols Sampling methods

To establish an appropriate and suitable sampling strategy for AMR surveillance in bioaerosols, an initial sampling plan was developed (Appendix B). The sampling plan was based on a rapid evidence assessment, along with M9 Technical Guidance Note (Environmental monitoring of bioaerosols at regulated facilities), using a decision support tree as originally developed within the Environment Agency report "Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms" (Environment Agency 2022) and in consultation with the EA project manager. The sampling plan included identification of sampling sites/ locations, sampling design, (number of samples, volume, duration) and bioaerosol sampling methods/ equipment to allow for culture- based and culture- independent AMR focused downstream analysis by UKHSA.

In order to test the Sampling Plan Viability, with respect to its constraints and suitability for the collection of a range of AMR target species in real-world scenarios, two sampling campaigns were conducted:

1. a pilot study which measured repeatedly over time at a single site (Appendix C), and
2. a field trial using a variety of background locations at six separate sites across England (Appendix D).

The pilot study aimed to test the effectiveness of a range of sampling methods in real-world conditions; these included methods involving filtration, impingement, and cyclone, for longer and shorter durations and varying collection efficiencies under varied flow rates to identify suitable methods for the field trials. The objective of the pilot study and field trials was to generate experience with a shortlist of possible active bioaerosol sampling approaches that could be used in surveillance for AMR, specifically considering practical deployment and cost implications.

Figure 2, below, illustrates key decision points to determine the development of sampling plans for both the pilot study and field trials.

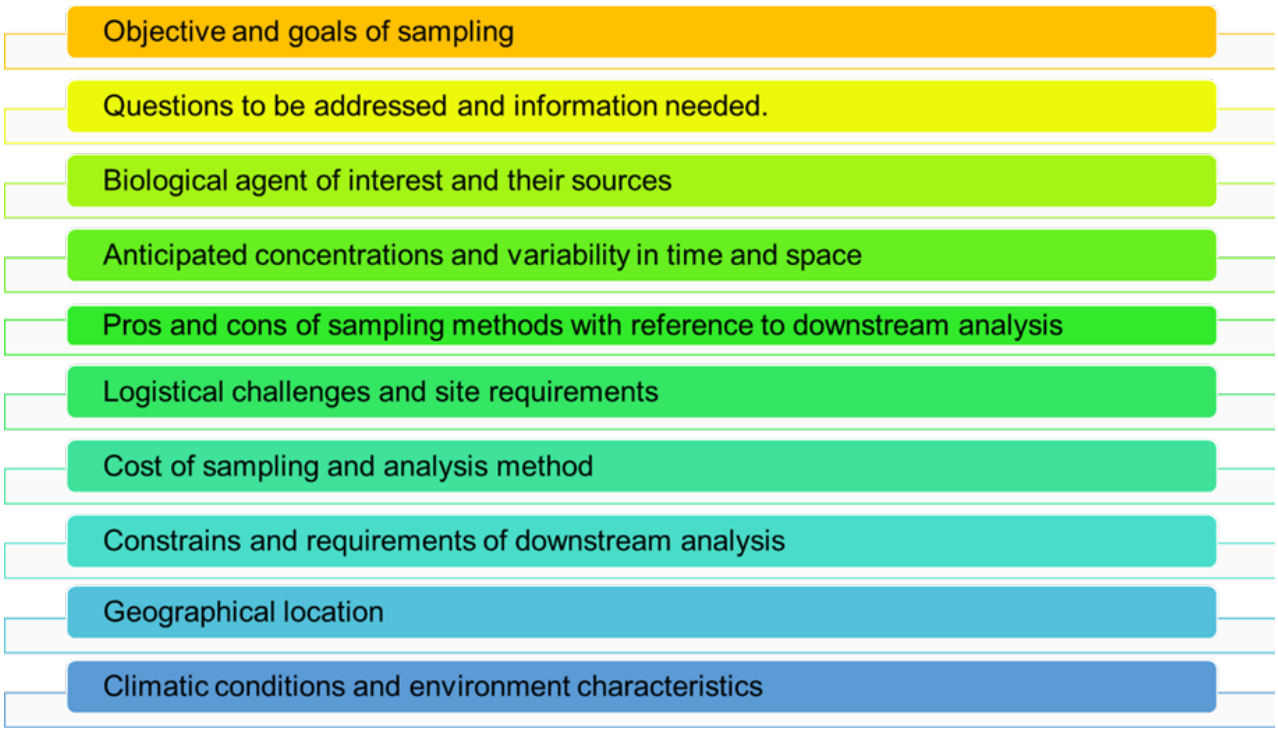

Figure 2. Key parameters of decision support framework to develop a sampling strategy for AMR in bioaerosols (adapted from Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms. Environment Agency, 2022).

Pilot trials

For the pilot study, sampling was conducted at the Cranfield sewage treatment works on multiple dates, including December 7th, 2022 and January 4th, 18th, 24th, and 31st, 2023. Two locations were selected for sampling: one near the trickle bed filters to represent the source and another upwind of the source to represent ambient air. Figure 3 displays the sampling setup adjacent to the source.

Figure 3. Pilot study sampler setup

These sampling methods involved filtration, impingement and cyclone for long and short durations, testing collection efficiencies for each of these under varied flow rates. The samplers used were filtration (IOM, Leckel with Bio inlet), impingement (SKC BioSampler) and cyclone (Coriolis Compact (dry cyclone)). Flow rates varied from 2l/min (IOM) to 50 l/min (Leckel with Bio-inlet, Coriolis Compact). Three repeated measurements were carried out for short (1) and long (2 hours) durations at the source and ambient location. In total, 48 samples were collected, excluding travel blanks as per the Sampling plan.

The collected samples were contained, labelled, and preserved in the fridge overnight and shipped next day to the UKHSA Laboratories (Porton Down) for analysis as per their requirements.

The results of the pilot study of source and ambient air sampling suggest that using filtration (gelatine filters) and liquid impingement were the best for capturing sufficient amounts of viable bacteria. However, liquid impingement was subject to losses through evaporation and the impingement instruments were found to be highly fragile in the field. The results also indicated a decline in viable bacteria counts from 1 hour sampling duration to 2 hours sampling duration, highlighting the loss of viability as sampling duration increases. Based on the pilot study, filtration (gelatine filters) and cyclones were selected for field trials.

Field trials

The objective of the field sampling trials was to evaluate the effectiveness and practicality of the set of shortlisted candidate sampling methods, and to refine them for use in diverse sample settings and multiple locations across England. Six different locations were selected, representing various settings such as rural and urban backgrounds, composting facilities, beef and dairy farms, and coastal areas. Figure 4, below, illustrates the sampling setup in a coastal setting.

Figure 4. Field trial sampling setup at a coastal location

From the pilot study, two chosen candidate sampling methods were selected. These were the Leckel with a Bio inlet (filtration method), and Coriolis Compact (dry cyclone method). Sampling was undertaken as dictated by weather conditions during the sampling period with an aim of sampling up to 1 hour on each round. In total, 36 samples were collected, excluding travel blanks. The collected gelatine samples were contained, labelled, and preserved in the fridge overnight and shipped the next day to the UKHSA Laboratories (Southmead, Bristol) for phenotypic fungal analysis as per their requirements. The dry cyclone samples were contained, labelled, and stored in a freezer for future molecular analysis should funds for this become available.

Sample storage

To ensure accurate and consistent analysis, it is recommended to store samples at between 0°C and 4°C for transport to the laboratory. This low storage temperature should greatly reduce the reproduction rate of the viable bacteria during transport prior to enumeration, without completely freezing the cells, which could lead to cell damage and death. DNA for metagenomic analyses is quite stable at this temperature.

Following isolation, single colonies may be stored long-term if they remain frozen at -70°C or below, and such freezer stocks may be regrown later for further characterisation of isolates. Established methods for this procedure for *E. coli* are described in the WHO Tricycle protocol (Annex 8: isolate storage); for MRSA, see Vitko and Richarson (2013). The Center for Disease Prevention and Control has detailed standard operating procedures for the preservation of fungal isolates (CDC).

Where DNA extraction is performed, extracted DNA should be stored at -70°C or below to prevent the degradation of the analyte.

Analysis options

Culture-dependent approaches

Culture-based approaches produce counts of total viable colonies and counts of resistant colonies. Combined with the volume of bioaerosol collected, the density of total and resistant colonies can be calculated, as well as the proportion of viable colonies that are resistant in each sample. Such data may be stored as spreadsheets (.xlsx or .csv).

Culture-independent approaches

Metagenomic sequencing will produce FASTA files, which may be large. If raw data files are to be stored long-term for re-analysis later then secure data repositories is required. The results of bioinformatic analysis (i.e., gene identities and their relative abundances (genes per 16S rRNA) can be stored as spreadsheets (.xlsx or .csv).

The results of HT-qPCR analysis (gene identities and their abundances) can be stored in spreadsheets (.xlsx or .csv). Gene counts should be converted to relative abundances (genes per 16S rRNA) for analysis.

Metadata and analysis options

Metadata should include at a minimum information about the sample, including:

- volume of air analysed,
- date and time,
- location,
- link to activity data for the site that is a putative bioaerosol source,
- local meteorology at time of sampling,
- instrument performance, and
- a description of the surrounding environment (e.g., type and number of sources of bioaerosols and their proximity to the sampler).

Collecting and storing additional data about the sampling location would be beneficial to interpret the results and understand the factors influencing dispersion. For example, data

from nearby air quality monitoring stations would be beneficial to explore correlations with other air quality parameters and weather conditions, to support modelling of airborne AMR.

Without knowing the precise nature of the data (such as amount, independence, skew and variability), it is difficult to recommend the most appropriate statistical approaches. Multiple linear regression is used by the Environment Agency's bathing water prediction team to estimate faecal indicator bacteria densities at designated bathing waters in England, with unique models being constructed for each location (as possible).

Alternative sampling method approaches

During the development of the AMR in Bioaerosols Surveillance Strategy, a number of alternative sampling approach options were explored, in addition to those recommended. These included:

- A dedicated Passive Sample Network
- Use of existing media as sample matrix for AMR, including air quality filter samples, car ventilation filters and HVAC filters
- Collection of deposited material on plant leaves

Passive samplers offer advantages in terms of cost, simpler design, ease of use and long sample duration. These are broadly based on gravitational settling or electrostatic capture. Passive samplers have been proven to be a useful tool when deployed alongside active collection devices (Manibusan and Mainelis, 2022) and in large scale spatial distribution measurement campaigns. However, they can only offer qualitative measurements since the volume of the sampled air is unknown and the relationship between airborne concentrations and particle deposition is weak. Whilst, the current project did not test any passive sampling method, their use as complementary methods adjunct to active sampling has the potential to allow long-duration sampling across multiple locations. This needs to be further explored in the context of AMR surveillance strategy. Because of the spatial coverage that might be achieved with passive methods they are likely to have a part to play in assessing spatial patterns (presence or absence to a given degree of confidence) of AMR in the atmospheric microbiome, as demonstrated by Shelton et al (2022)

Car cabin ventilation filters (Hurley, 2019) and HVAC filters (Möritz et al, 2001) have been sampled for presence of bioaerosols. Both car cabin and HVAC filters are anticipated to experience a significant particulate load which could influence the stability of any collected bioaerosol materials as well as the extraction efficiency of biological material from the filters. In addition, the extended duration that bioaerosol was collected onto the filters would lead to sample desiccation as well of degradation of the collected biological material. Data derived from the car cabin filters cannot be spatially mapped and is likely to have been influenced by multiple factors and sources, due to the filter being located in a moving vehicle, and not collected at a fixed location.

Analysis of deposited material on leaf surfaces has been a common method of characterising local air quality sources and potential sources (Mitchell, et al, 2010). However, as well as representing a deposition medium of particulate matter and

bioaerosols, leaf surfaces are also known sources of bioaerosols and microbiomes (Bowers et al. (2011, 2013); Manirajan et al. (2018)), and their analysis for AMR is likely to dominated by plant derived bioaerosol and not be representative of bioaerosols in the surrounding environment.

Data analysis and reporting of AMR in air samples

The requirements for data analysis and reporting of antimicrobial resistance (AMR) in air samples may vary depending on the specific outdoor environment and types of analysis carried out. Appropriate and consistent calibration of the sample collection equipment is important for all outdoor environments.

Culture dependent and culture independent methods for AMR in bioaerosols presents different challenges and opportunities for data analysis.

In the case of culturable methods, data analysis typically involves quantifying the number and types of antibiotic-resistant colonies obtained from the samples. This can include calculating the total colony forming units (CFUs) per volume of air sampled, as well as the proportion of resistant colonies among all colonies obtained. This information can be used to estimate the abundance and diversity of airborne antibiotic-resistant bacteria of the types favoured by a given culture medium and method, and to compare the levels of resistance across different sampling sites or time points.

Culture-independent methods have the potential to look for AMR markers across more of the atmospheric microbiome, including organisms not amenable to culturing. Quantitative PCR methods can screen samples for large numbers of specified AMR genes (ARGs), while metagenomic analyses can produce data that can be even more comprehensively screened. The former delivers results that can be used directly for assessment purposes while the latter generates large amounts of data, analysis of which requires expertise in the use of bioinformatic tools.

Data analysis can involve identifying and characterising the ARGs present in the samples, as well as assessing the diversity and abundance of the microbial communities, comparing the composition and structure of microbial communities across different samples and identifying potential sources of antibiotic resistance gene dissemination.

Using both culturable and non-culturable methods can provide complementary information on the levels and dynamics of airborne AMR. Combining the results from both approaches can provide a more comprehensive understanding of the abundance and diversity of antibiotic-resistant bacteria in bioaerosols, as well as the potential for dissemination of resistance genes through the air.

4. Design for an initial surveillance strategy for AMR within Bioaerosols in the UK

Dispersion and propagation of AMR microorganisms via bioaerosols in the environment is an understudied potential hazard. AMR surveillance encompasses a vast array of potential targets, covering thousands of resistance genes in millions of different species of microorganisms. AMR can be amplified in the environment, and resistance genes can be transferred between distantly related bacteria. These factors make surveillance of AMR in any environmental setting a considerable challenge. However, to understand the nature and scale of the problem, and reduce potential harm, the systematic collection of AMR data in bioaerosols is required.

Objectives of surveillance

Given the paucity of evidence on which to base a fully operational surveillance programme, in order to address critical knowledge gaps, we recommend the following steps are taken. In consultation with the EA two primary goals for an early-stage surveillance programme have been identified. These are to characterise the prevalence (% of determinants that are resistant to one or more antimicrobial agent) and density (resistant determinants per volume of air) of resistance determinants (microorganisms and/or their genes) at and/or around:

1. Potential AMR source sites in the UK
2. Potential AMR receptor (human exposure) sites in the UK

Securing basic information on airborne AMR in source and receptor environments in the UK will provide a basis (which does not currently exist in the scientific literature) for answering more complex questions about airborne AMR in the UK and the potential harm caused by it. For example, systematic surveillance of prevalence and density around potential sources might help better identify those sources that aerosolize AMR the furthest distances and therefore may need more mitigation. In addition, a detailed understanding of AMR at source sites may assist source-attribution in receptor environments. In parallel, surveillance of potential receptor sites might provide an estimate of the number of AMR microorganisms to which people in the UK are typically exposed, allowing proportionate mitigation measures to be taken and/or further measures to be taken once the health risks of such exposure are better understood. Finally, simultaneously characterising both sources and receptors might enable correlations to be made between the two, indicating where the airborne AMR that people are exposed to is coming from that can be investigated with more targeted surveillance in the future.

Thus, a three-part initial strategy is proposed, covering:

1) an overall AMR in bioaerosols sampling strategy for both sources and receptors,
2) specific recommendations for source-focussed surveillance strategy,
3) specific recommendations for a receptor (exposure)-focussed surveillance strategy.

General recommendations across source and receptor strategies

A consistent approach to measuring AMR in bioaerosols should be applied across both source and receptor-focussed surveillance strategies. Given the limited state of scientific knowledge on airborne AMR, ensuring there is consistency will provide the best opportunity for gaining an initial understanding of the baseline prevalence and densities of AMR in bioaerosols across sources and receptors. This will draw together potential links between sources and receptors, different sample substrates and media (air, land, water). Absolute consistency may not be achievable given that differences between air in source and receptor environments sometimes require different sampling approaches (e.g., different airborne particle concentrations require different sampling durations). However, there are opportunities where a general approach can be taken, and recommendations for the best approach for specific surveillance objectives are given below.

Bioaerosol sampling approach

The general bioaerosol sampling method used in both source and receptor environments should be chosen with three main priorities in mind:

1. Retaining both culture-dependent and culture-independent downstream options for characterising AMR (see 'Microbiological approach').
2. Enabling a quantitative evaluation of the density of AMR endpoints per unit of air.
3. Determining changing patterns in spatial distribution of AMR

The pilot study and field trials conducted as part of this project tested potential sampling methods which met the first two of these criteria in both source and ambient air. These trials covered a range of sampling methods involving filtration, impingement and cyclone for long and short durations.

The results of the pilot study of source and ambient air sampling suggest that using filtration (gelatine filters) and liquid impingement yielded the greatest counts of viable bacteria. The pilot study results indicated good preservation of culturable bacteria onto gelatine filters over a 1-hour duration at 50 l/min, though loss of viable bacteria becomes a risk, as sample volumes and sampling duration increases.

Based on the results of our field trials, the estimated labour cost of collecting a single AMR in bioaerosol sample would be £553, expenses £125 and additional courier fees (same day fee £350). Capital costs vary between £600 for the IOM sampler approach to £8,050 for the Coriolis sampler. (2023 figures) Specific detailed costings are outlined in Appendix G.

Microbiological approach

A surveillance strategy for airborne AMR in the UK should be able to capture more immediate threats (such as AMR in viable pathogens, as well as more potentially emergent

(e.g., AMR in microbial communities as a whole that might spread to organisms of health significance). In order to achieve this, it will be necessary utilise both culture-dependent approaches to monitor the prevalence of resistance in viable key airborne AMR pathogens (sentinel surveillance), and culture-independent approaches to monitor the potential threat of airborne AMR (broad surveillance).

Targeted surveillance of airborne AMR (culture-dependent component)

In order that key AMR pathogens are targeted, a surveillance strategy should characterise the prevalence of resistance to primary antimicrobials in key airborne pathogens. Whilst AMR is relevant beyond just resistant genes that occur among pathogens (AMR genes can be horizontally transferred from one microorganism species to another), there are direct and immediate potential health risks from airborne exposure to viable pathogens which are phenotypically resistant to clinically important antimicrobials. Therefore, focussing on key AMR pathogens is a priority when developing a surveillance strategy. There are many possible AMR pathogens that may be used as objective indicators of potentially consequential human exposure to airborne AMR, but the following three serve as good starting points:

- Azole-resistant *Aspergillus fumigatus*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli*

The suggestion of azole-resistant *A. fumigatus* and MRSA is guided by existing concerns about airborne transmission of these organisms. Where AMR pathogens are airborne and feature other characteristics listed below:

1) are resistant to key groups of antimicrobials critical to human health (azoles and beta-lactams), and appear on the WHO's lists of priority pathogens (WHO 2017; WHO 2022);
2) have known environmental reservoirs;
3) cause infections (including respiratory ones acquired via inhalation);
4) have been the focus of previous airborne AMR studies identified in the literature review (offering background knowledge on their likely distribution and baseline prevalence levels across environmental compartments).

The option of cefotaxime-resistant *E. coli*, by contrast, is guided by the fact that this microorganism and resistance trait is the indicator used in the WHO Global Tricycle Surveillance protocol to detect and measure the prevalence of AMR in humans, animals and the environment (Global Antimicrobial Resistance Surveillance System (GLASS), 2021). This indicator therefore has some precedent as a 'sentinel' microorganism/phenotype for AMR. By incorporating it into an airborne AMR surveillance strategy it would also offer a potentially insightful point of comparison to the other matrices covered in that protocol. Furthermore, although rarely a respiratory pathogen, *E. coli* has been commonly found in air in previous airborne AMR studies and has the potential to colonise the gut via an airborne route (e.g., via colonising mucus which is then swallowed).

All three sentinel AMR pathogens suggested above, have standardised analytical methods with suitable quality controls that can be used to culture and quantify resistance. Furthermore, culture-based analytical methods tend to be cheaper than molecular techniques and more readily deployable as less specialised equipment and skills are required. This would facilitate greater spatial and temporal coverage and greater sample numbers can then be achieved. This approach also allows for the interpretation of phenotypic measurements of resistance and relating them to human health outcomes. This is especially relevant where that minimum inhibitory concentration (MIC) is used to identify strains where there is a high likelihood of treatment success, as opposed to strains where treatment is more likely to fail (Turnidge and Paterson, 2007). One significant disadvantage is that samples need to be analysed within 24-48 hrs, and they provide a limited amount of information about AMR dynamics at the microbial community (or microbiome) level.

Analytical methodologies for sentinel species

For azole-resistant *A. fumigatus*, samples should be plated directly onto two types of agars: 1) selective Sabouraud dextrose agar supplemented with 200 mg/L penicillin and 400 mg/L streptomycin (non-selective agar) to characterise total density of *A. fumigatus*. And 2) Sabouraud dextrose agar made in the same way and supplemented with 1 mg/L itraconazole (the established EUCAST breakpoint for *A. fumigatus* resistance to itraconazole) to characterise the prevalence and density of resistant *A. fumigatus*. Samples should be incubated at 43°C for 48h (Shelton et al 2022)[1]. Itraconazole is a triazole antifungal commonly used in human medicine to treat aspergillosis.

For MRSA, samples should be plated directly onto two types of agars: 1) selective Mannitol Salt agar to characterise total density of S. aureus; 2) Mannitol Salt agar supplemented with 2 mg/L oxacillin (recommended for MRSA) to characterise the density of MRSA (The European Committee on Antimicrobial Susceptibility Testing, 2023). Agar plates should be incubated at 37°C for 18-24 hours. Oxacillin is similar to methicillin and the standard antibiotic used to test for MRSA and (by proxy) its beta-lactam resistance phenotype.

For ESBL-producing *E. coli* samples should be directly plated onto two types of agars: 1) TBX agar to characterise total density of *E. coli* and 2) TBX agar supplemented with 4 mg/L cefotaxime to characterise the density of resistant *E.coli* (The European Committee on Antimicrobial Susceptibility Testing, 2023). Agar plates should be incubated at 37°C for 18-24 hours.

[1] Note that this differs slightly from Shelton *et al.*, (2022)'s focus on tebuconazole, which was justified on the basis of this being one the third most sprayed azole in agricultural/horticultural fungicides in the UK. For the purposes of this surveillance network where human health is the primary concern, we believe it is more appropriate to focus on an antifungal commonly used in human health (to treat disease caused by the target organism). Furthermore, itraconazole has a EUCAST-established breakpoint for *Aspergillus spp.*, further facilitating the relation of resistance to human health.

Cefotaxime is a third-generation cephalosporin, which is a clinically important antibiotic class. These methods align with those proposed by the Tricycle protocol (Global Antimicrobial Resistance Surveillance System (GLASS), 2021).

The prevalence of resistance is then derived from the ratio of colonies counted on selective versus non-selective media. Once isolated, cultured cells can undergo further analyses to characterise AMR, or picked and stored long term in -70°C freezers. Such analyses may include, for example, antimicrobial susceptibility testing using disk diffusion or microbroth dilution; molecular detection of specific genes, or whole genome sequencing of isolates (GLASS), 2021).

The costs of consumables for all three culture-based approaches described above has been estimated at approximately £8.32 per sample (2023 prices). A breakdown of costs per assay are available in Appendix E - Costs and other Considerations for culture-dependent approaches. These indicative costings do not consider greater costs associated with infrastructure, specialist equipment, staffing, sample and data storage, or logistics.

Broad surveillance of airborne AMR (culture-independent component)

Fewer than 1% of microorganisms can be cultured, and there are many resistance genes that may be present on mobile genetic elements harboured by non-culturable microbes. Therefore, it is important to survey bacteria other than pathogens or culturable species due to the risk of horizontal gene transfer of antibiotic resistance genes between bacteria (this process does not occur in fungi). Culture-independent methods can detect and quantify resistance in complex microbial communities typically found in environmental matrices. Metagenomic sequencing and high throughput (HT) qPCR are particularly advantageous in their ability to characterise AMR across a very broad range of targets simultaneously, particularly of value when surveying for resistance genes harboured by bacteria. While metagenomic approaches can characterise all known resistance genes (thousands), it is limited in its ability to detect rare genes. HTqPCR can quantify fewer genes simultaneously (up to 384 genes per sample presently), but quantification is likely to be more accurate, and more sensitive for rare genes, such as those conferring resistance to last-resort antibiotics.

One approach may be to use metagenomics approaches to narrow down the number of key genes which are then accurately quantified using HTqPCR. All qPCR-based assays must include biomass markers for bacteria (16s rRNA) and fungi (18s rRNA) to confirm the presence of microorganisms in the sample and allow relative abundance to be determined to account for differences in densities of microorganisms in each sample.

Additionally, bioinformatic pipelines and HTqPCR panels can be used or adapted to characterise aspects of the microbial communities beyond just resistance genes. For example, pipelines are available for extracting taxonomic information that can be used to quantify microbial diversity (e.g., research-based tools like MetaPhlAan) or infer likely sources of the microorganisms contained in the sample (McGhee et al 2020; Linder et al 2022). Likewise, qPCR assays are developed to detect important taxonomic groups of bacteria including pathogens such as *Acinetobacter baumannii*, Staphylococcus, as well as

groups that indicate faecal origins e.g., *E. coli*, Bacteroidetes, among other microbial source tracking markers.

Once DNA is extracted from samples, sub-samples can then be sent for amplicon sequencing e.g., of 16S rRNA (bacteria) or ITS (fungi) to generate higher resolution data on microbial diversity and may assist with characterising rare microorganisms missed by metagenomic sequencing. Information gained from characterisation could then be used to identify sources. However, this type of analysis alone would not produce information about resistance potential and in order to be more exploratory would need to be used in combination with other assays such as HTqPCR.

In addition to the above an initial approach could be to focus on the total density of AMR genes in air (per m^3 air) or '*Simpson's diversity*'. The emerging data could then be used to determine any emerging patterns, providing species targets for future targeted surveillance.

In the first instance collection of representative samples across different sources and receptors could then undergo metagenomic analyses to gain an understanding of the diversity of resistance genes present before refining to a core panel of genes that are then analysed using HTqPCR for more accurate quantification.

In terms of costs, culture-independent approaches tend to be more costly per sample than culture based, but this is balanced by the quantity and quality of relevant information they produce. Outsourcing these services to commercial providers would be advantageous. For example, one provider of HTqPCR identified currently (March 2023) charges £2,450 for analysis of 384 genes in four samples (£612.50 per sample or £1.60 per data point)(2023 prices), and is able to customise the assays to cover a smaller number of gene targets across a greater number of samples, providing additional services such as DNA extraction, shipping and statistical analysis (see Appendix F - Costs and other Considerations for culture-independent approaches). **For next generation sequencing costs (yielding data on a conservative estimate of 2,000 resistance genes, and not including additional data outputs such as microbial diversity and source attribution), a sample may undergo DNA extraction and be sequenced for as little as £200 per sample. Bioinformatic analyses may cost £1,039 for a set of 100 samples (~£10 per sample), making the price per data point is £0.10. Sequencing more deeply to detect genes present at low abundance incurs a greater cost (£305 per sample but equates to only £0.15 per datapoint)** (2023 prices)**.** These costs become more cost effective if greater numbers of samples are sent for sequencing. It is recommended that samples are selected for metagenomic sequencing based on complementary culture-based and/or qPCR data for 16S rRNA, as this will screen out low-concentration samples which may fail to produce good sequencing results. Inclusion of negative controls in each batch of metagenomic sequencing assays is also recommended, given the microbial diversity of some of the bioaerosol samples could be high.

Source-focussed strategy

Primary goal

The primary goal of a source attribution-focused strategy is to estimate the levels of airborne AMR in source locations where a large number of antimicrobial resistant microorganisms are potentially being aerosolized. This could be achieved through a national surveillance strategy for AMR in bioaerosols, which would involve sampling around candidate representative source locations such as wastewater treatment plants (WWTPs), farms and composting facilities.

Sampling objectives and approaches

A framework for the overall sampling strategy for a source attribution-focused surveillance network for airborne AMR would involve the following steps:

1. Define specific objective of surveillance

Where detection of AMR from any site in a typical potential source category (biowaste, WWT etc.) occurs, or to detect change in the probability of occurrence, then all sites of that type would need to be sampled, perhaps in an extension of the current sampling regime for permitting purposes. Where a number of representative sample locations are selected, a statistically representative sample (based on prior knowledge of AMR occurrence frequency) would be required.

2. Determine which source categories will be considered

Should the objective be to determine the contribution of particular sectors to the atmospheric microbiome in order to determine risk and proportionate mitigation, then an iterative approach would be required. Such an approach could involve sampling a proportion of sites from each source category, and analysing the probability of emitted bioaerosols carrying AMR, through regular routine monitoring of all sites then applied only to those in the higher risk source categories.

3. Identification of the appropriate number of candidate source locations to meet the objective

By identifying candidate representative source locations for airborne AMR (such as WWTPs, farms, and composting facilities), which are hypothesised to aerosolise a large number of antimicrobial-resistant microorganisms.

4. Sampling frequency

Determine the frequency of sampling based on the characteristics of the source locations, the surrounding environment, and the risk of exposure to human populations. Sampling should be conducted periodically, focusing on periods of increased activity or risk (weather conditions that may increase aerosolisation).

5. Sampling duration

Determine the sampling duration based on the characteristics of the source locations (such as known rates of bioaerosol emission) and the surrounding environment. For example, longer sampling durations may be required for low-density airborne microorganisms or in areas with lower concentrations of the target microorganisms.

6. Sampling equipment

Select appropriate sampling equipment and locations based on the sampling duration, the type of microorganisms being targeted, the intended analytical endpoints and the characteristics of the environment. Sampling locations should be representative of the source and the surrounding environment and should be consistent across different sampling events to ensure comparability of data, taking into account the requirement to sample up and downwind of putative sources.

7. Sample management and transport to analysts

Protocol for sample treatment on collection and during transport. For example, sterile technique, temperature and storage media.

8. Sample analysis

Analyse the collected samples using appropriate laboratory methods to identify and quantify antimicrobial-resistant microorganisms.

Sample size

The sample size required for a complete surveillance programme of AMR in bioaerosols from different outdoor environments would depend on several factors, including the objectives of the surveillance programme, the statistical power desired, and the variability in the data. Generally, a larger sample size would provide more precise estimates of the levels of AMR in the different outdoor environments and increase the statistical power to detect differences between locations. However, a larger sample size also requires more resources and can be impractical in some situations. There is likely to be a de minimus sample number below which meaningful conclusions from data cannot be achieved.

A viable approach to estimate the required sample size is to conduct a regional pilot study to estimate the variability in the data and determine the effect size of interest (Yamamoto et al 2014). Based on this information, power calculations can then be performed to estimate the sample size required to detect the desired effect size with a given level of statistical power and significance level. In addition, it is important to consider the representativeness of the samples and the geographical coverage of the surveillance programme. Sampling locations should be chosen to be representative of the different outdoor environments of interest, and a sufficient number of locations and sampling times should be included to provide a comprehensive geographical coverage and understanding of variability.

Receptor-focussed strategy

A second key objective of a national surveillance strategy for AMR in bioaerosols is to assess the levels of AMR in receptor locations, particularly where humans are likely to be exposed and could suffer harm as a result. One approach to achieve this goal is to conduct nationwide sampling of airborne AMR in locations that represent typical ambient air conditions in the UK, which people are likely to encounter in their daily lives.

By following these recommendations, the implementation of a national AMR in bioaerosols surveillance strategy through a sampling network will provide valuable information for understanding the airborne transmission of AMR in the environment. This will then develop evidence-based strategies to prevent their spread and protect public health.

Primary goal

The primary goal of this strategy is to quantify the average airborne density (per m3 air) of microbial determinants (e.g. bacteria/fungi, 16S/18S genes) and AMR determinants (e.g. resistant cells/spores, AMR genes) in ambient air across the UK. Quantifying the average airborne density and its variation for these targets will help develop an initial understanding of typical exposure levels of the general UK populace. This information may be used in the future to understand human health implications more directly. As knowledge on the relationship between exposure to AMR in bioaerosols and human health impact becomes more developed, the sampling strategy could evolve to cover a range of UK locations. Further, it could be deployed on an annual or seasonal basis, in order to develop an initial understanding of spatial and temporal variation in levels of exposure to airborne AMR.

Sampling approaches

To achieve the above-mentioned goal, a possible starting point might be to leverage existing air quality sample networks that are aimed at understanding the exposure of the UK population to various air pollutants. The Automatic Urban and Rural Network (AURN), which consists of 170 operational stations across the UK at diverse urban and rural sites (Figure 5), is the largest such network. Sampling airborne AMR across some or all of the AURN stations would provide a comprehensive national perspective on airborne AMR while minimising duplication of effort in locating suitable sampling sites. Moreover, sampling across an existing air pollution network would enable potential correlation of airborne AMR data with other pollutant and meteorological data that these sites collect (e.g., PM10, temperature). This correlation could improve our understanding of the relationship between air pollution, meteorological parameters, and airborne AMR, which is currently a gap in the literature.

Deploying active bioaerosol samplers alongside existing, automated, and continuous air pollution samplers at AURN sites would be necessary to achieve this. This could be done either seasonally or annually, and all samples should be collected as close to simultaneous as possible, within a defined timeframe to ensure a degree of temporal consistency (e.g.,

within the same month) and over several days to ensure a comparable and representative picture of airborne AMR at each site.

Sample size

A key consideration in the design of any surveillance network is whether the sample size is sufficient to meet the aims of surveillance. Estimating an appropriate sample size where existing knowledge is scarce (as is the case here) can be difficult, though the fact that a common outcome of interest in AMR research is the prevalence of determinants which are resistant (as is the case here) makes this slightly easier, since proportion/percentage-based outcomes are restricted to a 0-1/0-100% range. It is possible to estimate whether a sampling network of a similar size to the AURN would be sufficient to calculate the average prevalence of resistance in airborne microorganisms. Careful thought should be given to the statistical power of sampling, and preliminary data-gathering is required in order to assess this.

Sentinel surveillance of airborne AMR (culture-dependent component)

In order to calculate the average prevalence of resistance in ambient UK air of sentinel AMR pathogens, the same power calculations can be used as those described in the Tricycle protocol (Global Antimicrobial Resistance Surveillance System (GLASS), 2021). In employing this approach, an individual sampling campaign would need to acquire at least 100 samples that are culture-positive for the target organism to estimate any prevalence of resistance (0-100%) with an absolute precision of 10% and level of confidence of 95%[2]. Were bioaerosol sampling to be deployed across the entire Defra Air Quality Site network of over 300 sites, this would require 33% of sites/air samples would need to be positive for the target organism to get a reasonable estimate of prevalence. Using the scenario that active bioaerosol sampling were deployed to sample large volumes of air, this approach is feasible to achieve for the sentinel AMR pathogens using 300 sites, given that:

- **For *A. fumigatus*,** Shelton *et al.*, (2022) found that 36-57% of UK sites/air samples were positive for *A. fumigatus* (depending on season), meaning that 169 air samples from a set of 300 would be expected to be positive. However, given that Shelton *et al.*, (2022)'s estimates are based on citizen scientists deploying passive samplers (6.8 x 8 cm squares of adhesive film) over 6-8 hours, these are likely to be conservative estimates. Therefore, with active sampling and/or targeting of seasonal peaks[3], it is likely that >169 culture-positive samples could be achieved.

[2] https://statulator.com/SampleSize/ss1P.html
[3] Shelton *et al.*, (2022) found that the highest prevalence of positive samples was in summer (57%)

- **For *S. aureus*,** this is likely to be feasible given the microorganism's ubiquity in ambient air. It is more difficult to get an exact estimate of prevalence from the current literature (let alone a UK-specific one), however Sivri *et al.*, (2016) were able to culture them for all 11 urban locations sampled in Istanbul, for example.
- **For *E. coli*,** this is likely to be feasible given the microorganism's ubiquity in ambient air. Again (compared to *A. fumigatus)*, it is more difficult to get an exact estimate of prevalence from the literature (let alone a UK-specific one), but de Rooij *et al.*, (2019) found evidence of *E. coli* presence in all samples of ambient air at residential sites in The Netherlands, which were actively sampled over 14 days. Therefore, with active sampling over several days, it is likely that >100 culture-positive samples could be achieved from the Defra Air Quality Site network of 300 sites

Broad surveillance of airborne AMR (culture-independent component)

For broad surveillance of airborne AMR via culture-independent methods, the application of power analysis would be much more complicated than surveillance of sentinels, and much larger sample sizes would be required given the number of response variables (gene abundances) generated by these methods (Ferdous *et al.*, 2022). Furthermore, it is probably not necessary in this context - given that for practical considerations (e.g., cost, expertise) these methods are likely to be deployed on a smaller scale for more exploratory surveillance of emergent threats. Generating such descriptive data (e.g., total density of AMR genes in air, typical diversity of AMR genes, prevalence of particular genes) may provide targets for more hypothesis-driven investigation in the future and inform future power analyses for these investigations. Furthermore, assuming a consistent approach to airborne AMR sampling between exposure focussed and source-attribution focussed strategies, multidimensional scaling could be employed to gain an understanding of the similarity between the airborne AMR composition of ambient air samples, potential sources, and changes in background locations. These are essential in order to generate initial hypotheses about where any airborne AMR in ambient air is coming from, and by what means it could be distributed.

Existing air quality monitoring operations

Currently ambient air is routinely monitored for a number of pollutants at background locations across the UK and at locations close to known air emission sources. Air quality is also periodically monitored by site operators at industrial and waste processing sites, which is required as part of the site's operating permit. Such existing air quality monitoring operations may serve as a foundation upon which airborne AMR surveillance may be built - for example, existing sites might provide access to power and telecommunication links, security and metadata recording local conditions. In this section, we consider whether these existing air quality monitoring operations might serve as the basis for an AMR surveillance network, in terms of practicalities and suitability of site locations.

Description of key national air quality monitoring networks

There are currently around 300 Environment Agency managed sites monitoring air quality across the whole of the UK (Figure 5). Ambient monitoring sites are currently managed by The Environment Agency on behalf of Defra and the Devolved Administrations. Local authorities across the UK also routinely monitor air quality as part of their Local Air Quality Management duties.

There are 17 individual networks, sub-divided into automatic and non-automatic networks, including the various sites making up the Automatic Urban and Rural Network (AURN), located at 170 of these sites. A number of the national air quality monitoring sites are specifically focussed on wider ecological and environmental impacts of air pollutants, such as the UK Eutrophying and Acidifying Pollutants (UKEAP).

Details of the seventeen national air quality monitoring networks are outlined in Table 1 and the AURN site location are illustrated in Figure 5 below.

Table 1 National Air Quality Network Types and Pollutants Monitored.

Network	Pollutants	Automatic or Non-Automatic
Automatic Urban and Rural	CO; NO_2; O_3; PM_{10}; $PM_{2.5}$; SO_2	Automatic
Locally managed automatic monitoring	CO; NO_2; O_3; PM_{10}; $PM_{2.5}$; SO_2	Automatic
Automatic Hydrocarbon	Non-Methane Volatile Organic Compounds	Automatic
Non-Automatic Hydrocarbon	Benzene	Non-Automatic
Poly-Aromatic Hydrocarbons	27 Poly-Aromatic Hydrocarbons	Automatic
Total Organic Micro Pollutants	Dioxins Furans and PCBs	Automatic
Black Carbon	Black Carbon (880nm), plus absorbance by particulate matter of varying wavelengths	Automatic
Heavy Metals	Arsenic; Cadmium; Chromium; Cobalt; Copper; Iron; Lead; Manganese;	Automatic

Network	Pollutants	Automatic or Non-Automatic
	Nickel; Selenium; Vanadium; Zinc	
Particulates	Total particle number concentration; Particle Count of 51 sizes from 16.6 nm to 604.3 nm. Elemental Carbon; Organic Carbon and Total Carbon in PM_{10}. Elemental Carbon; Organic Carbon and Total Carbon in $PM_{2.5}$.	Automatic
Stratospheric Ozone and UV	Total Ultraviolet radiation	Automatic
UKEAP: Precipitation Network	The following in precipitation; Ca^{+2}, Cl^-, K^+, Mg^{+2}, Na^+, PO_4^{-2} as P, NO_3^{+2} as N, NH_4^+ as N, SO_4^{-2} as S, Non-marine sulphate as S, F^-, Acidity; Conductivity; pH; Rainfall	Non-automatic
UKEAP: Acid gas and Aerosols	Particulate Ca^{+2}, Cl^-, K^+, Mg^{+2}, Na^+, NO_2^{+2}, NO_3^{+2} SO_4^{-2} Gaseous HCl, HNO_3, HNO_2; SO_2	Non-automatic Automatic
UKEAP: Rural NO2	NO_2; Corrected NO_2	Automatic
UKEAP: National Ammonia	Gaseous NH_4^+	Passive (non-Automatic)
UKEAP: Monitor for AeRosols and Gases in Ambient air (MARGA)	Ca^{+2}, Cl^- , K^+, Mg^{+2}, Na^+, $NH4^+$ $NO3^-$, SO_4^{-2} in PM_{10}; Ca^{+2}, Cl^- , K^+, Mg^{+2}, Na^+, NH_4^+ $NO3^-$, SO_4^{-2} in $PM_{2.5}$; Gaseous, HCl; N_2O; NO; NH_3; SO_2	Automatic
UKEAP: Automatic Mercury	Reactive Hg, elemental Hg: Hg in $PM_{2.5}$	Automatic
UK Urban NO_2 Network	NO_2 Corrected	Automatic

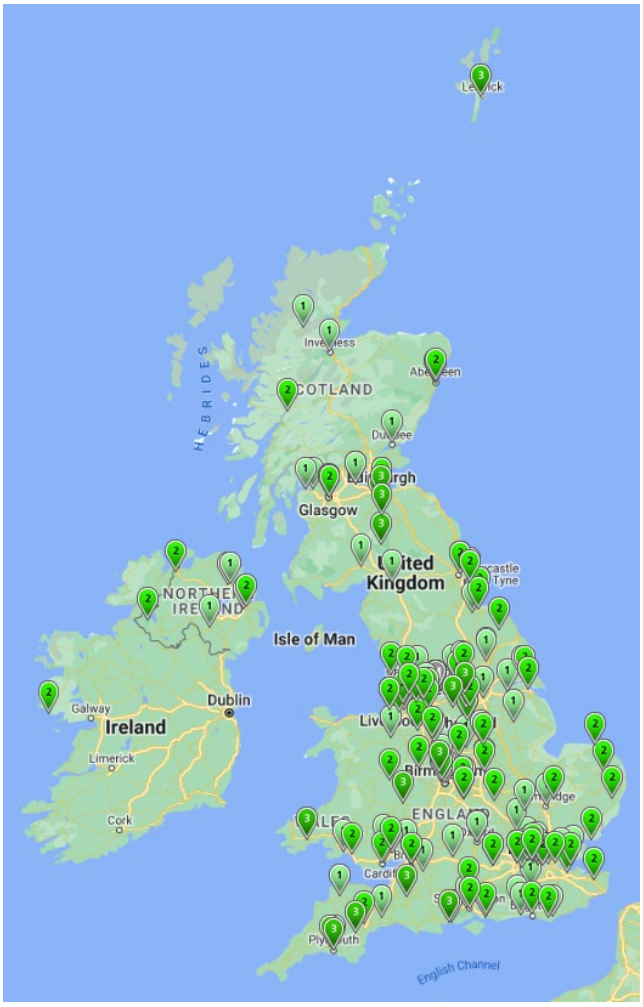

Figure 5. Automatic Urban and Rural Network (taken from Defra Interactive monitoring map: https://uk-air.defra.gov.uk/interactive-map?network=aurn, accessed 25-3-2023).

National pollen monitoring network

National monitoring of pollen and fungal spores in the UK is currently undertaken by a national Pollen Monitoring Network, which is a collaborative network made up of the Met Office and a number of universities. Monitoring results are input into the national pollen forecasting service run by National Pollen and Aerobiology Research Unit, at the University of Worcester.

There are currently 18 active pollen monitoring stations across the UK mainland, which largely utilise the Hirst principle of sampling (a sampler using a vacuum pump with a typical flowrate of 10/l/min, which deposits airborne material, including pollen grains, spores and other particles through impaction onto an adhesive tape), with two stations (Worcester and Manchester) using automatic monitoring methods (Figure 6).

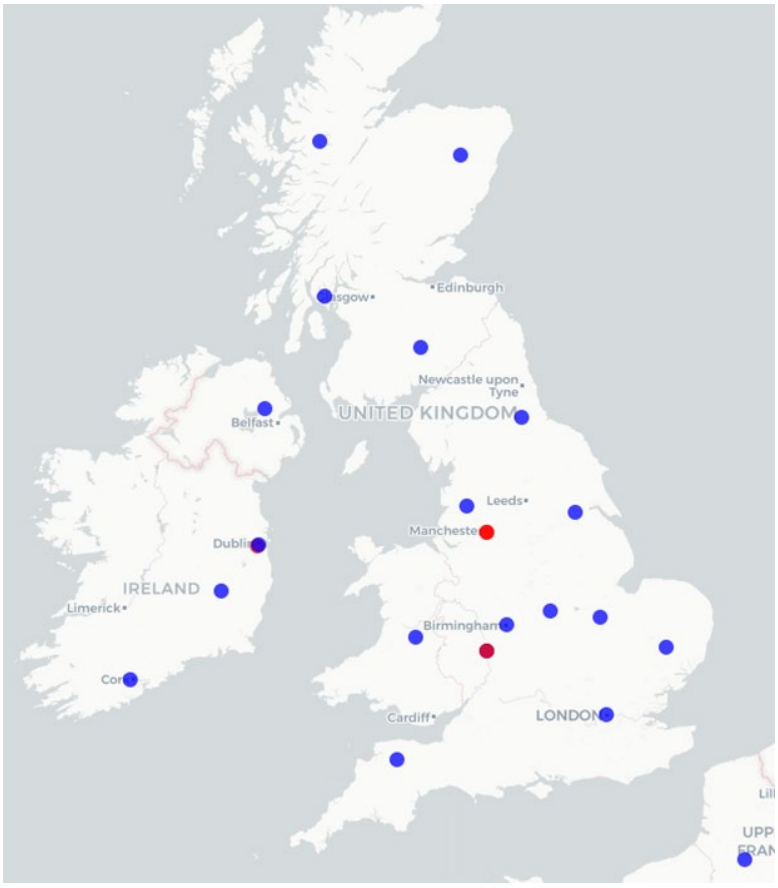

Figure 6. Pollen and Fungal Spore monitoring stations - blue dots (Hirst trap), red (Automatic station) (taken from EAACI Task Force TF-40108 'Inventory of pollen monitoring sites in the world).

Bioaerosol monitoring sites

Bioaerosols are routinely (every 3 to 6 months) monitored at permitted sites across the UK known to be a source of bioaerosols (e.g., composting, anaerobic digestion and mechanical biological treatment are known to be significant sources, where a risk of exposure is considered to be present). Sampling is by impaction, or filtration as set out in the Environment Agency's M9 guidance document, and samples are cultured to provide an estimate of colony-forming units (CFU) per cubic metre of air. Several hundred sites are routinely monitored each year.

Evaluation of re-use potential of existing operations

In considering the development of a national Surveillance Strategy for AMR in bioaerosols, use of the existing Defra air quality monitoring sites, the national pollen network monitoring sites and routine monitoring at permitted sites should be considered. The advantages and disadvantages of including existing Defra air quality monitoring sites, national pollen network monitoring sites and routine monitoring at permitted sites within the AMR sampling surveillance strategy are investigated below.

National air quality monitoring networks

The evolution of Defra's national air quality sampling network and its selected locations have occurred through a combination of the following:

- The requirement for an air pollutant sample to be collected and representative of air quality across a known area, location category, or to representative exposure of a known receptor.
- The benefit of co-locating other sampling networks together to generate mutually useful metadata.
- The logistical advantage gained through co-locating other sampling networks, reducing site management tasks, allowing single site visits to attend to multiple networks, and reducing site ownership and operational costs.

As sampling instruments to collect AMR in Bioaerosol are likely to be deployed over shorter durations than existing air quality instruments, using sampling techniques not currently deployed at existing Defra air quality monitoring sites, the benefits of including existing sites within an AMR sampling surveillance strategy need to be explored.

Advantages

- Allows the management and site access to be retained within the Environment Agency's control.
- Allows use of other data and meta-data that might inform analysis of bioaerosols and/or AMR measured for the site and factors that might influence or be related to bioaerosol or AMR concentrations. This could include particulate concentrations, concentrations of gases which might influence atmospheric lifetime of viable microorganisms (for example, ozone) and meteorological data, which are collected at a number of the Defra sites.
- Access to long-term air quality data and air pollutant trends at each site.
- Ensures sites are both well-managed and secure for the collection of AMR in bioaerosols samples.
- Ensures access to electrical power and other utilities for the operation of powered sampling devices.
- Provides a potential opportunity to use monthly visits by Local Service Operators to routinely collect bioaerosol samples.

Disadvantages

- Initial permission to gain approval of site use could be problematic, potentially delaying establishment of an AMR in bioaerosols sample networking.
- Access to site will require formal approval of site managers, potentially presenting minor barrier to ad-hoc or infrequent site attendance.
- Location of the established site may not bear any relationship to risk of exposure to AMR in bioaerosols and may be less than optimal in terms of representativeness of exposure risk to AMR in bioaerosols.

- Use of monitoring sites will be dependent upon the continued operation of these sites by Defra, and its representatives.
- Where multiple Local Site Operators are required to routinely collect AMR in bioaerosols samples, extensive training will be required in order to establish a consistent and robust quality of sample.
- Should a short sample duration be the preferred AMR in bioaerosols sampling approach, then there is no advantage in utilising established sample sites.

National pollen monitoring network

Advantages

Several advantages of using existing National Pollen Monitoring Sites are apparent, and these include:

- Benefits of utilising inherent knowledge within National Pollen Monitoring Sites regarding wider potential influences upon bioaerosols.
- Coordinated sample collection between National Pollen Sample Monitoring and AMR in bioaerosols could add value to both sample networks, highlighting coincidental influences where they may occur.
- Allows use of on-site meta-data for analysis of AMR in bioaerosols and influences, such as pollen counts, and meteorological data collected at a number of the Pollen Monitoring Network.
- Access to long-term pollen count data and pollen trends at each site.
- Ensures sites are both well-managed and secure for the collection of bioaerosol samples.
- Ensures access to electrical power and other utilities for the operation of powered sampling devices.
- Provides a potential opportunity to utilise daily visits by Pollen Network Monitoring staff who routinely collect bioaerosols samples.

Disadvantages

- Initial permission to gain approval of site use would require a memorandum of understanding between Defra and the Met Office.
- Access to site will require formal approval of site managers, potentially presenting minor barrier to ad-hoc or infrequent site attendance.
- Location of the established site may not bear any relationship to risk of exposure to AMR in bioaerosols and may be less than optimal in terms of representativeness of exposure risk to AMR in bioaerosols.
- Use of monitoring site will be dependent upon the successful and continued operation of site by the MET Office and its representatives.
- Where multiple Pollen Network Monitoring staff are required to routinely collect AMR in bioaerosols samples, extensive training will be required in order to establish a consistent and robust quality of sample.

- Where the preferred AMR in bioaerosols sampling approach is less than 24 hours in duration, then there may be no resource efficiency utilising Pollen Network Monitoring staff as Pollen Samples are collected over a 24 hour period.

Bioaerosol monitoring sites

Bioaerosol monitoring sampling is undertaken at permitted waste sites at regular intervals. The use of an existing monitoring programme at known sources of bioaerosols could provide insights into the prevalence of AMR in bioaerosols. The methods used for sampling are prescribed in the M9 sampling approach.

Advantages

There are several advantages of using Bioaerosol Monitoring Sampling of Permitted Waste Sites for AMR in bioaerosol sampling, including:

- Bioaerosol sampling of permitted sites uses M9 sampling approach, which is a standardised method for sampling of bioaerosols.
- Analysis of AMR in bioaerosols collected at permitted waste sites adds to knowledge regarding drivers for AMR in bioaerosol.
- M9 sampling requires meteorological data to be collected, which could prove of value in assessing AMR in bioaerosols at permitted waste sites.
- Access to retrospective bioaerosol monitoring data records at permitted waste sites could prove of value in assessing sources of bioaerosols.
- Waste site operator involvement in the collection of AMR in bioaerosols at permitted waste sites will allow information on site practices and operations to be available, which could influence the prevalence of AMR in bioaerosols and aid interpretation of AMR data obtained.

Disadvantages

- Sampling of permitted sites for bioaerosols is undertaken periodically, with a frequency of between every 3 to 6 months. Such a sampling frequency may be of too low a temporal resolution.
- Lack of buy-in from site operators, as bioaerosol samples are the responsibility of individual site operators, with limited budgets and no statutory obligation to collect samples for the purpose of an AMR in bioaerosol surveillance programme.
- Risk of inconsistent and / or variable sampling quality, as bioaerosols samples collected on permitted sites are undertaken by commercial laboratories, with a range of sampling expertise and sampling quality.
- Bioaerosol sampling of permitted sites is limited to culture-based methods.

Summary

Of the two existing sampling networks discussed above, and the existing periodic sampling of bioaerosols on-site, a strategic alignment alongside the AURN would likely represent the most viable and beneficial approach. However, aligning sampling of AMR in bioaerosols alongside the AURN would require additional resourcing and care would be needed to ensure that the sampling objectives of both AMR in bioaerosols and the AURN are both met, without compromise. This would initially require a parallel dedicated AMR in bioaerosols sampling team to work alongside the AURN resource, with a shared duty approach in the medium term as sampling AMR in bioaerosols methodologies are standardised. Consideration of sample site relevance and representation will need to be applied to the existing AURN network when evaluating which of the AURN sites are to be selected for inclusion in the AMR in bioaerosols sampling network. As not all sites would be representative of relevant AMR exposure or close to AMR sources.

Exploration of site and source specific sampling AMR in bioaerosols through sampling permitted processes would require a degree of co-operation from process operators, and third parties. This could present a significant risk of uncertainty in terms of data quality, due to inconsistent sampling practices and variable quality of post collection sample handling.

Decision Framework Outputs

A decision tree introduced in an earlier analysis of AMR sampling strategy (Environment Agency, 2022), was developed further with inputs from the sampling plan, pilot study and field trials. These assisted with informing the strategy options for AMR surveillance in bioaerosols. Decision categories, requirements and feasibilities were all addressed, and a set of findings were informing the approach to be taken in identifying an AMR in bioaerosols surveillance strategy options (Table 2 below).

Table 2 Decision framework informing options for an AMR in bioaerosols surveillance strategy.

Decision Category	Requirements and feasibilities	Findings
Targets	Single species and/or ensemble of species	In order to characterise AMR within bioaerosols more than one species is required to be targeted.
	Time dependent snapshot and/or long-term trends	Long-term trends of AMR in bioaerosols can be developed over time from frequent sampling at fixed locations. However, given the paucity of current information, a sample network is likely to be required to develop through a series of initial snap-shot samples.

Decision Category	Requirements and feasibilities	Findings
		Where sampling is close to known sources snap-shot samples will prove valuable in, for example, linking emissions with specific activities or processes.
	Single point and/or spatial assessment	Close to known sources a spatial array of samples could be collected (for example as set out in EA guidance M9). Single point samples could in some circumstances provide good quality repeatable sampling results where validated sampling methods are employed. Low-cost/ low-tech (passive) sampling methods could be employed at a greater number of sample locations to provide a higher resolution spatial assessment of airborne AMR.
	Source apportionment vs general surveillance	Both source apportionment, utilising bioaerosol sampling of permitted sites where available, and general surveillance are required to develop in parallel an understanding of source terms and exposure risks.
	Receptors of concern	Receptors of concern may include vulnerable or general human population or both.
	End users of airborne AMR concentration data	Human health risk of exposure to AMR in bioaerosols is to be undertaken by UKHSA.
	Target level of uncertainty required for surveillance to deliver results that are fit for purpose.	User uncertainty requirement will depend on what level of statistical uncertainty is required for evidence from surveillance to be sufficiently robust to increase confidence in decisions pertaining to AMR in bioaerosols. For example, what is the confidence to which measurements can detect the presence of AMR bioaerosol?
Network Geographical distribution	Number of sites	Determination of the optimal number of sample sites to meet surveillance objectives with specific statistical confidence should be based on prior knowledge of AMR bioaerosol occurrence. Where this information is not available then development of surveillance

Decision Category	Requirements and feasibilities	Findings
		network design is likely to require preliminary stages of measurement. Cost and finite resource may limit the number of sample sites to a level that would result in a confidence level below the target specified, and a decision must be taken as to whether lower confidence levels are acceptable to decision-makers or more resource or reduced costs must be identified in order to continue with development of a surveillance network.
	Rural and urban sites	Where population exposure and exposure risk are considered a priority objective then urban sample sites may be of significance in that they represent exposure of larger numbers of people than rural sites. However this presupposes both populations are exposed to similar sources and activity levels. This might be true for well-mixed ambient background levels of AMR-carrying bioaerosol. Depending on the heterogeneity and distribution of potentially AMR-carrying bioaerosol sources and patterns of dispersion (notably distance of transport), cumulative exposure of sub-populations in rural areas might exceed those in urban populations. For surveillance of site-specific emissions the critical target population is considered to be that at the nearest sensitive receptor location
	Sampling height	For human exposure assessment sample height should follow M9, which requires sampling position to be representative of the inhalation zone of an adult (typically taken as1.5m height.) For other purposes (such as determining emission rates) other heights might be chosen as appropriate (for example, to maximise air concentration).
	Meteorological data requirements	Recording micro-meteorological data such as local wind direction and velocity (and density turbulence characteristics) during sampling is required to, for example, support determination of potential sources, predict how emission may subsequently disperse to receiving

Decision Category	Requirements and feasibilities	Findings
		environments.
	Source distribution	Any known or suspected sources in the vicinity of sampling should be identify, and their activities noted. Ambient background sample sites should be located a number of kilometres from known or suspected nearby sources, bearing in mind that field measurements have shown that bioaerosol levels can remain above upwind background for several hundred metres downwind of sources and low numbers of site-specific microorganisms at a kilometre or more from the source.
Site(s) requirement	Power availability and data connectivity	Ideally continuous electrical power should be available at sites which require pumped samples of greater than 30 mins in duration, to avoid the logistical challenges presented by the need for batteries, generators or fuel cells. Where sites are to operate unmanned data connectivity (either cabled or access to wireless/mobile phone signal) allow performance to be monitored remotely and action taken in the event of, for example, equipment failure
	Risk to equipment	Locations which present a known or suspected security or environmental risk to equipment must be avoided unless appropriate measures for protection can be put in place.
	Installation of large or heavy instruments	Large or heavy instruments, unless long-term sampling is to be undertaken, should be avoided or appropriate handling measures must be provided to avoid injury to site technicians whilst moving instruments. Lighter instruments would be advantageous where possible, in particular for short time-period monitoring where equipment may need moving quickly in order be able to sample downwind of a site in changing conditions.

Decision Category	Requirements and feasibilities	Findings
	Manned vs unmanned stations	Use of sampling methods which require collection of very short duration (<6 hrs) samples may utilise manned stations. Where longer duration sampling is required, unmanned stations would be advantageous.
	Access	Sites should be selected on the basis of ease of sampling staff accessibility and owners access permission.
	Training of staff	All site sample staff should be specifically trained in the collection of bioaerosols including sterile technique and sample handling and storage, as well as any supporting instrumentation.
	Difficult environments	Difficult environments should be avoided, unless they are a specific known or suspected source of AMR in bioaerosols, which may justify its use.
	Sample storage	Samples storage should follow protocols appropriate to maintaining their integrity and suitability for the type of analysis they will subsequently undergo.
	Transport of samples to analytical centre	Samples should be kept at an appropriate temperature, stored in isolation, and transported to the laboratory in sufficient time to maintain their integrity and suitability for the type of analysis they will subsequently undergo.
Selection of sampling method	Analytical approach in laboratory	The type of laboratory analyses to be carried out determines the sampling methods to be used in order to provide suitable samples.
	Sampling medium	Sampling media should be selected on the basis of sampling method, duration and target endpoint analyses.
	Sample specifications and sampling efficiency	Sampling should be carried out at sufficient flow rate and/or duration to enable the target level of confidence in resulting data to be

Decision Category	Requirements and feasibilities	Findings
		achieved. Methods should take into account other factors which might impact on results; for example, high air shear in a sampler might compromise viability of organisms to be assessed by culture methods.
	Reliability	In order to ensure sampling reliability, methods should be chosen from those for which evidence of reliable performance is available, such as those which have a track-record if operational use. Reliability of passive sampling techniques is currently unknown.
	Labour costs	These are likely to comprise a significant part of the cost of a surveillance network and should be fully taken into account. Labour costs include not only those involved with installing and operating samplers and delivery of samples to analysts, but those involved with quality control and assurance processes such as instrument calibration.
	Capital costs; Instrument lifetime; Acquisition time of new instrument or reuse of instrument	Adopting sampling methods with low equipment attrition rates will reduce long-term capital costs. Sampling network capital expenditure will increase as sample network spatial resolution increases. Where surveillance is deemed to need only short-term sampling this will permit equipment to be used at multiple sites, reducing capital expenditure. There is no current national monitoring programme for bioaerosols. Therefore, new instruments will need to be acquired. In order to avoid cross contamination of sample instruments, it is recommended that instruments within the existing air quality network are not utilised to collect AMR in bioaerosols samples.
	Sample media costs	Sample media costs vary across the sampling methods but are marginal in comparison to the

Decision Category	Requirements and feasibilities	Findings
		current laboratory costs of analysing non-culturable AMR samples.
	Power requirements	Continuous electrical power will be required for sampling methods >0.5 hrs in duration.
	Housing requirement	Current sampling methods have no specific essential sample housing requirements. No housing is likely to be required for short-term sampling. For longer term installations appropriate housing should meet requirements for ventilation and temperature management. Particular attention should be applied to inlet conditions in order to ensure that representative samples are being captured without interferences (for example, losses in sample inlets).
Supplementary data	Weather data	Meteorological data for short-term sampling campaigns can include micrometeorological data and wind direction. Longer term exposure assessments may use validated meteorological data from the closest meteorological monitoring site, taking into account factors such as complex topography and built environment influences, which may serve to decouple local micrometeorology from that at sites some distance away. Numerical weather modelling may also be used to interpolate local weather from other sites and Met Office weather models.
	Modelling tools and assessment methods	Modelling data will be used to further develop surveillance objectives. Modelling and mapping of initial surveillance data will provide an understanding of potential receptor exposure, areas of hot-spots and likely AMR sources.
	Management or activity data	National sample network activity should be centrally managed by a national agency or delegated service provider, to ensure minimal

Decision Category	Requirements and feasibilities	Findings
		standard of service, acceptable data quality and to maximise data capture rates.
	Cover data and land use data	Sample sites should be selected on their coverage of major land use categories, areas of high exposure risk, and areas where AMR sources are known to exist. Accurate data should be recorded as to sample site locations and surrounding features such as land uses.

Risks and opportunities

Sampling and analysis of AMR in bioaerosols have both risks and opportunities. The major risks and opportunities have been identified and discussed below.

Risks

1. **Establishing a new network**: AMR in bioaerosols is a relatively new area of research that lacks an established network for understanding its background levels and dynamics. This presents a major challenge for developing effective strategies to monitor and mitigate the spread of airborne antibiotic resistance. Understanding the background levels of airborne AMR is crucial for identifying changes in their abundance and distribution that may signal the emergence or spread of new resistance determinants. Without establishing a new monitoring network, it will be difficult to establish such quantitative baseline data and capture the diversity of environmental and anthropogenic factors that influence the airborne microbiome and resistome.
1. **Analytical capability**: One of the main challenges with AMR in bioaerosols sampling and analysis is the complexity of the process. In order to sample and analyse the bioaerosols accurately, specialised equipment and trained personnel are needed. Errors during the sampling or analysis process can lead to incomplete or inaccurate data, which can limit the usefulness of the data. There is also a risk of cross-contamination during the sample collection and processing, which can introduce false positives and biases in the data. Finally, there can be a lack of standardisation in AMR analytical methods, which can make it difficult to compare results across different studies.
2. **Sampling objectives**: At this early stage, it can be challenging to define clear sampling objectives for AMR in bioaerosols sampling network. Without a clear understanding of what the sampling strategy aims to achieve, it can be difficult to design effective sampling strategies that yield meaningful data. For example, if the sampling is too infrequent or not collected in the right locations, it may not capture the full picture of antibiotic resistance in the environment.

3. **Standardised approaches**: There is currently a lack of standardisation in the methods used for AMR in bioaerosols sampling and analysis. This can lead to inconsistencies in the data and limit comparability between studies. For example, different studies may use different methods for sampling time, sampling conditions, storage of samples, and analysis, making it difficult to compare results across studies.
4. **Defining common end point**s: The interpretation of AMR in bioaerosols data can be complicated by a lack of common endpoints. Different studies may use different units of measurement or definitions for the abundance of antibiotic resistance genes, which can complicate the interpretation and comparison of results across different studies.

Opportunities:

1. **Improved understanding of antibiotic resistance spread**: Establishing a national or even international network of sampling sites that can capture the diversity of environments and conditions that influence the AMR in bioaerosols will provide valuable insights into the spread of antibiotic resistance in the environment. This can help identify areas of high risk and aid in the development of targeted control strategies. This network should use standardised sampling and analysis methods to ensure consistency and comparability of results, and it should be designed to capture the temporal and spatial variability of airborne ARGs.
2. **Early detection and response to emerging resistance**: AMR in bioaerosols sampling can help identify emerging antibiotic resistance, enabling early detection and response to prevent the spread of resistance. This can help limit the impact of antibiotic resistance on public health and support the development of new treatment strategies.
3. **Identification of potential sources of resistance**: AMR in bioaerosols sampling can help identify potential sources of antibiotic resistance in the environment, including specific bacterial strains and geographic regions. This can inform the development of targeted interventions to limit the spread of resistance.
4. **Development of new control strategies**: By providing insights into the spread and sources of antibiotic resistance in the environment, AMR in bioaerosols sampling can inform the development of new control strategies, such as improved sanitation protocols, targeted antibiotic use, or the development of new antimicrobial agents.
5. **Taking advantage of the growing using of genomics in public health**: The rising use of genomics in public health that occurred as a result of the pandemic could potentially have benefits for AMR surveillance in the UK, as some of the same testing technologies and infrastructure could be used to detect and monitor the spread of antibiotic-resistant bacteria. In addition, the data analysis and surveillance components of the PathSafe programme could also be adapted to support AMR surveillance efforts. The programme's emphasis on real-time data collection and analysis could potentially be used to track the spread of antibiotic-resistant infections and identify areas of high risk for AMR.
6. **Collaboration and knowledge sharing**: AMR in bioaerosols sampling can help build collaborations and knowledge sharing among different sectors, such as healthcare, agriculture, and the Environmental Agency, to develop effective

strategies for reducing antibiotic resistance. By providing a common understanding of the sources and spread of antibiotic resistance, AMR in bioaerosols sampling can facilitate collaboration and coordination between these sectors.

5. Conclusions

This report provides a template for an early-stage surveillance strategy for airborne AMR in the UK. Despite the currently limited scientific understanding of airborne AMR and the risk it poses to human health, a strategy which may act as a pragmatic starting point for the surveillance of airborne AMR in the UK is proposed. Such surveillance is important for filling the fundamental knowledge gaps regarding the prevalence and density of airborne AMR in different types of UK air, which will be vital for assessing and managing any risks it poses to the UK population.

Although the design for a UK surveillance strategy (detailed in the previous chapter) includes some specific recommendations, there are broader recommendations underpinning them which might be used more generally. These are:

1. **Bioaerosol sampling:** Use bioaerosol sampling techniques that allow for both culture-dependent and culture-independent methods of AMR characterization. Active sampling methods should be used to determine the actual densities of AMR microorganisms per unit volume of air. The specific bioaerosol sampling design (e.g., sampling method and duration) will need to be adopted to the specific environment being sampled, but maximising the consistency of the sampling approach between source and receptor environments is recommended to permit comparisons.
2. **AMR measurement:** Utilise both culture-dependent and culture-independent approaches to quantify AMR in bioaerosols. Cheaper, culture-dependent approaches can be used for larger scale, targeted surveillance of key ‘sentinel’ AMR pathogens - providing a basis upon which to estimate potentially harmful human exposure. More expensive, culture-dependent approaches (e.g., metagenomics) can be used to characterise AMR in bioaerosols more broadly to optimise AMR target selections and recognise emergent potential threats.
3. **Choice of sampling regime:** Leverage existing sampling regimes used in air quality monitoring (e.g., M9, AURN). This will enable maximum levels of spatial and temporal coverage to be achieved with the minimal duplication of effort. Furthermore, tapping into these existing regimes will open up opportunities for leveraging the wealth of historical and current air quality metadata being collected by them. This will enable choices about times and locations of sampling to be made that ensure a signal (e.g., peak emission times), and may also provide opportunities for better understanding the relationship between airborne AMR and other air pollutants.

Finally, it is important to state that the development of airborne AMR surveillance will in turn be shaped by the scientific development of new methods and techniques for detecting and quantifying AMR genes and microorganisms in complex environmental samples. A better understanding of the diversity of sources and pathways of AMR dissemination in bioaerosol is still needed for a comprehensive strategy to be developed, as is a better understanding of the potential health impacts of exposure to airborne AMR. Addressing these knowledge

gaps will require a multidisciplinary approach that combines expertise from the fields of microbiology, environmental science, public health, and epidemiology. Finally, it is crucial to translate findings into actionable measures to mitigate the spread of AMR in bioaerosols and protect public health. By addressing these challenges, we can move towards a better understanding of the role of bioaerosols in the dissemination of AMR in the environment.

Glossary

Air Resistome	The collection of antibiotic resistance genes (ARGs) and their bacterial hosts present in the air.
Airborne AMR pathogens	Pathogens that are resistant to one or more antimicrobial substance and are able to spread through the air.
Amplicon sequencing	A method in which a specific gene of interest is amplified using PCR before analysing the PCR product in terms of the sequence of nucleotide bases.
Antimicrobial resistance	A characteristic of microorganisms whereby they can survive and reproduce in the presence of antimicrobial agents which used to kill or inhibit them.
Automatic Urban and Rural Network	An existing air quality monitoring network in the UK that measures concentrations of various air pollutants at multiple sites across the country.
Azole resistant *Aspergillus fumigatus*	A fungus that causes infections and has developed resistance to a class of antifungal drugs called azoles.
Bioaerosols	Airborne particles containing biological material such as microorganisms, pollen, and spores.
Colony- forming Unit	A unit which estimates the number of microbial cells (bacteria, fungi, viruses etc.) in a sample that are viable, able to multiply via binary fission under the controlled conditions.
Culture based methods	Techniques for detecting and quantifying microorganisms that rely on growing them in culture media under controlled laboratory conditions.
Culture dependent	A set of microbiological analyses which involves growing microorganisms in the laboratory. Such assays give phenotypic information on analytes.
Culture independent techniques	Methods for detecting and characterizing microorganisms that do not rely on growing them in culture media, such as DNA sequencing and metagenomics.

ESKAPE	Acronym comprising the scientific names of six highly virulent and antibiotic resistant bacterial pathogens including: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacter species
Horizontal transmission	A process where bacteria can share antimicrobial resistance genes (ARGs) horizontally, acquiring them from other bacteria or the environment.
HTqPCR	High throughput quantitative polymerase chain reaction a software package amenable to the analysis of high density qPCR assays, either for individual experiments or across sets of replicates and biological conditions.
Metagenomic analysis	A culture independent approach that analyses DNA sequences of microorganisms in a sample.
Methicillin resistant *Staphylococcus aureus*	A bacterium that causes infections and has developed resistance to methicillin, a type of penicillin antibiotic.
Minimum inhibitory concentration	The minimum concentration of a drug (antimicrobial) that inhibits the observable growth of a particular target microorganism.
Multidimensional scaling	A statistical technique used to visualize and compare the similarities between samples based on multiple variables.
Passive sampling	A sampling method that does not involve an air mover but instead relies on gravitational settling, electrostatic attraction, or a combination of both, to collect particles onto a collection surface, such as a Petri dish with culture media.
Phenotypically	Observable characteristics of an organism
Phenotypically resistant	Refers to microorganisms that have developed resistance to antimicrobial agents through genetic changes that can be observed through changes in their observable characteristics.
PM10	Inhalable particles, with diameters that are generally 10 micrometres and smaller

Power analysis	A statistical method used to determine the minimum sample size needed to detect a particular effect size or prevalence with a given level of confidence.
Replication	The act of conducting multiple samples or experiments to ensure the results are consistent and reliable. Replication is important in bioaerosol sampling to account for variability in the data and to increase the accuracy and precision of the results.
Resistome	The collection of antimicrobial resistance genes and associated mobile genetic elements in microbial communities.
Routes of transmission	The pathways by which infectious diseases are transmitted from one person to another or from an animal to a person.
Sentinel antimicrobial resistant microorganisms	Bacteria or fungi that are commonly associated with antimicrobial resistance and can serve as indicators of the presence of AMR in the environment.
Source apportionment	The process of identifying and quantifying the contributions of different sources to a particular pollution problem.
Source-attribution	The process of identifying the source of AMR microorganisms in receptor environments.
Source- focussed strategy	A surveillance approach that aims to estimate the levels of airborne antimicrobial resistant microorganisms (AMR) in source locations where a large amount of AMR is likely to be aerosolized.
Vertical transmission	A process whereby genes are passed from parent to offspring.
Viable bacteria	Living bacteria that are capable of reproducing

List of abbreviations

AMR	Antimicrobial Resistance
ARB	Antibiotic Resistant Bacteria
ARF	Antifungal Resistant Fungi
ARG	Antimicrobial Resistance Genes
AURN	Automatic Urban and Rural Network
CAFO	Confined animal feeding operation
CFU	Colony forming unit
ESBL	Extended Spectrum Beta-Lactamase
GLASS	Global Antimicrobial Resistance Surveillance System
HTqPCR	High throughput quantitative polymerase chain reaction
HVAC	Heating, ventilation, air conditioning
MARGA	Monitor for AeRosols and Gases in Ambient air
MRSA	Methicillin-resistant Staphylococcus Aureus
PATH-SAFE	Pathogen Surveillance in Agriculture, Food and Environment
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
qPCR	Quantitative polymerase chain reaction

REPS	Rutgers Electrostatic Passive Sampler
UKEAP	UK Eutrophying and Acidifying Pollutants
UKHSA	UK Health Security Agency
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

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Appendices

Appendix A – Rapid Evidence Assessment for AMR in bioaerosols

Background

Antimicrobial resistance (AMR) is the resistance of bacteria, fungi and other microorganisms to the toxic effects of antimicrobial compounds. There are various mechanisms by which microorganisms tolerate or inactivate antimicrobials. These mechanisms are genetically determined, meaning that AMR is an inheritable characteristic in bacterial and fungal populations. In addition to vertical transmission of genes (from parent to offspring), bacteria can share antimicrobial resistance genes (ARGs) horizontally, acquiring them from other bacteria or the environment. Resistance characteristics in microorganisms that cause infections poses a significant threat to human health: these infections are harder to treat with available drugs, and so carry with them far greater risks of morbidity and mortality compared to susceptible microorganisms. Recent estimates of the impact of antimicrobial resistance demonstrate this threat, with approximately 1.27 million deaths occurring each year due to bacterial AMR (Murray et al., 2022). If current trends continue, there will be 10 million deaths globally each year attributed to antimicrobial resistance by the year 2050, with an estimated cumulative economic impact of 100 trillion USD by that time (O'Neill, 2016).

Identifying the routes via which AMR transmission to humans occurs is key to guiding legislative and regulative interventions that can reduce or prevent human exposure to drug-resistant microorganisms. As well as human-to-human and animal-to-human infection, environment-to-human pathways are being increasingly considered as a transmission route for AMR microorganisms (Stanton et al., 2022). Microorganisms and their genetic material are regularly recovered from airborne particles, and exposure to these bioaerosols has been associated with detrimental human health effects, including respiratory symptoms, gastrointestinal diseases, allergic and toxic reactions (Pearson et al., 2015; Searl, 2008; Stagg et al., 2010; Walser et al., 2015). Such health risks have already led to efforts to monitor bioaerosols at regulated facilities (Environment Agency, 2018). With rising AMR, the question of whether AMR in bioaerosols should be monitored is also being asked.

Airborne transmission is one of several routes via which people may possibly acquire AMR microorganisms, and bioaerosols may pose an important risk in terms of exposure to airborne AMR for two reasons. Firstly, air is an environmental compartment to which people are constantly and directly exposed through breathing - unlike other environmental compartments to which exposure is more sporadic or mediated by human treatment (e.g. natural water sources). Secondly, the diversity of microorganisms and genes recovered from bioaerosols is vast, with their exact composition changing according to local surface environments and meteorological conditions (Zhao et al., 2022). This elevates the potential AMR risk associated with bioaerosols and its ability to be effectively managed.

The aim of this review is to summarise the evidence on AMR in bioaerosols, including types of AMR associated with particular sources of AMR, their prevalence and factors affecting their dissemination. In addition, the evidence of risks to human health that exposure to AMR present in outdoor bioaerosols pose is summarised.

Methods

1. Review of studies of AMR in air associated with particular human activities.

The main part of this report is a review of the studies of airborne AMR, which takes a modular approach that focuses on particular human activities likely to aerosolise AMR (e.g. wastewater treatment, and farming), one at a time. The aim was to understand the methods used to detect and quantify airborne AMR, the types and prevalence of AMR in bioaerosols in different environments, as well as the factors governing aerosolised AMR dispersion. This main part of the review contributes evidence and data towards designing a strategy for measuring AMR in environmental air samples and was developed and agreed upon with the Environment Agency.

To conduct this main part of our review, the Web of Science Core Collection (A&HCI, ESCI, CPCI-SSH, CPCI-S, SCI-EXPANDED, SSCI) was searched using various search terms for antimicrobial resistance, bioaerosols and dusts, combined with search terms specific to each type of environment for review (e.g. terms such as ‘wastewater’ or ‘waste’ for wastewater treatment). Searches were limited to articles published since 2002. Reproducible search strategies are reported in the supplementary materials.

Following each search, the titles and abstracts of the returned records were imported into the Rayyan web-based software for screening against pre-defined selection criteria (Selection criteria in supplementary materials). One key criterion was that the studies must include samples of outdoor air. Indoor air samples were considered to be outside the remit of any potential Environment Agency activity. Results for one module (wastewater treatment plants) were double screened independently to identify and correct differences in how the two reviewers applied the selection criteria. All subsequent screening was performed by a single reviewer.

After filtering out the relevant studies, the study characteristics of these studies (year, location, AMR target, methods) were extracted and tabulated in separate tables for each module. More detailed data on AMR abundances were extracted only for wastewater treatment plant (WWTP) studies to assess whether meta-analyses would be feasible. This module was chosen to aid the interpretation of results from a parallel environmental sampling campaign for AMR in bioaerosols focussing on a single UK WWTP. Given the quality and quantity of data identified for this well-studied environment generating bioaerosols, it was decided that the value of repeating this activity across all modules was extremely limited. Therefore, information on study characteristics and findings were narratively summarised to answer specific questions about the nature of AMR in air associated with different types of human activity:

1. Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?
2. What are typical prevalences of antimicrobial resistance in bioaerosols sampled at source and ambient environments?
3. How do AMR targets in bioaerosols vary spatially and temporally in outdoor air?

These questions and the data summarised are intended to help guide the choice of suitable targets for AMR surveillance, the sampling and microbiological methods, and interpretation of the results of any sampling activity performed in the future. In this review, different types of air covering five distinct 'source' environments (i.e. human activities/sites likely to aerosolise AMR) as well as the category of 'ambient' air (i.e. that not associated with one particular human activity/site) were considered. In a series of 'mini-reviews' covering the four questions listed above, the following types of environments were reviewed:

1) Source 1: Air associated with wastewater treatment. This considered outdoor air collected at or near plants that treat sewage or wastewater. This source was considered given that WWTPs are 'mixing pots' of AMR and antibiotic-laden waste from humans and animals, and treatment of sewage involves various aerosolising processes.

2) Source 2: Air associated with livestock farming of pigs. This considered outdoor air samples collected on or near sites where pigs are raised for food. This source was considered given the use of antimicrobials (particularly antibiotics) in livestock-rearing practices.

3) Source 3: Air associated with livestock farming of poultry. This considered outdoor air samples collected on or near sites where poultry (chickens, turkeys, ducks, geese) are raised for food. This source was considered given the use of antimicrobials (particularly antibiotics) in livestock-rearing practices.

4) Source 4: Air associated with livestock farming of cattle. This considered outdoor air collected on or near sites where cattle are raised for food (dairy farms and beef farming). This source was considered given the use of antimicrobials (particularly antibiotics) in livestock-rearing practices.

5) Source 5: Air associated with arable farming. This considered outdoor air collected on or near sites where crops are grown. This source was considered given the use of antimicrobials (particularly antifungals) in arable farming.

6) Ambient air: Air that is not associated with a particular known source of bioaerosols or antimicrobial resistant microorganisms. These are typically environments in residential areas with many potential contributing sources, or in remote areas with few or uncharacterised sources contributing to air quality. This type of air was considered as it is potentially more reflective of the type of air to which the average person is exposed when going about their daily activities.

2. Review of human health effects of exposure to airborne AMR

This part of the review was intended to assess what is known about the health implications of exposure to airborne AMR described in the main part of the review, further informing the development of a surveillance strategy. PubMed was searched using terms covering antimicrobial resistance and bioaerosols, as well as potentially relevant health outcomes in humans, such as colonisation, transmission, and infection.

After downloading of search results, titles and abstracts were screened as before, applying similar eligibility criteria as above, but with three key adjustments. First, eligible studies had to either report on antimicrobial resistance in samples collected from human subjects, or quantify exposure to airborne AMR via inhalation. Second, studies that did not include data from an exposed population and unexposed population were excluded, since the availability of data from a control group is key to determining risk. Finally, included studies were not required to directly measure AMR in air samples, since the focus was on human health effects.

Study characteristics were extracted and key results summarised from selected studies. This information was used to answer specific questions about the evidence on risks to human health of exposure to AMR in bioaerosols:

1. Which antimicrobial resistance markers have been detected in samples taken from human subjects and how have these been measured?
2. What evidence exists for a relationship between exposure to airborne AMR and health effects?

Results

Air associated with wastewater treatment.

Nine papers describing eight separate studies of AMR in bioaerosols at wastewater treatment plants (WWTPs) were identified. Key characteristics of each study is presented in Table 1.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

Antibiotic resistant bacteria (ARB) are the most frequently studied markers of AMR in the studies performed at WWTPs. While fungi and moulds have been detected in bioaerosols analysed by the included studies (Bruni et al., 2019; Kowalski et al., 2017; Małecka-Adamowicz et al., 2017), assessment of their susceptibility or resistance to antifungals was not performed. A variety of microbiological methods were used to identify and characterise AMR, including culture-dependent methods, culture-independent methods, and combinations of the two approaches to identify the bacterial species or the anti-microbial resistent genes (ARGs) carried by cultured bacteria.

Air sampling methods: Various approaches to air sampling were reported, including different substrates, volumes (varying flow rates and durations), and sampling locations within WWTPs. Impaction onto a substrate is the most common type of method: agars, gelatine filters, polycarbonate substrate or polytetrafluoroethylene substrate. One paper described impingement into phosphate buffered saline solution. Reported volumes of air sampled ranged from 6L (Osińska et al. 2021) to 5040L (Gaviria-Figueroa et al. 2019). Reported flow rates ranged from 3L per min (Kozajda and Jeżak 2020) to 125L per min Gaviria-Figueroa et al. 2019), and reported duration of sampling ranged from 10 min (Kozajda and Jeżak 2020) to 2 hours (Kozajda and Jeżak 2020). Sampler locations in terms of heights and distance from source were rarely reported. However, Bruni et al. (2019) reported placing samplers 1.5m from source at a height of 3m. Osińska et al. (2021) described placing samplers at a height of 1.3m.

Culture-based methods to measure antibiotic resistance was commonly used, including disk diffusion, selective culture, or measurement of minimum inhibitory concentrations (Kozajda and Jeżak 2020; Korzeniewska and Harnisz 2013; Korzeniewska, Korzeniewska, and Harnisz 2013). Table 9 (Supplementary Materials) displays the bacterial species detected and their phenotypic resistance identified using culture-based methods only. Across all included studies, a diverse set of bacteria were phenotypically resistance to one or more of 31 different antibiotics were detect and/or quantified.

Culture-independent methods to measure antibiotic resistance included standard PCR, qPCR and high-throughput qPCR for multiple antibiotic resistance gene targets. These were combined with amplicon sequencing[4] of 16s rRNA gene (a universal bacterial gene used to distinguish species-like units of bacteria) to provide insights into the bacterial composition of bioaerosol samples (Gaviria-Figueroa et al. 2019). Table 10 (Supplementary Materials) presents data on the detection and/relative abundance of specific ARGs. Across all studies, 25 ARGs were detected or quantified.

Combining culture-dependent with culture-independent approaches allows one to determine the genes harboured by targeted bacterial species. Four papers combined both types of approaches, using culture-based methods to isolate culturable bacterial colonies, and molecular methods such as PCR, qPCR and amplicon sequencing of target genes (Korzeniewska and Harnisz 2013; Korzeniewska, Korzeniewska, and Harnisz 2013, Gaviria-Figueroa et al. 2019; Potorski et al. 2019). Table 8 (supplementary materials) displays the results of studies combining molecular with culture-based approaches. 20 named ARGs were detected or quantified across a handful of Enterobacteriaceae species (*E. coli, Klebsiella pneumonia, Citrobacter freundii*), or in unidentified cultured bacteria.

[4] In amplicon sequencing, a specific gene of interest is amplified using PCR before analysing the PCR product in terms of the sequence of nucleotide bases.

What are typical prevalences of antimicrobial resistance in bioaerosols sampled at source and ambient environments?

Differences in methodologies, settings, AMR targets and reporting prevent a comparison of prevalences across all studies. Supplementary tables 9 to 11 present data extracted from studies reporting on the abundance of AMR in bioaerosols.

Densities of genes per m^3 varied by gene, for example, 1-1000 copies per m^3 tetracycline resistance genes like *tet(A), tetB, tet(M)*, compared to 100-10,000 for beta-lactam resistance genes like *blaAMPC* and *blaTEM* (Osińska et al., 2021).

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Study designs included cross-sectional[5] and longitudinal studies[6], as well as transects[7]. Longitudinal transects were also reviewed (e.g. Małecka-Adamowicz et al. 2017; Osińska et al. 2021). Individual studies were limited in the amount of data available to be able to answer this question robustly. Studies report differences in the abundance of microbiological targets at different points of a WWTP, as well as seasonal differences in microbiological air quality, and correlations between targets and some meteorological parameters are also observed. However, these observations are sometimes contradictory between papers using different methods, are rarely made for antimicrobial resistance markers, and effect sizes of the correlations are not reported.

Temporal patterns: Several studies noticed higher viable microbiological abundances in warmer months compared to winter months (Małecka-Adamowicz et al. 2017; Kowalski et al. 2017, Kowalski et al. 2017). However, Osińska *et al.* (2021) used culture-independent techniques and reported higher abundances of the bacterial marker gene, 16s rRNA, in bioaerosols in the winter compared to samples collected in the spring, and higher abundances of the ARGs, blaTEM and blaAMP-C, in the winter. None of the studies analysed AMR at fine temporal scales, so variation in AMR markers in bioaerosols day-by-day, or week-by-week is unknown. Thus we could not draw conclusions about temporal variations finer than seasonal differences.

Meteorological parameters: Contradictory observations were also reported in studies investigating the relationship between temperature and AMR in bioaerosols and most of the

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[5] A cross-sectional study is one in which researchers collect data from many different individuals at a single point in time

[6] A longitudinal study is one in which researchers repeatedly examine the same individuals to detect any changes that might occur over a period of time

[7] A transect is a path along which researchers collect measurements (e.g. at several distances from a source)

relevant papers did not report data to distinguish whether difference were due to changes in AMR prevalence or changes in total bacteria: Kozajda and Jeżak (2020), Korzeniewska and Harnisz (2013) and Osińska *et al.* (2021) reported that temperature was positively correlated with the presence of AMR targets: *S. aureus* and the susceptibilities these colonies exhibited, beta-lactam resistant Enterobacteriaceae (Korzeniewska and Harnisz 2013), and chloramphenicol resistance genes e.g. cmlA (Osińska et al. 2021). Negative correlations were reported between temperature for 16s rRNA , beta-lactam and blaTEM. A positive correlation between humidity and 16s rRNA and blaTEM was found, but a negative correlation with cmlA chloramphicol (Osińska et al. 2021). Positive correlations were reported between wind speed with beta-lactam resistant Enterobacteriaceae and ESBL-resistant Enterobacteriaceae *(*Korzeniewska and Harnisz 2013). While Gaviria-Figueroa *et al.* (2019) did not explore correlations between AMR markers and wind, they modelled anti-microbial resistent gene (ARG) dispersion downwind in the absence of rainfall, with increasing windspeed expecting to result in higher abundances of ARGs dispersed within a 120km radius of the plant. This model made a number of important assumptions about bioaerosols emissions, precipitation, and local topography, and these predictions were not validated. None of the studies looked at the effects of precipitation on AMR emissions or dispersal.

Spatial: A few of the included studies found differences in the microbiological quality and abundance of AMR markers in bioaerosols at different parts of the sampled wastewater treatment plants depending on process and treatment applied (Osinka, Maleka). With higher abundances in bioaerosols found at locations performing primary screening, and those for biological processing (i.e. aerobic digestion). Kozajda *et al.* (2020) performed the largest cross-sectional study across 16 WWTPS in Poland, focussing specifically on *Staphylococcus aureus*. But antibiotic resistant *S. aureus* were detected in only two of the wastewater treatment plants sampled, and only one of these locations was described as being outdoors. The paucity of data on this AMR marker prevents a full understanding of spatial variation in AMR in bioaerosols and may indicate that *S. aureus* is not a sensitive marker for AMR in bioaerosols in this setting due to its low recovery in air samples. A simulation of ARG dispersion was produced by Gaviria-Figueroa *et al.* (2019), using an estimated ARG emission rate of 10,620 ARG per hour from the WWTP source, combined with the frequency of observed windspeed and direction to say how frequently different abundances of genes would be found across the different sectors surrounding the WWTP. In addition, they calculated dispersion in the absence of wind direction. Even at the lowest wind speeds (5 km/h), more than 220,000 ARGs are detected per day within a 10km radius. At the highest windspeeds (20 km/h), more than 220,000 ARGs per day would be detected within a 50 km radius, and more than 200,000 ARG per day will be detected within a 140 km radius.

Other environmental conditions: A few papers noted the typical sources of wastewater input (municipal, hospital etc), and surrounding activities (e.g. intensive livestock operations), (Kozajda et al. 2020), or terrain (coastal conditions) that may affect the results of AMR in bioaerosols from WWTPs receiving these types of inputs (Gaviria-Figueroa et al. 2019). Bruni *et al.* (2019) collected samples at two time points, but noted that the results of one of

the sets of samples was probably affected by an advection episode of air masses originating from deserts in North Africa.

Table 1 Key study characteristics of the included studies of antimicrobial resistance in air associated with wastewater treatment.

First author year	Year (season)	Country	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Bruni *et al.* 2019	2018 (spring)	Italy	Longitudinal at a single plant	ARB	Impaction onto polycarbonate substrate 10L/min (duration, volume NR). Sample height 3 m	Culture-based: culture followed by disk diffusion (20 antibiotics)
Gaviria-Figueroa *et al.* 2019	NR	United States	Transect (2 sites, 1 WWTP)	ARG-bearing bacteria; ARGs	Impaction onto gelatin membrane filters or PTFE filters 56 L/min 90min. 125 L/min 30min. Sample height NR	Culture-based methods combined with qPCR. Culture-independent qPCR on DNA extracted from total air sample. (HT-qPCR for 84 ARGs)

First author year	Year (season)	Country	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Korzeniewska & Harnisz 2013	NR	Poland	Transect (2 sites 1 WWTP)	ARB (Enterobacteriaceae bacteria producing ESBLs and Enterobacteriaceae harbouring plasmid-borne ESBL ARGs)	Impaction directly onto Chromocult Coliform Agar Rate, duration NR. 400 L per sample. Sample height NR	Culture-based: Selective culture, assessment of MIC (2 antibiotics); amplicon sequencing of 5 common ESBL gene families
Korzeniewska *et al.* 2013	NR	Poland	Transect (2 sites 1 WWTP)	ARB (*Escherichia coli* producing ESBLs and *E. coli* harbouring plasmid-borne ESBL ARGs)	Impaction directly onto Chromocult Coliform Agar Rate, duration NR. 400 L per sample Sample height NR	Culture-based: Selective culture, assessment of MIC (2 antibiotics); amplicon sequencing of 5 common ESBL gene families
First author year	Year (season)	Country	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods

First author year	Year (season)	Country	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Kowalski *et al*. 2017	Winter (year not reported)	Poland	Cross-sectional (5 WWTPs once each)	ARB	Impaction directly onto Trypticase Soy Agar 28.3L/min, 8 min. Sample height 1.5 m	Culture-based: culture followed by disk diffusion (20 antibiotics)
Kozajda *et al*. 2020	2017 (summer)	Poland	Cross-sectional (16 WWTPs once each)	ARB (*Staphylococcus aureus* bacteria resistant to antibiotics)	Impaction onto agar or gelatin filter Agar: 20 L/min for 10 min Filtration (gelatin) 3 L/min over 1.5-2h. Sample height NR	Culture-based: culture followed by automatic method to assess MIC (21 antibiotics)
Malecka-Adamowicz *et al*. 2017	2014-2015 (spring, summer, autumn, winter)	Poland	Longitudinal transect (single WWTP, 6 locations, 5 times)	ARB (Antibiotic resistant *Staphylococci*)	Impaction onto Chapman nutrient medium 50-100 L air sampled (duration, rate, height NR)	Culture-based: culture followed by disk diffusion (8 antibiotics).

First author year	Year (season)	Country	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Osinsksa *et al.* 2021	2019 (winter and spring)	Poland	Longitudinal transect (single WWTP, 2 locations, 2 times)	ARGs	Impingement into PBS solution Height 1.3 m 6 L per sample (duration, rate NR)	Culture-independent: PCR (19 ARGs) and qPCR (8 ARGs)
Potorski *et al.* 2019	2018 (summer)	Poland	Transect (1 WWTP, 2 sites)	ARB, and the ARG they carry	Impaction directly onto LB agar and LB supplemented with antibiotics Sampling NR	Culture-based: Selective culture (3 antibiotics), PCR (13 ARGs)

Acronyms: NR not reported; ARB antibiotic resistant bacteria; ARF antifungal resistant fungi; ARG antibiotic resistance genes; ESBL extended spectrum beta-lactamase; HT-qPCR high-throughput quantitative PCR; MIC minimum inhibitory concentration; PBS phosphate buffered saline; PCR polymerase chain reaction; PTFE polytetrafluoroethylene WWTP wastewater treatment plant

Air associated with livestock farming (pig)

Seven papers describing six separate studies presented research into AMR in bioaerosols collected on or near pig rearing facilities were identified. Key characteristics of each study are presented in Table 2 below.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

All the papers reviewed investigated antibiotic resistant bacteria, with no papers measuring antifungal resistant fungi. Many papers chose to focus on particular pathogens such as staphylococci, *E. coli, Salmonella, or Enterococcus*, apparently due to a concern about the hazard these present to humans (particularly those working on farms or consuming farm

produce) being colonised with resistant bacteria. Enumeration of these was achieved by impaction of air samples onto agar, followed by culture-based methods antimicrobial susceptibility testing to a range of antibiotics. These antibiotics covered major classes of antibiotics, including beta-lactams, macrolides, quinolones, aminoglycosides, tetracyclines, among others. Standard PCR was commonly employed to identify the presence of a few specific genes representing resistance to two antibiotic classes: beta-lactams, and oxazolidinones (linezolid). One study (Ruiz-Ripa et al., 2020b) used PCR for 37 ARG targets extending beyond these two groups of antibiotics.

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Of all the papers, Gibbs et al., (2006) provide the best indication of the prevalence of antibiotic resistance among bacterial isolates cultured from air. Data is presented for percentage resistance to six antibiotics among four groups of cultured bacteria at five outdoor locations (one site upwind, four downwind sites). Among *S. aureus*, the most frequent resistance observed was to lincomycin (78% - 95% of isolates), and the least frequent resistance observed was to ampicillin (21% - 75%). Similar prevalences were noted among Group A streptococci: 75% - 100% colonies were resistant to lincomycin, and 17%-50% resistance to ampicillin. Resistance to lincomycin was also the most frequently observed resistance among faecal coliforms and total coliforms (66% - 100% and 100% respectively). Resistance to oxytetracycline was least common among faecal coliforms (33% - 67%), whereas resistance to tetracycline was least common among the isolated total coliforms (0%-50%). Data presented in other papers is limited by small sample sizes, low levels of detection of the AMR target, or by methods where a relative abundance or the density of AMR targets cannot be quantified.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Data from studies of pig farms follows the general observation that total culturable bacteria, including AMR bacteria, decrease in abundance downwind of hypothesised sources of bioaerosols (distance-decay function). AMR bacteria were detected up to 150 m downwind of source (Gibbs et al., 2006; Ruiz-Ripa et al., 2020a). Gibbs *et al.* (2006) quantified non-linear relationships between distance from the facility, and the density of antibiotic resistant bacteria per m^3:

1) Density of bacteria resistant to at least two different classes of antibiotics (CFU/m^3)

$= –612.25\ln(x) + 3171.8$

2) Density of bacteria resistant to resistant to all four antibiotic classes (CFU/m^3)

$= –98.936\ln(x) + 528.33$

Insufficient data were reported from studies with longitudinal designs, due to low levels of detection (von Salviati et al., 2015; Ferguson et al., 2016) or data were summarised across all time points (Gibbs et al., 2006, 2004).

Some studies suggest that swine-rearing practices may influence the levels of AMR detected in bioaerosols emitted from pig farms, including antibiotic treatment regimes and animal housing (animal densities and access to outdoor spaces). Davis et al., (2018)'s pilot study noted detection of multidrug-resistant *S. aureus* at industrial pig operations reporting conventional use of antibiotics and higher densities of confined animals, whereas *S. aureus* isolates were not recovered from air samples collected at antibiotic free pig operations, which concurrently reported lower animal densities, and that animals were kept in open pastures).

Table 2 Key study characteristics of the included studies of antimicrobial resistance in air associated with pig farming

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
(Arfken et al., 2015)	NR	North Carolina, United States	Transect (2 sites on a single farm, 1 time point)	ARB (Kanamycin-resistant bacteria and methicillin-resistant *S. aureus*)	Passive sampling: agar plates exposed for 10 minutes Impaction onto nitrocellulose filter. 34L per min for 10 min. Sample height 2 m	Culture-based: Selective culture (2 agars, 2 antibiotics)

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
(Davis et al., 2018)	2015 (summer)	North Carolina, United States	Cross sectional (4 farms sampled, 1 time point)	ARB (methicillin-resistant and multidrug-resistant *S. aureus, S. aureus* harbouring beta-lactamase ARGs)	Impaction onto gelatin filters. (4 L/min for 80-100 min) Impingement into PBS (12.5 L/min for 40-60 min) Impaction onto Chromagar Staph aureus plates 28.3 L/min, sequential collection at 5, 10, 20 min Sample height 0.9 m – 1.5 m	Culture-based: Isolation followed by disk diffusion (15 antibiotics). PCR (2 ARGs)
(Ferguson et al., 2016)	2010 (autumn, winter)	United States	Longitudinal (1 site, 3 times)	ARB (methicillin-resistant *S. aureus*)	Impaction onto agar plate 28.3 L/min and 1.5 L/min. Duration, volume, and height of sampling NR	Culture-based: isolation followed by antimicrobial susceptibility testing (antimicrobials NR). PCR (1 ARG)

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
(Gibbs et al., 2004)	2000	United States	Cross-sectional Transect (2 sites, 5 outdoor locations at each)	ARB (*Salmonella*, *Staphylococcus*, coliforms and faecal coliforms)	Impaction onto tryptic soy agar and malt extract agar 28.3 L/min, volume sampled ranged from 0.028 to 0.4245 m3 Height 1 m	Culture-based: replica plating onto species-specific agars, followed by disk diffusion (6 antibiotics)
(Gibbs et al., 2006)	2003 summer	United States	Longitudinal transect (1 sites, 5 outdoor locations, over 4 time points)	ARB (*S. aureus*, faecal coliforms, Group A streptococcus, coliforms)	Impaction onto tryptic soy agar 28.1 L/min for between 15 seconds – 5 min. Sample height 1.3 m	Culture-based: plating onto species-specific agar, followed by disk diffusion (6 antibiotics)
(Ruiz-Ripa et al., 2020a)	NR	Spain	Transect (1 site, 12 locations)	ARB (*Enterococcus faecium*)	Passive sampling: agar plate exposed over 4 hours Height NR.	Culture-based: isolate followed by disk diffusion (18 antibiotics) and MIC determination using E-test (3 antibiotics). PCR for 3 linezolid ARGs

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
(Ruiz-Ripa et al., 2020b)	NR	Spain	Transect (1 site, 12 locations)	ARB (antibiotic resistant Stapylococci, and resistant *S. aureus*)	Passive sampling: agar plates exposed over 4 hours Height NR	Culture-based: Staphylococcus colonies picked for disk diffusion (13 agents). *S. aureus* tested for resistance to 9 antibiotics using Microscan. MIC determination using E-test (2 antibiotics). PCR for 37 ARGs.
(von Salviati et al., 2015)	2011-2012 winter, spring, summer, autumn	Germany	Longitudinal transect (1 site farm, 2 locations, 3 times	ARB (ESBL/AmpC-producing *E. coli*)	Impingement into PBS 11.5-13 L/min for 90 min Sampling height NR	Culture-based: selective (1 antibiotic). Confirmed *E. coli* tested using disk diffusion (6 antibiotics) PCR for 4 beta-lactam ARGs.

Acronyms: NR not reported; ARB antibiotic resistant bacteria; ARF antifungal resistant fungi; ARG antibiotic resistance genes; ESBL extended spectrum beta-lactamase; HT-qPCR high-throughput quantitative PCR; PBS phosphate buffered saline; PCR polymerase chain reaction

Air associated with livestock farming (poultry)

We identified four relevant studies of antimicrobial resistance in air collected at or near poultry farming operations. The characteristics of these papers are presented in Table 3.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

A wide range of antimicrobial resistance determinants (i.e. phenotypes and genotypes) have been measured and detected in air associated with poultry farms, covering all major antibiotic classes including beta-lactamases, tetracyclines, penicillins and cephalosporins. Three studies tested for resistance in a diverse set of antibiotics (Brooks et al., 2010; Sanz et al., 2021; Vela et al., 2012), whilst the remaining two focussed more on particular organisms with particular resistance phenotypes/genotypes (Friese et al. 2013). Both of the more focussed studies investigated on airborne MRSA (methicillin-resistant *Staphylococcus aureus)* and targeted the beta-lactam resistance gene *mecA*, either in isolates (Friese et al. 2013).

Although some studies enumerated fungi, none measured fungal resistance in air associated with poultry farms.

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Three of the four studies (Brooks et al., 2010; Sanz et al., 2021; Vela et al., 2012) did not measure and/or report resistance outcomes in a way that made it possible to quantify prevalence. Of the studies that did estimate prevalences, Friese et al. (2013) reported MRSA prevalences of 0.07% (impingement) and 0.08% for filtration among total *Staphylococcus* spp. counts across all air samples taken outside nine turkey/broiler farms.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Some information on spatial and temporal variations in AMR abundances can be gleaned from studies reporting such data. Friese et al. (2013) did not identify clear distance-decay relationships in MRSA concentrations with distance from turkey/broiler farms, reporting very low densities of 7-93 CFU/m^3 air 50m outside and 11-24 CFU/m^3 air 150m outside (indoor concentrations were not reported). However, this lack of a relationship may be associated with the high variance associated with such low densities. Total *Staphylococci* concentrations at these distances were not reported, precluding a description of changes in prevalence. Although they did not adequately report resistant bacterial densities, Brooks et al. (2010) found total bacterial densities of ~10,000 cells/m^3 10m outside a broiler CAFO compared to ~1,000,000 cells/m^3 inside; whilst Sanz et al. (2021) found very low outdoor (50-150m North/South/East/West of a broiler farm) densities of all bacterial types considered (<6 CFU/m^3 air) - compared to densities of 100,000CFU/m^3 inside.

Although they did not adequately report temporal trends in resistant bacteria, another interesting temporal observation from Vela et al. (2012) is that the total amount of bacteria in outdoor air increases through the production cycle (not detectable-200 CFU/m^3 day 1, ~10^4 at 14 days, ~10^5 at 35 days), with no obvious diurnal trends. Sanz et al. (2021) also only reported on total bacteria, finding no seasonal trends either in densely populated indoor or outdoor air.

Table 3 Key study characteristics of the included studies of antimicrobial resistance in air associated with livestock farming (poultry).

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Brooks et al. (2010)	2006-2007 (summer, autumn, winter, spring)	Mississippi, United States	Single-point; Single-site	ARB (Staphylococci/ Salmonella/ coliforms/ enterococci resistant to antibiotics)	Impingement into saline solution	Culture dependent: isolation followed by disk diffusion (12 antibiotics)
Friese et al. (2013)	2011 (summer - autumn)	Germany	Cross-sectional (1 barn in 9 locations); Longitudinal	ARB *(Staphylococcus* resistant to beta-lactams (MRSA))	Impingement into unspecified medium	Culture dependent: isolation followed by confirming mecA gene/MRSA subtype
Sanz et al. (2021)	2019 (summer) and 2020 (winter)	Spain	Single-site	ARB *(Enterococcus hirae* resistant to antibiotics)	Impaction directly onto Mannitol salt agar (MSA) and Chromocult coliform agar (CCA) plates	Culture dependent: isolation followed by disk diffusion (7 antibiotics)
Vela et al. (2012)	NR	Western Canada, Canada	Single-site; longitudinal	ARB *(Staphylococcus xylosus* and *Staphylococcus aureus* resistant to antibiotics)	Impaction directly onto phenylethl alcohol agar (PEA; Staphylocci/Streptococci selective) agar	Culture dependent: isolation followed by disk diffusion (21 antibiotics)

Acronyms: NR not reported; ARB antibiotic resistant bacteria; ARGs antibiotic resistance genes; MRSA methicillin-resistant Staphylococcus aureus

Air associated with livestock farming (cattle)

We identified seven relevant studies of air collected at or near beef or dairy farming operations. The characteristics of these papers are presented in Table 4.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

A wide range of antimicrobial resistance markers have been measured and detected in air associated with cattle farms, covering all major antimicrobial classes including beta-lactamases, tetracyclines, penicillins and cephalosporins for bacteria. There was some preference towards measuring resistance to tetracyclines and penicillins when studying cattle, perhaps because these are commonly used antibiotics in cattle farming. Five of the seven reviewed studies used culture-based methods whilst three used qPCR to enumerate antimicrobial genes. Tetracycline, beta-lactamase, erythromycin and sulphonamide resistance genes were the targets of the three qPCR studies identified. These targets were almost always detected to some extent across the studies but varied in their relative abundance. Some studies isolated fungi from air samples, but none measured fungal resistance to antifungals among these.

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Substantial differences in study designs (e.g. season of measurement, distance from cattle farm) and their reporting (especially not reporting results per units of air and/or bacteria sampled) made it difficult to deduce typical prevalences. However, Sancheza *et al.* (2016) reported 750 and 810 copies of sulphonamide and beta-lactamase ARGs per m^3 air sampled in air near (3m from the boundary of) conventional farms versus no detection/lower detection of different genes (210 for an erythromycin ARG near (3m from the boundary of) organic farms. During 30 minutes of passive sampling onto glass-fibre filters, Wooten, Mayer, and Smith (2019) reported collecting ‘copy counts’ (assumed to mean ARGs but unclear) of ~1000 (0-2.5km) upwind and between ~10,000 and ~1,000,000 (0-5km) downwind of beef feedlots - but relative abundance (i.e. gene copy numbers per 16s rRNA or per unit of volume of air) was not reported.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Three studies had a transect designs that sought to understand spatial patterns of antimicrobial resistance in air associated with beef and dairy cattle farms. Navajas-Benito *et al.* (2017) detected resistance to chloramphenicol, trimethroprim-sulfamethoxazole, tetracyclines, nalidixic acid and ciprofloxacin 100m downwind of a dairy cattle farm. However, the authors only isolated and tested a few *E. coli* strains and did not estimate total abundance of resistant *E.coli* in air. McEachran *et al.* (2015) and Wooten, Mayer, and Smith (2019) provide convincing evidence that tetracycline resistance gene copies densities are up to a thousand-fold higher in air downwind of beef cattle feed yards (of feedlots in the

United States), compared to air samples collected upwind of these activities - potentially persisting at high levels at least 2km from the feed yard. However, poor reporting (especially not directly reporting copies per unit of air) and the lack of other similar studies precludes a more detailed description of spatial patterns.

Temporal patterns were hard to deduce because there were only three longitudinal studies and per-season data on AMR were not reported in two of them. Alvarado et al. (2009) found a higher proportion of ampicillin and penicillin resistance in dairy confined animal feeding operation (CAFO)-associated air in the United States in summer and autumn, and higher cefaclor resistance in autumn. It was not however possible to directly relate this to antibiotic use on the farm, and there was no replicated sampling.

Only Bayle *et al.* (2021) and Lenart-Boroń, Drab, and Chrobak (2021) discussed how the spatial and/or temporal patterns observed might relate to environmental parameters (e.g. temperature, humidity) that they also measured. However, they only discussed indoor changes and their correlations with environmental parameters, which is beyond the scope of this review of outdoor airborne AMR.

Table 4 Key study characteristics of the included studies of antimicrobial resistance in air associated with livestock farming (cattle).

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Alvarado *et al.* (2009)	2006 (spring, summer, autumn)	New Mexico, United States	Longitudinal; Single-site	ARB *(Staphylococcus aureus* bacteria resistant to antibiotics)	Impaction directly onto Malt Extract agar	Culture dependent: isolation followed by disk diffusion (3 antibiotics)
Bayle *et al.* (2021)	2018 (summer and autumn)	Rhône-Alpes, France	Longitudinal; Single-site	ARB *(Staphylococcus* and *Aerococcus* bacteria resistant to antibiotics)	Impaction directly onto Columbia (non-selective) and ChromID ESBL (extended spectrum beta lactamase	Culture dependent: isolation followed by disk diffusion (12 antibiotics)

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
					resistant bacteria selective) agar	
Lenart-Boroń, Drab, and Chrobak (2021)	NR (spring, summer, autumn, winter)	Krakow, Poland	Longitudinal; Single-site	ARB *(Staphylococcus* bacteria resistant to antibiotics)	Impaction directly onto Trypticase Soy Agar	Culture dependent: isolation followed by disk diffusion (7 antibiotics)
McEachran *et al.* (2015)	2012 (summer-winter)	Texas, United States	Transect (upwind and downwind); Cross-sectional (10 commercial feedlots)	ARGs	Impaction onto glass fibre filters (active, pump-assisted sampling)	Culture independent; qPCR (5 ARGs)
Navajas-Benito *et al.* (2017)	2012 and 2013 (winter and summer)	Spain	Transect (inside and outside downwind)	ARB *(Escherichia coli* bacteria resistant to antibiotics)	Impaction directly onto agar plates (air	Culture dependent: isolation followed by disk diffusion (14 antibiotics)

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
					sampled-assisted)	
First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Sanchez a *et al.* (2016)	2013 (summer)	California, United States	Cross-sectional; paired design (3 pairs of conventional and organic farms)	ARB & ARGs	Impingement into 10% glycerol (air sampled-assisted)	Culture dependent: isolation followed by disk diffusion (6 antibiotics) Culture independent: qPCR (3 ARGs).
Wooten, Mayer, and Smith (2019)	2011 (summer-winter)	Texas, United States	Cross-sectional (61 residential sites in the vicinity of poultry farms)	ARGs	Impaction onto glass fibre filters (active, pump-assisted sampling)	Culture independent: qPCR (2 ARGs)

Acronyms: NR not reported; ARB antibiotic resistant bacteria; ARGs antibiotic resistance gene

Air associated with arable farming activities.

We identified two relevant studies of antimicrobial resistance in air associated with arable farming activities. Note that one of these was also considered under ‘ambient’ air. Table 5 summarises the characteristics of these studies.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

Finn et al. (2021) targeted all known resistance genes (using metagenomic sequencing) and did not target any fungal resistant determinants. Fraijee *et al.* (2020) measured tebuconazole resistance in airborne *Aspergillus fumigatus* and detected it using culture-based methods.

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Finn et al. (2021) measured airborne AMR in dryland air in the vicinity of arable farming and reported ARG prevalences of between 0 and ~70% of the abundance of 16S rRNA gene copies. The highest prevalences observed were for chloramphenicols, tetracyclines, sulphonamides and aminoglycosides, though there was substantial seasonal variation. Despite the active air sampling method, absolute quantities of resistance genes per m^3 air were not reported. Fraaije *et al.* (2020) was the only study to quantify antifungal resistance in *A. fumigatus* and estimated 4% of the isolates to be azole resistant.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Given only two studies were found, no generalisations can be made about spatial and temporal patterns.

Regarding spatial patterns, Fraaije *et al*. (2020) reported similar resistance profiles (distributions of minimum inhibitory concentrations) in populations of *A. fumigatus* in Germany, France and the UK. Other locations from which samples were collected were not studied in such a level of detail so as to enable a comparison of resistance profiles across locations. Finn *et al.* (2021) sampled only one location, precluding an analysis of spatial patterns.

For temporal patterns, Finn *et al.* (2021) reported spring and winter peaks of ARG diversity, related to a spring peak in chloramphenicol resistant prevalence (~70% 16S abundance) and a winter peak in tetracycline resistance prevalence (~70% 16S abundance), respectively. Rather than being the result of an arable farming source, the authors' speculated that the chloramphenicol peak may be associated with a nearby wastewater treatment plant, whilst the tetracycline peak was likely associated with the nearby feedlot (though there was no direct source attribution). Fraaije *et al.* (2020)'s cross-sectional study design did not allow an analysis of temporal patterns.

Only Finn *et al.* (2021) attempted to link bioaerosol patterns to environmental parameters measured, finding that aerosolised cotton densities (18S copies m^3 air) was the best correlator with plant pathogen densities (other variables considered included temperature, humidity and precipitation). However, they did not report specifically on how environmental parameters correlated to antimicrobial resistance prevalence changes.

Table 5 Key study characteristics of the included studies of antimicrobial resistance in air associated with arable farming activities.

First author year	Year (season)	Location	Study design	AMR target category (subtype)	Air sampling method	Microbiological methods
Finn *et al.* 2021	2009-2010 (winter)	Arizona, US	Longitudinal; single site	ARG-bearing bacteria	Impaction onto quartz-fibre filters (active, pump-assisted sampling)	Culture independent: HT-qPCR (384 ARGs)
Fraaije et al., (2020)	2018-2019 (winter, spring)	Germany; France; United Kingdom; Hungary; Austria; Belgium; Netherlands	Cross-sectional; 3 sites	ARF (Tebuconazole-resistant *Aspergillus fumigatus)*	Impaction directly onto Sabouraud dextrose agar (SDA) (active, pump-assisted sampling)	Culture dependent: isolation followed by dilution method (agar) antimicrobial susceptibility testing (8 antifungals)

Acronyms: NR not reported; ARF antifungal resistant fungi ARGs antibiotic resistance genes; HT-qPCR high-throughput quantitative PCR

Ambient air

Ten studies met the agreed selection criteria for these types of environments and were summarised. The characteristics of each study (year, country, study design, and methods) are summarised in Table 6.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

A wide range of antimicrobial resistance markers have been measured and detected in ambient air, covering all major antimicrobial classes including aminoglycosides, beta-lactamases, glycopeptides, macrolides, fluoroquinolones, sulphonamides, and tetracyclines for bacteria, and azole-resistant fungi. Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are the most commonly assessed AMR in bioaerosols, with only two studies looking at antifungal resistant fungi. Half of the studies used culture-based methods whilst the rest used molecular techniques, usually qPCR for one or more antibiotic

resistance genes. Targets of PCR-based techniques included genes conferring resistance to various antibiotics, such as beta-lactams, tetracyclines, vancomycin, sulphonamides, macrolides, chloramphenicol, aminoglycosides and quinolones. ARGs were almost always detected to some extent across the studies that used PCR-based techniques.

Two papers detected antimicrobial resistance among fungi cultured from ambient bioaerosols: Azole-resistant Aspergillus fumigatus (Shelton et al., 2022) and cycloheximide-resistant fungi (Raisi et al., 2013).

All but one study sampled bioaerosols by impaction onto a substrate. The rates, durations, and total volumes of air sampled varied greatly between studies (when reported), with some sampling as little as 0.48 m^3, and others as much as 700 m^3).

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Ambient environments sampled represented very different settings, including outdoor air in urban and suburban residential locations, residential areas in rural regions, coastal sites, drylands, parks, and mixtures of these. There is insufficient data of each AMR type, in each setting type to determine typical prevalences for them.

For bacteria, de Rooij *et al.* (2019) performed a survey of ambient air in 61 rural residential locations and quantified the average concentration of antibiotic resistance genes across sites ranged between 3,284 and 63,038 copies per m^3 for *tetW* and 1.5 and 315 copies per m^3 for *mecA*. Donderski *et al.* (2005) estimated penicillin resistance among bacteria isolated from urban residential areas to be between 20% and 50%, erythromycin resistance to be between 14% and 63%, streptomycin resistance to be between 11% and 58%, tetracycline resistance to be between 9% and 29%, and vancomycin resistance to be between 4% and 23%. Echeverria-Palencia *et al.* (2017) sampled air near urban parks, finding *blaSHV* present at all sites at densities ranging between 0.5 and 1.8 gene copies per L air. *Sul1* was detected at some locations, and gene densities (when detected) ranged between 2 $x10^{-4}$ copy numbers per L and 0.03 copy number per L.

For fungi, a large-scale citizen science surveillance study passively sampled air from undescribed ambient settings (Shelton et al 2022). Tebuconazole-resistant fungal isolates were detected in 4% of air samples and at low densities of 1 - 2 resistant isolates from each filter (Shelton et al. 2022). The findings are consistent with measurements taken at Rothamsted Research station in 2016, suggesting prevalence was relatively stable across recent years (Fraaije et al., 2020). The passive sampling employed in this study by citizen scientists enabled a large number of samples to be collected from a wide geographic area (UK-wide) but does not allow the volume of air sampled to be estimated, precluding a calculation of AMR density for comparison between sites. Samples were returned in the post, which may have affected the viability of the microorganisms for the culture-based techniques used.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

The study reported by de Rooij *et al*. (2019) reported variation in ARG concentrations both over time and space: Although the gene targets were detected both close to and far from hypothesised sources of bioaerosols in ambient environments (up to 3 km away), concentrations of the markers decreased with increasing distance from livestock farms. The most influential variable associated with both AMR markers (*tetW* and *mecA*) was the number of farms weighted to distance within a 3 km buffer around the sampling locations. The livestock types most associated with concentration of these genes (after adjustment for temporal autocorrelation and other animals in the vicinity) were poultry and pigs.

Most of the studies without a hypothesised source of bioaerosols were conducted at a single site or did not explore spatial patterns (Finn et al. 2021; Gandolfi et al. 2011; Raisi et al. 2013; Li et al 2018). Other studies of ambient air collected a small number of samples and lacked power to deduce spatial patterns or their causes, even where environmental variables were measured (Echeverria-Palencia et al., 2017; Liu et al., 2022; Sivri et al., 2016). The Shelton *et al.* (2022) citizen science study had larger sample sizes collected within a smaller geographic area (the United Kingdom). The authors found some evidence for a negative correlation between total (susceptible + resistant) *A. fumigatus* densities and distance to the nearest composting facility (i.e. a distance-decay relationship). However, they were not able to identify any spatial patterns in resistant *A. fumigatus*, probably due to the low detection rate and low densities of tebuconazole-resistant *A. fumigatus* in the samples.

Similarly, consistent temporal patterns of antimicrobial resistance in ambient air were hard to identify, despite the fact that several studies took samples from multiple points in time and attempted to attribute them to other spatially-varying factors. Four studies suggested that total numbers of microorganisms were higher in summer than in winter (Donderski, Walczak, and Pietrzak 2005; Shelton 2021; Sivri et al. 2016), but one study found higher densities in winter (Gandolfi et al., 2011) and two other studies found no evidence of changes in microorganisms over the seasons sampled (Finn et al., 2021; Raisi et al., 2013). For resistant organisms, there is even less evidence for consistent seasonal patterns. Finn *et al.* (2021) reported spring and winter peaks of ARG diversity, related to a spring peak in chloramphenicol resistant prevalence (~70% 16S abundance) and a winter peak in tetracycline resistance prevalence (~70% 16S abundance), respectively. The authors' speculated that the chloramphenicol peak may be associated with a nearby wastewater treatment plant, whilst the tetracycline peak was likely associated with the nearby feedlot. Aside from the results reported by Finn *et al*. (2021), changes in airborne antimicrobial resistance prevalence and/or diversity in other included articles appeared to be more sporadic, and did not indicate seasonal trends in AMR abundance or diversity. Authors frequently suggest that changes in the antimicrobial resistant fraction of bioaerosols were related to particular, sporadic events such as human activities (e.g. agricultural activities) or varying rates of bioaerosol emission from nearby sources, the weather, or dust storms. However, these suggestions were based on contextual information, rather than direct source attribution.

Taken together, the research currently suggests that patterns of AMR in ambient air are not currently easily predictable or generalisable, and that one must consider, on a site-by-site basis, the particular local activities, topography and events that could contribute to (or reduce) airborne AMR.

Table 6 Key study characteristics of the included studies of antimicrobial resistance in ambient air.

First author and year	Year (season)	Location (setting)	Study design	AMR target category (AMR subtype)	Air sampling method	Microbiological methods
De Rooij *et al.* 2019	2015 – 2016 (spring, summer, autumn, winter)	The Netherlands (rural residential)	Cross sectional and longitudinal (61 locations over 26 weeks)	ARGs (*tetW* and *mecA)*	Impaction onto Teflon filters 10L/min for 15min per hour per day for 14 days (50,400 L per sample). Sample height 1.6 m	Culture-independent: qPCR (2 ARGs). qPCR primers included for *E. coli, S. aureus* and *Campylobacter jejuni*
Donderski, Walczak, and Pietrzak (2005)	2005 (spring, summer, autumn, winter)	Toruń, Poland (urban residential)	Cross-sectional (12 points in 2 locations); longitudinal (monthly basis for 12 months)	ARB (heterotrophic mesophilic, and Enterobacteriaceae resistant to antibiotics)	Impaction directly onto agar (medium NR) Rate/volume/ duration of sampling NR. Sample height 1.3 m	Culture dependent: isolation followed by disk diffusion (5 antibiotics)
Echeverria-Palencia *et al.* (2017)	2015 (winter)	California, United States (parks)	Cross-sectional (9 locations across 4 cities)	ARGs (*sul1, blaSHV*)	Impaction onto polycarbonate filters 2 L/min for ≥4 h (~480 L	Culture independent: qPCR (2 ARGs)

First author and year	Year (season)	Location (setting)	Study design	AMR target category (AMR subtype)	Air sampling method	Microbiological methods
					per sample). Sample height 1 m.	
Finn *et al.* (2021)	2010 (spring, summer, autumn, winter)	Arizona, United States (mixed-use drylands)	Longitudinal; Single-site over four seasons	ARGs	Impaction onto quartz filters 1m per min (duration NR) Sample height NR	Culture independent: HT-qPCR (384 ARGs), metagenomic 16S rRNA amplicon sequencing
Gandolfi *et al.* (2011)	2008-2009 (summer, winter)	Milan, Italy (urban)	Longitudinal Single-site (3 times)	ARB (*Staphylococcus*, *Pseudomonadaceae, Enterobacteriaceae, Acinetobacter, Enterococcus*); *Staphylococcus* bearing ARGs	Impaction onto nitrocellulose filters 38 L/min, 24-48 h Sample height 1.3 m	Culture dependent: isolation followed by disk diffusion (10 antibiotics), and PCR (5 ARGs) on phenotypically resistant Staphylococcus isolates
First author and year	Year (season)	Location (setting)	Study design	AMR target category (AMR subtype)	Air sampling method	Microbiological methods

First author and year	Year (season)	Location (setting)	Study design	AMR target category (AMR subtype)	Air sampling method	Microbiological methods
Li *et al.* (2018)	2016-2017	Global (urban)	Cross-sectional (174 points across 19 cities and 13 countries)	ARGs (conferring resistance to beta-lactams, macrolides, quinolones, tetracyclines, aminoglycosides, vancomycin, and mobile elements *tnpA,* and *IntI1*)	Impaction onto automobile air-conditioning filters Duration, rate and volumes, heights of samples NR	Culture independent: HT- qPCR for 39 ARGs and metagenomic 16S rRNA amplicon sequencing
Liu *et al.* (2022)	2017 (winter, spring)	Switzerland (urban, suburban)	Cross-sectional (5 cities)	ARGs (*floR* and *blaTEM*)	Impaction onto air pollution monitoring filters 720 m^3 per day Height NR	Culture independent: electrochemical DNA biosensors (2 ARGs) and PCR (same 2 ARGs)
Raisi *et al.* (2013)	2008 (spring) and 2009 (spring and winter)	Crete, Greece (suburban residential, close to sea)	Longitudinal; (Single-site, 41 days)	ARB (streptomycin-resistant heterotrophic bacteria) and ARF (cycloheximide-resistant fungi)	Impaction directly onto Tryptone Soy agar (medium not reported) 100 L/min, duration NR. 100 L collected for fungi and actinobacteria, 250 L for heterotrophic bacteria. Height NR	Culture dependent: sub-culturing following by liquid antimicrobial susceptibility testing (1 antibiotic and 1 fungicide)

First author and year	Year (season)	Location (setting)	Study design	AMR target category (AMR subtype)	Air sampling method	Microbiological methods
Shelton *et al*. (2022)	2018-2019 (spring, summer, autumn, winter)	United Kingdom	Cross-sectional	ARF (Tebuconazole- resistant *Aspergillus fumigatus*)	Passive sampling onto adhesive film 6-10 h at height of 1 m	Culture dependent: isolation followed by agar antimicrobial susceptibility testing. Amplicon sequencing of *cyp51A*.
Sivri *et al.* (2016)	2009 (winter)	Istanbul, Turkey (urban, close to sea)	Cross-sectional (11 locations)	ARB (methicillin-resistant *Staphylococci); staphylococci* harbouring *mecA*	Impaction directly onto agar (medium not reported) Details of air sampling rate, duration volume NR	Culture dependent: isolation followed by disk diffusion (10 antibiotics)

Acronyms: NR not reported; ARB antibiotic resistant bacteria; ARF antifungal resistant fungi; ARG antibiotic resistance genes; ESBL extended spectrum beta-lactamase; HT-qPCR high-throughput quantitative PCR; MIC minimum inhibitory concentration; PCR polymerase chain reaction

What is known about the human health effects of exposure to air containing AMR microorganisms or genes?

Six epidemiological studies were identified that directly measured AMR in samples collected from human subjects exposed to different types of air, the characteristics of which are summarised in Table 7.

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

Most studies measured upper respiratory tract colonisation (nasal or throat swabs) by resistant opportunistic pathogens, particularly antibiotic resistant bacteria, such as staphylococci and enterococci (Rosenberg Goldstein et al., 2017, 2014; Zieliński et al., 2020). All studies used culture-dependent methods to detect AMR in human-derived samples, with the exception of one, which used culture-independent methods (qPCR) to measure 23 different ARGs present on swabs taken from the upper respiratory tracts of human subjects (Zieliński et al., 2021). One study investigated the association between

bioaerosol exposure and ESBL-producing Enterobacterales in stool samples (Rodríguez-Molina et al., 2021). One study collected dermal swabs as well as nasal swabs to analyse them for the presence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) or vancomycin-resistant enterococci (Rosenberg Goldstein et al., 2017). No studies that analysed the relationship between exposure to AMR in bioaerosols and infections or death were identified. No studies were found that addressed the health effects associated with exposure to airborne antifungal resistant fungi.

What evidence exists for a relationship between exposure to airborne AMR and colonisation?

The nature of the relationship between exposure to airborne AMR and colonisation by AMR in humans is not clear. Evidence assessing the association between airborne AMR exposure and human health effects was limited to observational study designs, such as cross-sectional, case-control studies[8]. One study reported statistically significant differences between populations exposed to hypothesised sources of AMR and unexposed control populations in terms of AMR carriage (Rosenberg Goldstein et al., 2017). However, several studies found no such effects. Rodríguez-Molina et al., (2021) reported no evidence of an significantly increased risk of carriage of ESBL-producing *E. coli* in WWTP workers compared to residents living far from the WWTPs, or between residents living close to the WWTPs and the residents living far from the WWTPs. Similarly, Rosenberg Goldstein et al., (2014) reported no difference in the proportion of irrigation sprayers at a reclaimed water facilities who were nasal carriers of multidrug resistant *Staphylococcus aureus* compared to the unexposed population (office workers). Schinasi et al., (2014) identified living in regions with medium densities of swine was a statistically significant risk factor for nasal carriage of MRSA. However, living in areas with high densities of swine was not a risk factor for nasal carriage of MRSA, nor was living in rural areas. This observation could be due to differences in different types of swine production, or the influence of uncharacterised sources of AMR in the study population.

In addition, inhalation of outdoor aerosolised AMR as a route of exposure is not well established in these studies: None of the reviewed studies collected and analysed air samples for AMR to assess whether the genes or organism detected in samples from human subjects is airborne, nor were any quantitative exposure estimates found. Five of the six studies recruited participants based upon occupational exposures, which may involve inhalation of bioaerosols in both indoor and outdoor settings, as well as a mixture of other exposure routes (e.g. direct contact with the source of bioaerosols).

[8] A case–control study (also known as case–referent study) is a type of observational study in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute.

Table 7 Key study characteristics and main findings of the epidemiological studies relevant to airborne transmission of AMR.

Study	Country	Bioaerosol source environment	Study design	AMR outcome (method of detection)	Population	Control group	Main findings
Rodriguez-Molina *et al.* 2021	Germany, the Netherlands, Romania	WWTPs	Cross-sectional	Faecal carriage of ESBL-Enterobacterales (culture-dependent)	WWTP workers, and local residents	Distant residents (>1km away)	No evidence of an increased risk of carriage of ESBL-producing *E. coli* in WWTP workers or nearby residents, compared to the general population.
Rosenberg Goldstein *et al.* 2014	US	Reclaimed water	Cross sectional	Nasal carriage of MRSA, MDR MSSA, or vancomycin resistant Enterococci (culture-dependent)	Reclaimed water spray irrigators	Office workers (academic)	No MRSA or VRE were isolated from nasal swabs. While a higher proportion of irrigation workers tested positive for MDR MSSA compared to office workers, the difference between these two populations was not significant.
Rosenberg Goldstein *et al.* 2017	US (Mid-Atlantic)	Waste water treatment plant	Cross-sectional	Nasal and dermal carriage of MRCoNS (culture-dependent)	Reclaimed water spray irrigators	Office workers (academic)	16% of irrigation workers tested positive for nasal colonisation by MRCoNS, and 0% of office workers. And a higher proportion of irrigation workers were colonised by

Study	Country	Bioaerosol source environment	Study design	AMR outcome (method of detection)	Population	Control group	Main findings
							CoNS isolates resistant to cefoxitin or tetracycline, but after adjusting for confounders, these differences were not statistically significant. Conversely, a greater proportion of office workers were colonised by erythromycin-resistant CoNS compared to irrigation workers.
Schinasi *et al.* 2014	US (North Carolina)	Swine density	Case-control	Nasal carriage of MRSA (culture-dependent)	People with MRSA detected in nasal swabs	People without MRSA in nasal swabs	Nasal carriers of MRSA were more likely to living in areas with medium densities of swine than people without MRSA. However, living in areas with high densities of pigs was not a significant risk factor.
Study	Country	Bioaerosol source environment	Study design	AMR outcome (method of detection)	Population	Control group	Main findings

Study	Country	Bioaerosol source environment	Study design	AMR outcome (method of detection)	Population	Control group	Main findings
Zielinski *et al.* 2020	Poland	WWTP	Ecological study (swabs from the exposed population were pooled into a composite sample, as were swabs from the control group)	Nasal and throat carriage of staphylococcus strains harbouring antibiotic resistance (*nuc, mecA, vanA, qacA/B*) and virulence genes (*sasX, pvl, tst1, hla, sec*) (culture-dependent)	WWTP workers	Healthy individuals not WWTP employees	Staphylococci harbouring antibiotic resistance and virulence genes were detected in WWTP workers and control groups. Different prevalences of resistance genes were detected in the strains isolated from exposed and unexposed populations: mecA was detected in strains isolated from WWTP worker swabs, but was not detected in the control group. VanA was detected in strains isolated from swabs collected from WWTP workers, and was detected in nasal swabs from controls (but strains isolated from control throat swabs). A greater proportion of strains isolated from control subjects

Study	Country	Bioaerosol source environment	Study design	AMR outcome (method of detection)	Population	Control group	Main findings
							harboured qacA/B compared to strains isolated from WWTP employees. Risk metrics cannot be quantified.
Zielinski *et al.* 2021	Poland	WWTP	Ecological study (swabs from the exposed population were pooled into a composite sample, as were swabs from the control group)	Nasal and throat carriage ARGs (various) (culture-independent qPCR)	WWTP workers	Healthy individuals not WWTP employees	Higher concentration of ARGs were measured in swabs from WWTP employees compared to control group. Risk metrics cannot be quantified.

Acronyms: MRCoNS methicillin- resistant coagulase negative staphylococci; OR odds ratio; 95%CI = 95% confidence interval; MRSA methicillin resistant staphylococcus aureus; WWTP wastewater treatment plant; CoNS coagulase negative staphylococci; VRE vancomycin resistant enterococci; ESBL extended-spectrum beta-lactamase; MDR multidrug resistant

Summary of findings

Research on antimicrobial resistance (AMR) in outdoor bioaerosols across several different setting types (ambient, wastewater treatment plants, livestock farming, and arable farming environments) has been reviewed to assess what is known about the types of AMR present, their abundance, and factors affecting the distribution of AMR markers disseminated in the air. Furthermore, several of the studies from which evidence was gathered were quite old, meaning that some of the data on AMR prevalences may not reflect current levels. For example, recent initiatives to reduce antibiotic use in humans and animals may have decreased AMR levels in environments considered. Nevertheless, such data may be useful to consider the environmental factors influencing the dispersion of AMR in bioaerosols.

Existing research has focused on characterising the bacterial (and antibiotic resistant) components of bioaerosols, particularly potentially pathogenic bacteria (such as *Staphylococcus aureus* and *Escherichia coli)*. The study of antifungal resistant fungi in this matrix is not as advanced, and as such, data about antifungal resistant fungi in several important settings is not available.

While there are more studies and more data on airborne antibiotic resistant bacteria and antibiotic resistance genes compared to fungi, the number of studies on particular types of air is small and methodologically diverse. Thus, a robust understanding of the processes governing the dispersal of AMR in outdoor air, and typical prevalences and compositions of resistant organisms, is still not possible. Nonetheless, observations from the reviewed literature may serve to inform a strategy for environmental surveillance of airborne AMR, and indicate key knowledge gaps that need to be addressed.

Review of studies of AMR in air associated with particular human activities

Which antimicrobial resistance markers have been detected in bioaerosols and how have these been measured?

We found that diverse AMR markers have been measured in bioaerosols, covering phenotypic resistance to a large range of antibiotics and antibiotic classes, as well as antibiotic resistance genes and gene subtypes. Antifungal resistance markers are limited to *Aspergillus fumigatus* resistant to tebucanazole (Shelton et al 2022, Fraijee et al 2022) and cycloheximide-resistant fungi (Raisi et al., 2013). In ambient air, investigators typically targeted diverse sources or ones associated with particular AMR threats that were of considered emerging epidemiological threats at the time of study (e.g. MRSA or azole resistance). In source-associated environments, the choice of AMR markers measured in bioaerosols appeared to reflect the antibiotics used and/or the material being aerosolised (often cited as justifications by study authors for their selection of analytes in their studies). For example, studies of wastewater treatment plant-associated air targeted quite diverse AMR markers given they receive waste from diverse sources and are widely considered to be hotspots for AMR (Korzeniewska et al., 2013), whereas AMR markers targeted in studies of farming activities often focussed on a narrower range of targets, such as MRSA, and resistance to antibiotics used in veterinary medicine (e.g. tetracyclines and macrolides). The choice of microbial targets may also reflect the nature of the aerosol sources. For example,

bioaerosols generated at WWTPs originate from the mixing and aeration of the liquid fraction of faecal waste, and therefore enteric bacteria are a commonly targeted group of microorganisms. Airborne dust in farming-associated environments was also a particular focus of most studies, with airborne AMR hypothesised to originated from the aerosolization of dried faeces, animal feed and soil. Airborne AMR associated with lagoons storing animal waste was considered to a limited extent (Arfken et al., 2015). However, the number of studies included in each module was small and, in all cases, there were studies that targeted many resistance markers.

There were three approaches described for the collection of air samples to analyse airborne AMR: passive sampling (leaving culture medium exposed to air for microorganisms to colonise); active sampling by impaction (air is passed onto solid culture medium or filter at a defined rate of flow), or by impingement (air is passed into a liquid medium at a defined rate of flow). The most frequently used method for sampling microorganisms in air was impaction followed by culture-based methods to grow bacterial isolates, which were then tested for their sensitivity to one or more antibiotics, usually by disk diffusion. Disk diffusion is a widely used method, since thresholds for interpreting zones of inhibition for specific bacteria are readily available – antibiotic combinations have been established and published by international agencies, like the European Committee on Antimicrobial Susceptibility Testing, and the Clinical and Laboratory Standards Institute).

Entirely culture-independent methods are used in more recently published articles, and high-throughput qPCR in particular offers quantitative data across a large range of genes. However, culture-independent approaches (PCR and qPCR) make up a small proportion of the overall evidence base, and none of the studies identified described using metagenomic approaches to quantify AMR targets in bioaerosols, although metabarcoding, i.e. the sequencing of 16S rRNA amplicons in complex mixtures to determine bacterial taxa present, is described by several studies.

Overall, AMR marker selection by authors is likely to have been biased toward pathogens and/or genes posing a public health risk, and restricted in scope by resources available to researchers. Methods for AMR target measurement are diverse across studies, and each method has inherent advantages and disadvantages for quantifying AMR, which need to be considered in combination with practical considerations.

What are typical prevalences of antimicrobial resistance detected in bioaerosols sampled?

Many of the included studies quantified some measure of AMR in bioaerosols, but reported prevalences in different ways. This ranged from reporting percentage of total isolates that were phenotypically resistant to an antimicrobial, to the density of resistant isolates in a given volume of air, or to the relative abundance of ARGs (ARGs per 16S rRNA copy number, or per ng DNA). Despite an abundance of data in the papers, therefore, the variation in reporting alongside limited availability of raw data in the published studies makes it difficult to determine typical prevalences of AMR for any of the settings reviewed.

Nonetheless, the prevalence of AMR in bioaerosols is likely to represent (to a certain extent) the composition of resistant organisms in the source material, which may reflect local

antibiotic use practices. This was demonstrated by the transect study performed by Gaviria-Figueroa *et al*. (2019), that included analysing source material (WWTP sludge), as well as upwind and downwind locations, for AMR. Several AMR markers that were present in sources were not detect upwind, but were detected downwind. However, analysis of downwind samples did not reflect the full AMR profile obtained from sources (Gaviria-Figueroa et al., 2019) indicating either differential aerosolization or issues with limits of detection in air samples. Evidence on AMR profiles in bioaerosols reflecting antibiotic use come from Li et al., (2018), who reported some evidence that the prevalence of resistance to different classes of antibiotics is correlated with the quantities used of each of those classes globally. On a smaller scale, some of the studies observed a difference in the detection and prevalence of antibiotic resistant bacteria between farms using different rearing practices (intensive and non-intensive operations), which are associated with different antibiotic use patterns, the housing of animals, and the densities at which animals are kept (Davis et al., 2018). However, consistent patterns are hard to identify and even within the same study, prevalences between different samples can vary widely.

How does antimicrobial resistance in bioaerosols vary spatially and temporally?

Among the included studies were examples of longitudinal, cross-sectional, and transect study designs, which can provide data on the spatial and/or temporal variation in AMR target abundance in different environments. Some consistent trends are identified across studies and environments, whereas some of the results from different studies seem to contradict each other or demonstrate no trends.

For transect studies analysing air at potential bioaerosol sources, a consistent observation of a distance-decay relationship was made, whereby the abundance of AMR markers decreases with increasing distance from the source. Furthermore, downwind samples tended to have higher AMR abundance compared to upwind samples, with some studies indicating detection of AMR over 100 metres downwind from source (Gaviria-Figueroa 2019, Gibbs et al 2006, Ruiza-Ripa et al 2020a), or even many kilometres away (Gaviria-Figueroa et al 2019, de Rooij et al 2019). Wind speed and direction is expected to strongly influence the location and extent of AMR dispersal relative to the source. While not directly investigated by any of the included studies, topography in the local area was often discussed in relation to interpreting study findings. The natural and artificial features of the local landscape could serve to promote, or reduce, air circulation and thus AMR dispersal.

Longitudinal study designs provide some data on how AMR prevalences vary over time. The frequency of sample collection varied across studies, with some papers reporting data on AMR variation over short time frames (days or weeks), whereas most performed sampling to capture and compare seasonal variations in AMR. Findings on seasonality were inconsistent across studies, though there was some indication that lower densities of ARGs tend to be reported in winter, compared to warmer months. The findings are likely to be influenced by the methods used to measure AMR: Microorganisms are differentially sensitive to environmental factors like ultraviolet radiation, humidity and temperature. Thus culture-dependent methods will not detect non-viable microorganisms. Another consideration is the different rates of aerosolization at different times of year being influenced by the weather: dry conditions (usually experienced in the summer in temperate

regions like the UK) may promote aerosolization of dusts, and precipitation is expected to facilitate wet deposition of airborne particles. However, if material is too dry, microorganisms are more likely to die due to desiccation. Very few studies attempted to correlate meteorological parameters, such as temperature and humidity, to the abundance of AMR in bioaerosols, and the results are contradictory across AMR targets and studies. None of the studies reviewed collected air samples when it was raining, due to the effect of rain on sampling equipment. Therefore, the effects of rain on the abundance and diversity of AMR in bioaerosols are not yet understood. However, research on non-AMR aerosol dissemination suggests we should expect reduced AMR abundances in the air when it rains.

The hazard airborne AMR poses to human health

Few relevant studies were found that directly measured AMR in samples collected from populations exposed to environments where aerosolised AMR have been detected. The evidence base on this topic was expected to be small, based on the results of Stanton et al., (2022)'s map of evidence on human exposure to and transmission of antibiotic resistance from natural environments. Most of the reviewed studies focussed on human colonisation by a narrow selection of bacterial opportunistic pathogenic bacteria in highly exposed study populations (occupational exposures). While colonisation by AMR may be a precursor to infections by AMR pathogens, no studies were found that investigated the association between inhalation of airborne AMR and morbidity or mortality caused by treatment-resistant infections. Overall, the epidemiological evidence on the role aerosolised AMR plays in the dissemination of AMR to humans is not clear, with some papers reporting statistically significant associations between exposure and colonisation by AMR, and others finding no different between exposed and unexposed populations in terms of AMR colonisation.

Nevertheless, there being no evidence of an association is not the same as evidence of no association. While outside the scope of this review, various indirect and analogous evidence indicates a plausible but understudied transmission route for airborne AMR (Gwenzi et al., 2022; Shimonovich et al., 2021). For example, there is evidence of airborne transmission of non-AMR pathogens (Fernstrom and Goldblatt, 2013) including in some of the environments relevant to this review like farming environments, wastewater treatment plants (Gonzalez-Martin, 2019; Kataki et al., 2022). Furthermore, occupational exposures to environments with high risk of exposure to faecal waste material tend to receive more attention. People working in WWTPs are at greater risk of symptoms of infection (Muzaini et al., 2021), and working on livestock farms has been linked with nasal colonisation by livestock-associated MRSA (Bos et al., 2016; Franceschini et al., 2019; Schmithausen et al., 2015).

Quantitative and qualitative risk of exposure assessments can provide an understanding of the extent and scale of exposure to AMR in environmental media. While no studies were found that were relevant to the environments and geographies considered in this review, such estimates have been calculated in other geographies (Gwenzi et al., 2022).

Some of the studies in this review have identified shared features of AMR across AMR source matrices, bioaerosols and AMR analysed in human samples (Gaviria-Figueroa et al., 2019; Zieliński et al., 2020). In addition, Korzeniewska and Harnisz, (2013) demonstrated

the mobility of beta-lactamase ARGs in Enterobacteriaceae isolated from air samples collected at wastewater treatment works in Poland by performing conjugation assays, and concluded that all *bla* genes were located on mobile genetic elements. This highlights the possibility for ARGs in airborne microorganisms to transfer to human commensals (Ashbolt et al., 2013). Bruni et al., (2019) used an animal model of innate immunity (*Caenorhabditis elegans*) to evaluate the pathogenicity of multidrug resistant isolates recovered from air samples collected at a wastewater treatment plant, and demonstrated pathogenic potential in the multidrug-resistant bacteria isolated from bioaerosol samples. While the contribution of environmental bioaerosols to the development of resistant infections is so far uncharacterised, the occurrence of azole-resistant respiratory infections caused by fungi (such as *Aspergillus fumigatus)* in patients without previous azole use indicates inhalation of AMR fungal spores, known to be present in the environment (Dauchy et al., 2018; Jeanvoine et al., 2020).

Conclusions

This review summarises the methods and results of primary research conducted to assess antimicrobial resistance in bioaerosols across a range of important source and receptor environments in Europe and North America. All studies indicate that various antimicrobial resistant microorganisms or genes are present in the studied environments, and thus setting up a surveillance programme for airborne AMR is warranted. Studies investigating the AMR health effects associated with exposure to airborne AMR is a small, complex, but emerging field of study.

Method supplementary materials: Selection criteria

Table 8 Justified selection criteria and how these were applied.

Include	Exclude	Justification
Language: Studies written in English	Studies not written in English	Insufficient time and resources to translate papers not written in English
Sample locations: Studies that involve collection of air samples outdoors. Covered structures outdoors will also be eligible (e.g. livestock barns where 1 or more wall is missing)	Studies that collect only indoor air samples (for example, but not limited to hospitals, schools, homes, offices, toilets, nursing homes)	Indoor air quality is out of scope for this review

Include	Exclude	Justification
Sample types: Studies that collect aerosolised or airborne samples	Studies that collect rain or snow samples	Aerosol does not include snow or rain (https://en.wikipedia.org/wiki/Aerosol) but does include dust (https://www.nasa.gov/centers/langley/news/factsheets/Aerosols.html)
Study designs: Studies that investigate the spatial and/or temporal variation in AMR in bioaerosols	Exclude studies where samples are taken at a single time point and single location	Longitudinal studies will enable exploration of effects such as prevailing weather (e.g. windspeed) on AMR to be detected. Likewise, studies collecting data from a variety of locations or along a transect will provide valuable data to answer the research question.
Dates: Studies published between 2002 and now	Studies published prior to 2002	Insufficient time and resources to screen all published studies. Only recent studies will be included
Methods Empirical studies of microbiological air quality. Relevant Systematic reviews (i.e. ones that meet these eligibility criteria)	Exclude clinical (human) trials and non-systematic reviews, or outputs of modelled bioaerosols	This selection criterion will exclude studies on humans and narrative reviews.
Geography: Studies conducted in Europe, United States or Canada	Exclude studies conducted outside Europe, United States or Canada	To inform UK policy and practice, it would be advantageous to limit the evidence to places with similar geographies and roughly similar regulations in terms of risk that they have to deal with.

Method supplementary materials: search strategies

Web of Science searches

Core search terms for AMR in bioaerosols used across all modules.

#1 TS=((aerosol* OR air* OR bioaerosol* OR dust))

#2 TS=((bact* OR fung* OR microb* OR antibiotic* OR antifung* OR antimicrob* OR drug* OR multidrug*)

#3 #1 AND #2

Module-specific search terms were combined with core search terms using AND:

WWTPs

#3 AND (TS=(waste* OR sewage))

Cattle

#3 AND (TS=(beef OR dairy OR cattle OR cow* OR steer* OR bovine OR calf OR calves))

Pigs

#3 AND (TS=(pig* OR swine* OR pork* OR sow*))

Poultry

#3 AND (TS= (poultry OR chicken* OR duck* OR fowl* OR turkey* OR broiler* OR hen* OR goose OR geese))

Arable

#3 AND (TS=(arable OR crop*))

Ambient

#3 AND (TS=(atmos* OR ambient* OR urban OR city OR cities OR suburb* OR rural OR resident*))

Human Health (PubMed search)

#1 airborne OR aerosol* OR bioaerosol* OR dust

#2 (antibiotic resistan*) OR (antimicrobial resistan*) OR (antifungal resistan*) OR (antibacterial resistan*)

#3 (health) OR (illness) OR (infect*) OR (coloni*) OR (inhal*) OR (transmi*) OR (expos*))

#1 AND #2 AND #3

Method supplementary materials: Data tables

Commonly used acronyms: CFU = colony forming unit; DNQ = Detected but not quantified' NR = not reported; UCB = unidentified cultured bacterium

Table 9 Resistance phenotypes and identity of resistant species (when determined) detected in the literature using only culture-dependent techniques. The relative abundance, as prevalence (e.g. CFU/m3) or percentage is given where available

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
Amikacin	Stentorophomonas maltophilia	DNQ	Bruni et al. (2019)
	Escherichia coli	47.4-60%	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
Ampicillin	UCB	20-41 CFU/m^3	Potorski et al. (2019)
	Agrobacterium fabrum	DNQ	Bruni et al. (2019)
	Acinetobacter iwoffii	DNQ	
	Kocuria rhizophila	DNQ	
	Spingomonas hankookensis	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Staphylococcus aureus	0.1 CFU/m3* could be an indoor sample	Kozajda and Jeżak (2020)
Aztreonam	Staphylococcus warneri	DNQ	Bruni et al. (2019)
	Kocuria polaris	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Agrobacterium fabrum	DNQ	
	Bacillus pumilus	DNQ	
	Bacillus mycoides	DNQ	
	Bacillus licheniformis	DNQ	
	Staphylococcus hominis	DNQ	
	Paenarthrobacter nitrguajacolicus	DNQ	
	Micrococcus luteus	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Microbacterium oxydans	DNQ	
	Stenotrophomonas maltophlia	DNQ	
	Moraxella osloensis	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Aquabacterium parvum	DNQ	
	Staphylococcus epidermidis	DNQ	
	Micrococcus alaeverae	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
Benzylpenicillin	Mannitol-positive staphylococci	~50% of isolates	Małecka-Adamowicz et al. (2017)
Carbenicillin	Kocuria polaris	DNQ	Bruni et al. (2019)
	Agrobacterium fabrum	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Microbacterium oxydans	DNQ	
	Stentrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Aquabacterium parvum	DNQ	
Cefotaxime	E. coli	97-100% of E. coli	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
	Kocuria polaris	DNQ	Bruni et al. (2019)
	Agrobacterium fabrum	DNQ	
	Acinetobacter iwoffii	DNQ	
	Bacillus pumilus	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Bacillus mycoides	DNQ	
	Bacillus licheniformis	DNQ	
	Microbacterium oxydans	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Stentrophomonas pavanii	DNQ	
	Aquabacterium parvum	DNQ	
Cefoxitin	Mannitol-positive staphylococci	~5%	Małecka-Adamowicz et al. (2017)
Cefpodoxime	E. coli	80-84.2% of E. coli	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
Ceftazidime	E. coli	80-94.7% of E. coli	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
	Kocuria polaris	DNQ	Bruni et al. (2019)

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
Cefuroxime	Pseudomonas oryzihabitans	DNQ	
	Agrobacterium fabrum	DNQ	
	Acinetobacter iwoffii	DNQ	
	Bacillus pumilus	DNQ	
	Bacillus mycoides	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Aquabacterium parvum	DNQ	
Cephalothin	Pseudomonas oryzihabitans	DNQ	Bruni et al. (2019)
	Agrobacterium fabrum	DNQ	
	Acinetobacter iwoffii	DNQ	
	Staphylococcus hominis	DNQ	
	Sphingomonas hankookensis	DNQ	
	Stenotrophomonas pavanii	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Stenotrphomonas maltophilia	DNQ	
	Microbacterium phyllosparae	DNQ	
	Aquatbacterium parvum	DNQ	
Clindamycin	Staphylococcus aureus	0.2CFU/m3	Kozajda and Jeżak (2020)
Chloramphenicol	E. coli	15.8-20%	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
	UCB	5-168 CFU/m^3	Potorski et al. (2019)
Clindamycin	Staphylococcus aureus	0.2 CFU/m3 *could be indoors	Kozajda 2020
	Staphylococcus warneri	DNQ	Bruni et al. (2019)
	Kocuria polaris	DNQ	
	Pseudomonas oryzihabitans	DNQ	
	Agrobacterium fabrum	DNQ	
	Acinetobacter iwoffii	DNQ	
	Kocuria rhizophila	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Bacillus licheniformis	DNQ	
	Moraxella osloensis	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Aquabacterium parvum	DNQ	
Doxycycline	UCB	10-38 CFU/m^3	Potorski et al. (2019)
Erythromycin	Pseudomonas oryzihabitans	Not quantified	Bruni et al. (2019)
	Agrobacterium fabrum	Not quantified	
	Kocuria rhizophila	Not quantified	
	Stenotrophomonas pavanii	Not quantified	
	Stenotrophomonas maltophilia	Not quantified	
	Mannitol-positive staphylococci	20% of strains	Małecka-Adamowicz et al. (2017)
	E. coli	4	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
ESBL (cefotaxime)	Enterobacteriaceae	4	Korzeniewska and Harnisz (2013)
ESBL (cefpodoxime)	K. pneumonia	1	
	E. coli	4	
	Enterobacteriacae	5	
ESBL (ceftazidime)	E. coli	2	
	C. freundii	1	
	Enterobacteriaceae	3	
Fosfomycin	Staphylococcus warneri	DNQ	Bruni et al. (2019)
	Kocuria polaris	DNQ	
	Pseudomonas oryzihabians	DNQ	
	Agrobacterium fabrum	DNQ	
	Acinetobacter iwoffii	DNQ	
	Kocuria phizophia	DNQ	
	Bacillus pumilus	DNQ	
	Bacillus mycoides	DNQ	
	Bacillus licheniformis	DNQ	
	Moraxella osloensis	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Staphylococcus hominis	DNQ	
	Paenarthrobacter nitroguajacolicus	DNQ	
	Micrococcus luteus	DNQ	
	Microbacterium oxydans	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Stenotrophomonas pavanii	DNQ	
	Aquabacterium parmvum	DNQ	
	Staphylococcus epidermidis	DNQ	
	Microoccccus aloeverae	DNQ	
Gentamicin	E. coli	55.3-60% of E. coli	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
	Stenotrophpomonas maltophila	DNQ	Bruni et al. (2019)
	Microbacterium phyllospaerae	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Stenotrophomonas pavanii	DNQ	
	Aquabacterium parvum	DNQ	
	Mannitol-positive staphylococci	~5% of strains	Małecka-Adamowicz et al. (2017)
Imipenem	E. coli	0-2.6%	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
Levofloxacin	Mannitol-positive staphylococci	~1% of strains	Małecka-Adamowicz et al. (2017)
Mezlocillin	Agrobacterium fabrum	DNQ	Bruni et al. (2019)
	Kocuria rhizophila	DNQ	
	Microbacterium oxydans	DNQ	
	Stenotrophomonas maltphila	DNQ	
	Stenotrophomas pavanii	DNQ	
Oxacillin	Kocuria polaris	DNQ	Bruni et al. (2019)
	Pseudomonas oryzihabitans	DNQ	
	Agrobacterium fabrum	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
	Acinetobacter iwoffii	DNQ	
	Kocuria rhizophila	DNQ	
	Moraxella osloensis	DNQ	
	Spingomonas hankookensis	DNQ	
	Stenotrphomonas pavanii	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Microbacterium phyllospaerae	DNQ	
	Aquabacterium parvum	DNQ	
Penicillin	Staphylococcus warneri	DNQ	Bruni et al. (2019)
	Agrobacterium fabrum	DNQ	
	Microbacterium oxydans	DNQ	
	Stenotrophomonas maltophilia	DNQ	
	Stenotrophomans pavanii	DNQ	
	Staphylococcus aureus	0.1 cfu/m3*could be an indoor sample	Kozajda and Jeżak (2020)

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
Piperacillin/Tazobactam	E. coli	13.2-20% of *E. coli*	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
Rifampicin	Mannitol-positive staphylococci	~10% of strains	Małecka-Adamowicz et al. (2017)
Rifamycin	Pseudomonas oryzihabitans	DNQ	Bruni et al. (2019)
	Stenotrophomoasn maltophilia	DNQ	
	Stenotrophomonas pavanii	DNQ	
Streptomycin	Stenotrophomonas maltophilia	DNQ	
Tetracycline	Microbacterium phyllospaerae	DNQ	
	Mannitol-positive staphylococci	~5%	Małecka-Adamowicz et al. (2017)
Tobramycin	Microbacterium phyllospaerae	DNQ	Bruni et al. (2019)
	Stenotrophomonas pavanii	DNQ	
	Aquabacterium parvum	DNQ	

Antibiotic	Species of resistant organism	Prevalence (unit)	ref
Trimethoprim/sulfamethoxazole	E. coli	18.4-20% of *E. coli*	Ewa Korzeniewska, Korzeniewska, and Harnisz (2013)
Vancomycin	Pseudomonas oryzihabitans	DNQ	Bruni et al. (2019)
	Moraxella osloensis	DNQ	
	Stenotrophomonas pavanii	DNQ	

Table 10 Antibiotic resistance genes (and the antibiotic group to which they confer resistance) detected and quantified by culture-independent methods only, specifically qPCR and PCR. *Figure 4 in the paper displays data as a heatmap, colours in colour key are not true to main figure and hard to interpret. NB only ARGs that have been detected are displayed, ARGs screened for but not detected are listed in supplementary.

Antibiotic resistance class	Gene name	Prevalence (unit)	Reference
Aminoglycoside	aadA1	2.36 (copies ng^{-1} DNA)	Gaviria-Figueroa et al. (2019)
Beta-lactam	blaAMPC	10^2-10^3 (copies/m^3)	Osińska et al. (2021)
	blaTEM	10^4 (copies/m^3)	
Beta-lactam (class A)	GES	1.3 (copies ng^{-1} DNA)	Gaviria-Figueroa et al. (2019)
	TLA-1	2.1 (copies ng^{-1} DNA)	
Beta-lactam (class B)	IMP-12	3.5 (copies ng^{-1} DNA)	

Antibiotic resistance class	Gene name	Prevalence (unit)	Reference
Beta-lactam (class C)	FOX	0.37 (copies ng^{-1} DNA)	
	MIR	0.45 (copies ng^{-1} DNA)	
Beta-lactam (class D)	OXA-2	1.3 (copies ng^{-1} DNA)	
	OXA-10	2.5 (copies ng^{-1} DNA)	
	OXA-60	4.1 (copies ng^{-1} DNA)	
Chloramphenicol	cmlA	NR*	Osińska et al. (2021)
	florR	NR*	
	fexA	NR*	
	fexB	DNQ	
	catA1	DNQ	
Fluoroquinolone	Aac(6)-Ib-cr	2.62 (copies ng^{-1} DNA)	Gaviria-Figueroa et al. (2019)
	QnrB-5	1.47 (copies ng^{-1} DNA)	
	QnrS	3.11 (copies ng^{-1} DNA)	
	ermB	2.3 (copies ng^{-1} DNA)	

Antibiotic resistance class	Gene name	Prevalence (unit)	Reference
Macrolide-Lincosamide-Streptogramin_b	ermC	2.4 (copies ng^{-1} DNA)	
	mefA	2.36 (copies ng^{-1} DNA)	
Tetracycline	Tet(A)	2.3 (copies ng^{-1} DNA)	
		10^2-10^3 (copies/m^3)	Osińska et al. (2021)
	Tet(B)	10^0-10^2 (copies/m^3)	
	Tet(M)	10^0-10^2 (copies/m^3)	

Table 11 Results of studies that used a combination of culture-dependent and molecular methods to detect or quantify antimicrobial resistance.

Antibiotic resistance gene class	Gene	Species	Prevalence (unit)	ref
Aminoglycoside	Unspecified genes	UCB	20% of genes	Gaviria-Figueroa et al. (2019)
Beta-lactam (class D)	Unspecified genes	UCB	20% of genes	
Beta-lactam	blaCMY-2	UCB	9.5-25% of strains	Potorski et al. (2019)
	blaAMP-C	UCB	19-25% of strains	
	blaTEM	UCB	8.4-9.5% of strains	

Antibiotic resistance gene class	Gene	Species	Prevalence (unit)	ref
	blaTEM-1	E. coli	4/10 E. coli in air samples	Korzeniewska and Harnisz (2013)
	blaSHV	UCB	0-4.8% of strains	Potorski et al. (2019)
Beta-lactam (extended spectrum)	BlaCTX-M-1	E. coli	2/10	Korzeniewska and Harnisz (2013)
	BlaCTX-M-3	E. coli	2/10	
	BlaCTX-M-9	E. coli	2/10	
	BlaSHV-2	K. pneumoniae C. freundii	1/1 K. pneumoniae 1/1	
	BlaSHV-5	E. coli Enterobacteriaceae	1/10	
	BlaTEM-49	E. coli	1/10	
Chloramphenicol	cmlA	UCB	9.5-16.6% of strains	Potorski et al. (2019)
	floR	UCB	4.8% of strains	
	fexA	UCB	4.8% of strains	

Antibiotic resistance gene class	Gene	Species	Prevalence (unit)	ref
	catA1	UCB	14.3-25% of strains	
MLS	Unspecified	UCB	60% of genes	Gaviria-Figueroa et al. (2019)
Tetracycline	Tet(A)	UCB	19-25% of strains	Potorski et al. (2019)
	Tet(B)	UCB	4.8-8.4% of strains	
	Tet(M)	UCB	38-45% of strains	
	Tet(X)	UCB	23.4-33.3% of strains	

Appendix B – Sampling plan

Introduction

The Environment Agency (EA) is undertaking a research project as part of a cross-governmental project on how to carry out environmental surveillance of Antimicrobial Resistance (AMR). From initial research, whilst limited, it is clear that there are potential sources and pathways for AMR to reach a range of possible receptors including some that represent airborne risks[Error! Bookmark not defined.].

A project team consisting of WSP UK Limited, Cranfield University, and the University of Exeter (The Project Team) have been commissioned by the EA to develop surveillance strategy options and sampling strategies for both point source and ambient atmospheric AMR in bioaerosols and then through a series of field based trials test a range of methods and techniques, identified in the recently completed review**Error! Bookmark not defined.**. The methods and techniques will be tested to verify their performance and costs.

The purpose of this work is to help in the next step to investigate the atmospheric component of the environmental microbiome in relation to AMR. A review of methods and assessment options for environmental AMR in airborne microorganisms as well as a survey of antifungal residues applied to biosolids to land, has been completed[Error! Bookmark not defined.]. This task aims to develop a range of specified and costed options for carrying out surveillance of resistant organisms in aerosols at a regional and national scale, and will be informed by the recent review report[Error! Bookmark not defined.] and the field work but also by any other relevant programmes.

Information from the field trails and tests will assist in define the shape and nature of a potential future surveillance network, which would incorporate sampling variables including sample proximity to source, near to receptor, network size, network method, sampling density and frequency.

The above work will inform the development of a wider sampling strategy for AMR in the environment across the landscape and for all matrices/compartments.

Objectives

As per tender document

The broad objectives for this tranche of work are to develop:

- A range of specified and costed options for carrying out surveillance of resistant organisms in aerosols at a regional and national scale
- Field based trials equipment/ techniques based on the recently completed review to verify performance/ costs

In developing the surveillance strategy options, approaches and results of previous studies testing near to source and near to receptor will be used. Temporal and spatial variation will be explored, along with effects of prevailing conditions on AMR within bioaerosols.

Where information does not exist to support the development of surveillance strategy options, a conservative approach to the surveillance strategy will need to be considered.

This has been further broken down into three objectives to deliver the requirements of this tranche of work. They are:

1. Development of surveillance strategy options for aerosolised antimicrobial resistance;

2. Development of a sampling plan for field trials through a pilot study; and
3. Running of field trials.

Scope of this appendix

This document sets out an initial sampling plan to detect AMR in Bioaerosols, setting out the initial sampling selection, approach to be taken by The Project Team, including the long-list of bioaerosol sampling methods selected to be used in a pilot sampling exercise, how the project team aim to undertake pilot sampling exercise, AMR in bioaerosol analytical recommended methods, and the criteria to be applied when selecting the short-list of bioaerosol sampling methods selected to be used in the field trial.

In addition, specific constraints associated with sampling AMR within Bioaerosols have been set out, and how these may determine the optimum sampling approach.

2.1 Literature Review

An initial literature review will be undertaken on AMR in Bioaerosols, and the results of the summarise studies, including (where available) the number, frequency and duration of sampling, as well as the prevalence of AMR endpoints studied in those reports.

The review will aim to summarise approaches and results of previous and current studies which have undertaken AMR in bioaerosol testing near to source and near to receptor, where information to support this is available. The following will be taken into account:

- Sample temporal and spatial variation;
- Frequency of sampling;
- Effects of prevailing conditions upon AMR in bioaerosols
- Prevalence of AMR endpoints studied.

However, the extent of the AMR in Bioaerosols evidence base is currently unknown, and sensible limits may need to be placed upon the scope of the literature review to ensure a satisfactory depth and breath of the summary.

2.2 Pilot study

Part of the test bed in selecting a ‘long-list’ of AMR in Bioaerosol sampling methods, will be an initial sampling pilot study. The pilot study will allow the long-list of AMR in bioaerosol sampling methods to undergo initially trails, ensuring latter field testing will be informed and any unsuitable sampling methods can be eliminated at an early stage.

2.3 Field trials

Upon completion of the pilot study, a ‘short-list’ of AMR in Bioaerosol sampling methods will be compiled. This short-list will then be further tested in several field trials where all short-list bioaerosol sampling methods will be deployed simultaneously within close proximity to a number of potential AMR sources.

During the field testing, the number, frequency and duration of source-term sampling during the field tests, will be determined, and shall be dependant upon:

a) optimal sample design at varying process operation sites and (e.g. wastewater treatment, intensive livestock, arable farming, ambient environments);

b) sampling equipment and methods used to collect bioaerosol samples available for different culture-based and culture-independent AMR focused post-collection analysis.

The sampling methodology and longer term sampling strategy shall both inform appropriate options for surveillance strategies at a regional and national level.

2.4 Analytical techniques

The various analytical methods available to identify AMR within biological samples can be split into two groups:

- Culture based methods (microscopy, plate count, staining methods, MALDI-TOF);
- Culture Independent (Flow cytometry, qPCR, NGS and metagenomics).

A review published by the EA highlights that due to shortcomings in the molecular methods a combined approach of both techniques would be required for a comprehensive analysis of fungal spores and bacteria. This is due to a gap in existing gene databases covering both bacteria and fungi.

2.5 Potential sources

There are a number of known potentially significant sources of AMR, such as wastewater treatment plants, agricultural environments (e.g. slurry application/spraying), land fill, composting sites.

2.6 Targeting sources

There are many possible sources of air harbouring antimicrobial resistant organisms. A 2020 Environment Agency review (Jones 2020)[9] found that existing studies had mainly targeted confined animals feeding operations (CAFOs; pig, cattle and poultry) and wastewater treatment plants (WWTPs), focussing on AMR in airborne bacteria. Since this review, a major citizen science study surveying triazole-resistant *Aspergillus fumigatus* in UK garden soils has also been published, extending the picture to fungi (Shelton et al. 2022).[10].

In this study, we will initially focus on wastewater treatment plants, for several reasons:

[9] Jones, Matt L. 2020. 'Review of Airborne Antimicrobial Resistance'. Environment Agency. https://www.gov.uk/government/publications/review-of-airborne-antimicrobial-resistance.

[10] Shelton, Jennifer M. G., Roseanna Collins, Christopher B. Uzzell, Asmaa Alghamdi, Paul S. Dyer, Andrew C. Singer, and Matthew C. Fisher. 2022. 'Citizen Science Surveillance of Triazole-Resistant

1. The Jones (2020) review identified WWTPs as harbouring a medium-to-high level diversity of antimicrobial resistance. Studies of CAFOs in North America generally found to have higher levels of diversity, but since these highly confined indoor operations are not common in UK agriculture, we consider that this is probably overestimated.
2. Since wastewater treatment plants receive wastewater from several different sources (e.g. residential, agricultural, hospital), they are a good first source to target especially when trying to guarantee a signal in pilot studies.
3. The study team have access to an existing WWTP facility at Cranfield University, from which various sampling configurations and combinations can be tested at close quarters to the Cranfield Project team workshop, whilst overcoming a number of potential obstacles to the sampling programme, such as liaising with third party site owners, seeking third party site approvals and the logistical challenges in transporting sampling equipment and instruments, whilst dealing with sampling uncertainties faced at unfamiliar sites.

UK agricultural environments (including arable alongside livestock farms) and gardening/composting environments are likely future sources to target, especially as these may be more likely to capture antifungal resistance (since fungi are prominent pathogens and degraders in such environments)[11].

2.7 Assessing exposure risk

Though outside the scope of this study, consideration of the risk of human exposure to airborne AMR via inhalation will eventually enable the surveillance framework to be applied to determine direct influences of airborne AMR upon human health. As inhalation of AMR in bioaerosols poses a health risk to humans in various source-adjacent and downwind environments. and may support the business case for sustained surveillance in this area.

2.8 Development of AMR in the wider biome

In addition, the study will contribute to a greater understanding of risks from AMR developing in plant and animal pathogens, and also AMR in non-pathogens that might be transferred horizontally to pathogens.

Exposure to AMR in bioaerosols may result in colonisation, and infection, with vulnerable and immunocompromised individuals at increased risk of severe disease and death. For example, previous research has reported an association between living close to livestock rearing operations and nasal colonisation by the antibiotic resistant bacteria, methicillin resistant *Staphylococcus aureus*[12].

[11] Jones, Matt L. 2020. 'Review of Airborne Antimicrobial Resistance'. Environment Agency. https://www.gov.uk/government/publications/review-of-airborne-antimicrobial-resistance

[12] https://ehjournal.biomedcentral.com/articles/10.1186/1476-069X-13-54

The current gap in understanding of airborne antimicrobial resistance determinants, the related risk of exposure[13] and their spatial distribution is not confined to the UK,. Previous research on assessing human exposure risk to airborne AMR has been conducted in China. One method estimated the average exposure dose via inhalation (copies per day per kg) by measuring the average density of ARG in air samples (copies per m^3) and applying this to standard inhalation rates suggested by United States Environmental Protection Agency protocols[14,15]. Another study used a similar approach and refined exposure estimates according to age, body size and exposure frequency[16].

These studies demonstrate the utility of quantitative approaches to measuring AMR in bioaerosols. Similar assessment of human exposure risks can be achieved by collecting data on the density of AMR organisms in bioaerosols to calculate the number of AMR organisms inhaled per person per day.

2.9 Identifying uncertainties and constraints

Uncertainties and constraints shall be explored and mapped throughout pilot sampling, field trials and development of analytical methods for AMR in Bioaerosols. This will include uncertainties at both a regional and national scale, in order that margins of uncertainty can be derived for population exposure estimates and thereby target ways of designing surveillance options that could elements of uncertainty.

3.1 Sampling techniques

In relation to sampling techniques, the overall project objectives include:

a) development of sampling design for optimal sampling locations at sites differing in operations and process (e.g. wastewater treatment, intensive livestock, arable farming, ambient environments).
b) identification of potential sampling equipment and methods to collect bioaerosol samples available for different culture-based and culture-independent AMR focused post-collection analysis by UKSHA.
c) Use of a series of field trials to establish design options for a regional and national AMR in Bioaerosol surveillance monitoring.

A wide range of sampling techniques have been identified which are considered to potentially fulfil the above two project objectives. Our approach in selecting these methods

[13] https://environmentalevidencejournal.biomedcentral.com/articles/10.1186/s13750-022-00262-2

[14] https://pubs.acs.org/doi/10.1021/acs.estlett.7b00561

[15] https://pubs.acs.org/doi/10.1021/acs.est.8b04630

[16] https://www.sciencedirect.com/science/article/pii/S0048969719336897?via%3Dihub

is discussed in our Methodological approach section, and individual sampling methods are detailed further below.

3.2 Methodological approach

A range of sampling methods are available to collect bioaerosols and broadly fall into impaction, impingement, filtration, and cyclone. The advantages and disadvantages of these methods have been widely discussed in the literature[17,18,19]. Building on rapid evidence assessment along with M9 Technical Guidance Note (Environmental monitoring of bioaerosols at regulated facilities) and consultation with the EA project manager, we will apply the decision tree framework to identify an optimal sampling plan involving the identification of sampling locations at each site, distances from the source, sampling method/equipment, frequency, duration, volume and number of samples. The decision tree framework considers the below to ensure high throughput and representative sample capture:

- Objective and goals of monitoring
- Questions to be addressed and information needed (Concentration, identification, average concentration, worst-case concentration, particle size distribution)
- Biological agent of interest and their sources
- Importance of specific microbial groups or species (e.g. bacterial and fungal pathogens, viruses)
- Anticipated concentrations and variability in time and space
- Target genes or biomarkers (e.g. Antimicrobial resistance genes)
- Suitability, logistical challenges, and cost of sampling method
- Pros and cons of sampling methods with reference to post collection analysis (e.g. filter types, collection efficiencies, flexibility of analysis, reliability, ease of operation, remote operation capability, particle size discrimination, post- collection analysis options, cost)
- Constraints and requirements of post collection analysis (e.g phenotyping of organisms grown on selective media or qPCR /metagenomic)
- Geographical location and environment characteristics

[17] Mainelis, G. (2020). Bioaerosol sampling: Classical approaches, advances, and perspectives. *Aerosol Science and Technology*, *54*(5), 496-519.

[18] Kathiriya, Twinkle, Abhishek Gupta, and Nitin Kumar Singh. "An opinion review on sampling strategies, enumeration techniques, and critical environmental factors for bioaerosols: An emerging sustainability indicator for society and cities." *Environmental Technology & Innovation* 21 (2021): 101287

[19] Gollakota, A. R., Gautam, S., Santosh, M., Sudan, H. A., Gandhi, R., Jebadurai, V. S., & Shu, C. M. (2021). Bioaerosols: characterization, pathways, sampling strategies, and challenges to geo-environment and health.*Gondwana Research*, *99*, 178-203.

The sampling plan will be comprised of a pilot study (7.1) and field trials (7.2). During the pilot study a suite of sampling methods involving filtration (IOM, Leckel Bio inlet), impaction (Andersen eight stage impactor), impingement (BioSampler) and cyclone (Coriolis Compact (dry cyclone) will be deployed. We will test these for both long and short durations testing collection efficiencies to optimise methods for bulk and size-fractionated sample capture. The findings based on EA/ UKSHA response from post-collection analysis will inform the identification of optimal equipment and design for the field trail. The key focus will be to ensure that the sampling strategy is suitable for both point source and ambient atmospheric AMR in bioaerosols.

Following a recent review and analysis of techniques to sample AMR in Bioaerosols[Error! Bookmark not defined.], along with accrued knowledge of the AMR in Bioaerosols Project Team, a brief review of the Pro's and Cons of potential sampling approaches has been outlined in the following sections covering the four most common techniques of:

1. Impaction
2. Filtration
3. Cyclone
4. Impingement

3.3 Impaction

Impaction relies on the inertial impaction of particles onto the collection medium. Air is pumped through the sampler and particles varying in size will impact on to a collection surface depending on their inertia. Various single and multi-stage impactors are available (e.g. Andersen single, 6 and 8 stage impactors) with differing particle size fractionation. Multi-stage impactor collects and aerodynamically segregates the particles into different size ranges.

It is proposed that an eight-stage non-viable Andersen impactor loaded with polycarbonate filters will be used to collect aerodynamically size segregated aerosol samples from 0.4 to >10 µm. Eight-Stage Non-Viable Andersen Sampler is a multi-orifice cascade impactor and collects aerodynamically size-fractionated particles at different stages (each with a different 50% "cut-size" as the value of d_{ae} (i.e., d_{ae}−50%) achieving size segregation from 0.4 to >10 µm. (Stage 0: 9.0 - 10.0 µm, Stage 1: 5.8 - 9.0 µm, Stage 2: 4.7 - 5.8 µm, Stage 3: 3.3 - 4.7 µm, Stage 4: 2.1 - 3.3 µm, Stage 5: 1.1 - 2.1 µm, Stage 6: 0.7 - 1.1 µm, Stage 7: 0.4 - 0.7 µm). The sampler operates at a flow rate of 28.3 l/min through a calibrated vacuum pump. The air enters the inlet cone of the sampler and cascades through the succeeding stages with successively higher velocities from stage 0 to stage 7, inertially impacting the particles according to their sizes at different stages.

Table 3-1 - Size fractions of particles impacted onto stages of the Anderson impactor at 28.3 l min^{-1}

Stage number	Cut point Aerodynamic diameter (µm)
0	9.0
1	5.8
2	4.7

3	3.3
4	2.1
5	1.1
6	0.7

Advantages

- Ease of use
- Portable and reliable
- Ability to provide size fractionation of particles
- Particle sizes can be linked to different regions of the human respiratory system on inhalation.
- Ease of sterilization and transport
- Choice of impaction surface depending on analytical needs
- Choice of options for post-collection analysis (both culture-based and molecular analyses)

Disadvantages

- Loss of bio-efficiency due to desiccation and shear forces
- Loss of cell viability
- Particle bounce
- Particle build up on collection surfaces and altered collection efficiencies
- Sampling efficiencies affected by wind speed
- Remote operation capability - Access to power

3.4 Filtration

Filtration involves the collection of bioaerosols samples onto a porous material, usually a filter. Air is drawn through the filter through a calibrated pump, capturing particles onto filter media. Various filtration-based sampling methods are available differing in flow rates and filter media. In the current project, two filtration-based samplers will be used.

i) Institute of Occupational Medicine (IOM) sampler.

IOM sampler draws air through a 25mm porous polycarbonate or quartz filter (pore size of 0.8µm) housed in a filter cassette. This method is also one of the EA proposed sampling methods for bioaerosol monitoring at regulated facilities.

ii). Leckel sampler with Bio inlet.

Leckel sampler with bio inlet is a sampling system with a disposal filter holder for the sampling of airborne moulds in ambient air according to VDI 4252 part 2 using 80 mm gelatine filter with a polycarbonate filter laid under the gelatine filter.

Advantages

- Ease of use
- Good collection efficiencies
- Portable and reliable
- Ease of sterilization and transport
- Choice of filter media depending on analytical needs
- Range of options for post collection analysis (both culture-based and molecular analyses)
- Good recovery for biological materials for molecular analysis
- Longer sampling periods

Disadvantages

- Loss of bio-efficiency due to desiccation
- Loss of cell viability
- Loss of recovery during particle extraction from filter media
- Sampling efficiencies affected by wind speed
- Remote operation capability - Access to power (For Leckel sampler)

3.5 Impingement

Impingement involves the wet-based collection of bioaerosols and relies on bubbling air through a collection fluid, retaining particles in the fluid during sampling. In the current project, SKC BioSampler will be deployed. This is a glass collection device containing three tangential nozzles. Operating at 12.5 L/min air flows through the nozzles, and particles are collected in a fluid (e.g. water) by the upward swirling of the liquid on the inner walls of the collection vessel.

Advantages

- High collection efficiencies
- Less physical stress on collected microorganisms
- Low re-aerosolisation of particles
- Ease of transport
- Range of options for post collection analysis (both culture-based and molecular analyses)
- Good recovery for biological material for molecular analysis
- Liquid collection media - no overloading
- Ease of post collection analysis

Disadvantages

- Loss of collection fluid due to evaporation during long duration sampling
- Sterilisation of the sampler in between the samples
- Sampling efficiencies affected by wind speed

Constraints

Remote operation capability - Access to power

3.6 Cyclone

Cyclone samplers collect bioaerosols through the use of centrifugal forces in a wet or dry collection system. The current project will deploy Coriolis® Compact (Bertin Instruments). This is a dry cyclonic sampler which aspirates the airborne particulate matter in the ambient air with a 50 l/min flow rate and centrifuges the particles in a collection cone. After the sampling, the collected particles are removed by adding appropriate suspension liquid to the cone.

Advantages

- High collection efficiencies
- Reduced particle loss through re entertainment/bounce
- High volume sampling
- Ease of transport and sterilisation
- Remote operation capability – battery powered
- Compatible with multiple post collection analysis (both culture-based and molecular analyses)
- Good recovery for biological material for molecular analysis
- Ease of post collection analysis

Disadvantages and constraints

- Loss of viability due to desiccation and shear forces
- Sampling efficiencies affected by wind speed

4.1 Development of sampling strategy using decision support tree

The sampling strategy/approach has been developed using the decision support tree as presented in “Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms” (Environment Agency 2022)[Error! Bookmark not defined.].

The decision tree sampling selection tool will continue to be applied to progress sampling selection during the pilot study and reach completion at the end of the field-based trials.

Criteria within the decision tree will form the outputs for which this sampling plan aims to provide.

Table 4-1 - Decision tree analysis

Decision Category	Requirements and feasibilities	Findings
Objectives and Hypothesis	• Single species vs ensemble of species, • Time dependent vs long term trends,	

	• Single point vs spatial assessment, • Source apportionment vs general surveillance. • Receptors of concern • End users of airborne AMR concentration data • User uncertainty requirements	
Network Geographical distribution	• Number of sites, • Rural vs urban, • Sampling height, • Micrometeorological air flow, • Nearby sources	
Site(s) requirement	• Power availability, • Risk to equipment, • Installation in particular large or heavy instruments, • Manned vs unmanned stations, • Access, • Training of staff, • Difficult environments, • Sample storage, • Transport of samples to analytical centre	
Instrument& analysis requirements	• Sample analytical approach in laboratory, • Sampling viability of sampling medium, • Sample specifications and sampling efficiency, • Reliability, • Labour costs, • Capital costs, • Acquisition time of new instrument or reuse of instrument, • Instrument lifetime, • Sample media costs, • Power requirements, • Housing requirement	
Supplementary data	• Weather data (local v nearby v modelled), • Modelling tools and assessment methods, • Management or activity data, • Cover data and land use data.	

5. Sample analysis

The objectives of the sample analysis review is to assess the feasibility of the monitoring techniques in order to meet the objectives of the sampling strategy.

5.2. Analytical approaches to quantify and characterise AMR

5.2.1. Culturing bioaerosols, including antimicrobial resistance (AMR)

AMR can be tested for phenotype by growing cultures of micro-organisms with antimicrobial agents. Depending on the sample collection approach both fungal spores and bacteria can be enumerated. It should be noted that the reported number of culturable fungal spores/bacteria is only a fraction of the culturable microorganisms present at the time of sampling. The review[Error! Bookmark not defined.] also s hows that the large variety of culturable bioaerosols have a wide range of optimal growing conditions and therefore the choice of species to investigate is key for setting the analytical approach.

Overall, the culture approach is cost effective and much cheaper than the molecular approaches. In addition, the culture approach allows for simultaneous detection of many different species. The key downside of the approach is with it only detecting the culturable quotient of the bioaerosols present.

5.2.2. Molecular methods for analysing AMR

Culture-independent methods can be used in combination with, as well as without, a culturing step, and thus can provide broad and deep information about the antimicrobial resistance characteristics of a sample. Depending on the method, they can provide a fast and accurate characterisation of resistance.

DNA-based methods: There are many methods for extracting and amplifying DNA from environmental samples. This is predominantly done using kits for extracting the DNA with the amount of DNA extracted being a key factor. PCR-based approaches amplify target genes, and can therefore detect genes that are present at low levels in the sample. Assays generate results relatively quickly (same day) and are specific for certain genes. Prior knowledge of the genes to be targeted is needed. Methods are available for the quantification of genes (qPCR). Several techniques, such as metagenomics and metabarcoding, do not require gene targets to be selected before analysis and can identify thousands of different genes in a single sample. They do, however, require large quantities of DNA, and may miss genes that are present in the sample at low abundance. There are several databases for antibiotic resistance genes that can be searched (e.g. CARD, ARGD, ARG-OAP), but fewer antifungal resistance databases (e.g. MARDy).

Other molecular methods: Techniques such as MALDI-TOF mass spectrometry and enzyme-linked immunosorbent assays (ELISA) can also generate information about the identity of cultured cells without DNA extraction.

5.3. Target biological agent

As outlined in section 2.8, a greater understanding of AMR in bioaerosols will assist in understanding risks of AMR developing in plant and animal pathogens, and whether AMR in non-pathogens could be transferred horizontally to pathogens. The study will assist in a better understanding of the environmental drivers of AMR so that these might be mitigated.

Surveillance targets may be chosen based on their clinical importance, their presence and spatiotemporal variation in abundance, or based on their association with anthropogenic pollution

(Klumper et al 2022[20]). Selection of appropriate biological agents for surveillance may consider alignment with existing surveillance efforts internationally or nationally, and/or consider targets known to occur in relevant environments according to published research.

Examples of culture-based AMR endpoints to consider

- Cefotaxime-resistant E. coli. The One Health surveillance for extended spectrum beta-lactamase producing E. coli Tricycle project collects data on cefotaxime-resistant E. coli across several One Health compartments. E. coli is also one of the ESKAPE pathogens surveyed by GLASS. E. coli are gram negative bacteria, that are present at high abundance in the guts of warm-blooded animals, and also in sewage and slurry applied to agricultural land.
- Ampicillin-resistant E. coli. Ampicillin resistance is more common, and research in bathing waters shows it is associated with cefotaxime resistance among E. coli. Ampicillin may offer a more sensitive proxy for resistant bacteria, and thus reduce the volume of sample required for a detection.
- Methicillin-resistant Staphylococcus aureus is a gram-positive bacterium capable of causing invasive infections, and has been detected in bioaerosols from wastewater treatment plants and farm environments.
- Azole-resistant Aspergillus spp. Azole resistance poses a challenge to the treatment of invasive infections, and these compounds are used widely in non-clinical settings (e.g. agriculture). Aspergillus (e.g. A. fumigatus) are found in diverse environments, including wastewater treatment plants.

Specific targets for qPCR are to be identified within this project, as well as suggestions of potential species which may present a risk, though not able, for varying reasons, to be included with this study. Once further data from a literature review has been assessed. Possible resistance genes endpoints to consider include (DNA can be extracted and stored prior to selecting specific gene targets):

- Class 1 integron-integrase gene (intI1) is a mobile genetic element and has been proposed marker for anthropogenic pollution and is prevalent among multidrug resistant organisms.
- Specific resistance genes against sulphonamides, beta-lactams, macrolides, tetracyclines and quinolones (e.g. ermB, tetW, qnrS, lnuA, blaTEM, blaCTX-M, sul1)

5.4. Constraints associated with techniques

A number of constraints have been identified with analytical techniques to identify potential AMR, these include:

- The use of culture-based techniques will pose constraints on the transport and storage of samples. To maintain the viability of cells, samples must be chilled immediately upon collection, and ideally analysed upon arrival at the laboratory. If samples to be cultured need

[20] https://www.jpiamr.eu/app/uploads/2022/08/Towards-developing-an-international-environmental-AMR-surveillance-strategy_report-2022-08-04.pdf

to be stored long term, it is recommended that 20% sterile glycerol solution is added before freezing at -80oC.

- PCR-inhibiting substances (e.g. fats, proteins, humic acids, polysaccharides, metal ions) that are found in sewage, and may be present in bioaerosols at sufficient levels to interfere with qPCR assays. The effects of inhibiting substances can be eliminated or reduced in a variety of ways e.g. sample dilution, use of sample clean up kits/protocols to extract nucleic acids from the sample.
- Different methods and surveillance targets will have different limits of detection. Preliminary data collection as part of the pilot field trial will help to determine appropriate volumes of air to sample for different surveillance targets.
- Metagenomic methodologies are less standardised compared to culture-based methods and qPCR, which are used routinely for surveillance activities. These methods also require appropriate computing facilities and expertise to analyse and interpret the data.
- Metagenomic methods are unlikely to detect rare genes. If rare but important genes are selected for surveillance, qPCR will improve their detection.
- Collection of sufficient material for analysis
- Consider using DNA extraction kits that allow the isolation of DNA from both fungi and bacteria from a single sample to reduce costs.
- Molecular methods not requiring analysis of DNA (e.g. MALDI-TOF and ELISA) can be used to confirm the identity of cultured cells. This can also be achieved using DNA-based techniques, which moreover can detect resistance genes. Further, MADLI-TOF mass spectrometry requires an appropriate database against which to compare spectra for species identification.

6.1 Constraints around sampling and analysis

In identifying and developing the initial long-list optimum sampling methods to successfully capture AMR in Bioaerosol samples, a number of potential constraints have been identified, these include:

- Temporal Constraints
- Logistical Constraints
- Level of Expertise Required
- Contamination and Potential Secondary Influences
- Sensitivity of Method to Sampling Conditions
- Costs of Sampling
- Sample Spatial Coverage

6.2 Temporal constraints

In order for an airborne sampling programme to achieve basic data quality objectives, a sample collection programme needs to be representative of temporal variation in concentrations, or at least determine ‘what is an appropriate averaging time and frequency of sampling’. For bioaerosols, this could be as low as every minute for concentrated episodic sources. For area-wide continuous sources temporal variation in concentrations could be over several hours.

Ideally, bioaerosol sampling methods will need to be capable of recording changes in bioaerosol concentrations at similar intervals to the source under investigation. Sampling methods which collect samples over a duration which is considerably longer than the period in which major changes in bioaerosol concentrations occur, could potentially misrepresent any changes in bioaerosol concentrations. Sampling method which collect samples over a short duration is at risk of not collecting sufficient yield for analysis.

6.3 Logistics

Ease of deploying the bioaerosol sampler within the field and the staff requirement to operate sampling methods will be assessed. Any sampling method which require significant logistical commitments or staff inputs, will be considered to be significantly constrained and an non-optimal method.

6.4 Requirement of field staff expertise

The staff and resources requirement for each of the continuous bioaerosol sampling methods will be appraised for suitability and whether they represent an optimal method in terms of proportionality to the project objects. Resources such as requirement for highly skilled field staff, in order to operate or continually change out and preserve samples.

6.5 Sampling conditions

It is known that bioaerosol sampling requires samples to be undertaken during relatively low wind, dry ambient conditions. However, any bioaerosol sampling method which has greater dependence upon particular sampling conditions, and is too sensitive to typical conditions, could be considered to be significantly constrained and a non-optimal method.

6.6 Costs

Bioaerosol sampling methods vary considerably, similarly the costs of various sampling methods and their operation. The higher-cost sampling methods could represent low cost-benefit analysis. However, where a potential method enables project objectives to be achieved and supports the establishment of a functioning surveillance network. be achieved, could be considered optimal, even where that sampling method may be a higher cost technique.

6.7 Spatial coverage

In order to effectively determine the presence and influence of AMRs in bioaerosol in the UK, the recommended preferred sample method will need to be capable of being deployed across a number of sample location at a single site. Restrictions on spatial coverage of sampling methods are typically linked to costs, sampler size, degree of specialism required to operate.

7.Target sampling locations

7.1 Pilot study

The aim of the pilot study will be to initially field test the effectiveness of the sampling plan, explore its practicality and verify representativeness of the samples collected.

Sample results will be reviewed and finding shall inform the approach to be taken in the wider strategy of monitoring AMR in Bioaerosols, and the environment generally.

7.1.2. Sample design

Cranfield sewage treatment works facilities are located immediately adjacent to a small, wooded area and arable land. These are considered secondary bioaerosol sources, though such bioaerosol sources could be considered to form part of the profile of bioaerosol in ambient air. A time and activities diary will be collected during each sampling period to record on-site and surrounding area activities.

Sampling will be undertaken at the Cranfield sewage treatment works facilities located at the Cranfield University site. Cranfield WWTP received water from Chicheley brook which is a watercourse that runs through a predominantly rural catchment including the villages of Cranfield, Hardmead, Chicheley, North Crawley and Newport Pagnell. It is a tributary of the River Great Ouse and has a number of smaller streams and brooks discharging into it. Its national grid reference is SP94sw and its water body ID is GB105033038040. Flow is largely dependent on rainfall. Land use in the catchment is predominantly small-scale cropping, horticultural and livestock farm holdings including one dairy farm, two beef farms, two sheep farms, three grass-keeping farms and two large arable farms. The site is surrounded by agricultural fields and woodland (Figure 7-1).

We anticipate completing the sampling campaign in 5 days. Table 7-2 describes the proposed sampling strategy and the number of samples.

Figure 7-1 - The landscape of Cranfield sewage treatment works facilities

7.1.3. Instrumentation

A suite of sampling methods involving filtration (IOM, Leckel Bio inlet), impaction (Andersen eight stage impactor), impingement (BioSampler) and cyclone (Coriolis Compact (dry cyclone) will be deployed. These methods are selected to provide a range of post-collection

analysis options for both point source and ambient atmospheric AMR in bioaerosols. Table 1 provides a summary of different sampling methods, sampler and possible post-collection analysis.

Table 7-1 - Summary of different sampling methods and post-collection analysis options

Sampling method	Sampler	Collection medium	Flow rate (l/min)	Post collection analysis
Impaction	Eight-stage non-viable Andersen impactor	Polycarbonate filters (81mm)	28.3	Culture based (e.g. microscopy, plate count, staining methods, MALDI-TOF); Culture Independent (e.g. Flow cytometry, qPCR, NGS and metagenomics).
Filtration	Institute of Occupational Medicine (IOM) sampler Sven Leckel sampler with Bio inlet. (Sampling of fungi)	Polycarbonate filters (25mm) Gelatine filters (80mm)	2 50	Culture based (e.g. microscopy, plate count, staining methods, MALDI-TOF); Culture Independent (e.g. Flow cytometry, qPCR, NGS and metagenomics).
Impingement	SKC Bio Sampler	Liquid	12.5	Culture based (e.g. microscopy, plate count, staining methods, MALDI-TOF); Culture Independent (e.g. Flow cytometry, qPCR, NGS

				and metagenomics).
Cyclone	Coriolis® Compact (dry cyclonic air sampler)	Particles collected in a cone	50	Culture based (e.g. microscopy, plate count, staining methods, MALDI-TOF); Culture Independent (e.g. Flow cytometry, qPCR, NGS and metagenomics).

7.1.4. Sample location and position

Sampling will be conducted at the source and upwind (ambient air). At the source, samples will be taken between the trickling filter beds and the upper sludge storage tank (See Figure 7-1). This is to capture the maximum possible aerosolised biological load. Upwind samples will be taken 100 meters away from the sources. The upwind site will be chosen to represent ambient air, reflecting different emission sources in comparison to wastewater treatment.

7.1.5. Duration and extent of methods

Sampling will be undertaken over a working day for both long (3 hours) and short exposures (1 hour) at the source and upwind site. For each site, three repeated measurements will be carried out during the daytime at a height of 1 m by proposed sampling methods. Sampling for long (3 hours) and short durations (1 hour) will allow testing collection efficiencies and sample duration to optimise methods for bulk and size-fractionated sample capture for post-collection analysis.

Table 7-2 – Summary of sampling strategy

Day	Sampler	Sampling site	Duration	Number of measurements	Number of samples (see Note 1)
Day 1	1.Eight-stage non-viable Andersen impactor 2.IOM sampler 3.SKC Bio Sampler 4.Coriolis® Compact	At source Ambient (Upwind)	1 hour	2 (at source) 2 (Ambient)	22 22
Day 2	1.Eight-stage non-viable Andersen impactor 2.IOM sampler 3.SKC Bio Sampler 4.Coriolis® Compact	At source Ambient (Upwind)	1 hour	1 (at source) 1(Ambient)	11 11
Day 3	1.Eight-stage non-viable Andersen impactor 2.IOM sampler 3.SKC Bio Sampler 4.Coriolis® Compact	At source Ambient (Upwind)	3 hour	1 (at source) 1(Ambient)	11 11

Day 4	1.Eight-stage non-viable Andersen impactor 2.IOM sampler 3.SKC Bio Sampler 4.Coriolis® Compact	At source Ambient (Upwind)	3 hour	1 (at source) 1(Ambient)	11 11
Day 5	1.Eight-stage non-viable Andersen impactor 2.IOM sampler 3.SKC Bio Sampler 4.Coriolis® Compact	At source Ambient (Upwind)	3 hour	1 (at source) 1(Ambient)	11 11

Note 1 - Eight-stage non-viable Andersen impactor will produce 8 size fractionated samples for each measurement.

7.1.6. Preservation, dispatch and analysis of bioaerosol samples

The collected samples will be contained and preserved, labelled and shipped immediately to one of the UKHSA Laboratories (Porton Down, Colindale & Bristol) for analysis as per their requirements. The transportation matrix of the collected samples ((filters, liquid and cyclone cones) will be confirmed after consultation with the UKHSA laboratory.

The findings from the pilot study based on EA/ UKSHA response from post-collection analysis will inform the identification of optimal equipment and design for the field trial. The key focus will be to ensure that the sampling strategy is suitable for both point source and ambient atmospheric AMR in bioaerosols.

7.1.7. Sample records

A time and activities diary shall be collected during each sampling campaign.

7.1.8. Meteorological data measurements

Real-time measurements of meteorological conditions (wind speed, wind direction, temperature, relative humidity, atmospheric conditions) will be collected.

7.1.9. Potential influence of secondary bioaerosol sources

Cranfield University on-site waste-water treatment plant is located immediately adjacent to a small wooded area and adjacent to arable land. These are be considered secondary bioaerosol sources, though such bioaerosol sources could be considered to form part of the profile of bioaerosols in ambient air. A record of the immediate surroundings and activities will be collected as sampling meta data to ensure any indirect influences or provide an opportunity for known factors, but unknown sources to be explored retrospectively.

7.1.10. Sampling programme and timelines

Once the Sample Plan has been reviewed and approved by the client team, mobilisation of pilot sampling is anticipated to take not more than 3 weeks from receipt of approval, pending supply of the limited sampling equipment being supplied by third parties.

Though currently in planning stage, the anticipated approach is that three repeated measurements are undertaken over short and long duration with 4 methods at upwind and at source.

7.2 Field trials

The objective of the field trails will be to test suitability and viability of a short-listed set of candidate sampling methods.

The field trails will build upon findings and outcome from the pilot study and the literature review work. The field trial will aim to optimise a number of shortlisted candidate sampling methods across a longer sample study at multiple locations and differing sample settings.

7.2.2. Number of field trials locatins

It is anticipated that throughout the field trials, bioaerosol samples shall be collected from between 4 to 6 differing sample locations where all shortlisted candidate sampling methods will be deployed.

The final field trial sampling sites are to be determined by the Environment Agency (EA).

7.3 Preservation, dispatch and analysis of bioaerosol

During both the pilot and field trial, bioaerosol samples will contained and preserved, labelled and shipped immediately to one of the UKHSA Laboratories (Colindale & Bristol) for analysis as per their requirements. Analysis methods will be determined by the UKHSA laboratory.

The transportation matrix of the collected samples will be confirmed after consultation with the UKHSA laboratory.

Appendix C – AMR in bioaerosols pilot sampling report

Introduction

In order to inform the sampling strategy for AMR surveillance in bioaerosols, a pilot study sampling plan was developed. The sampling plan entailed identification of sampling site/location, sampling design (number of samples, volume, duration) and bioaerosol sampling methods/equipment to allow culture based and culture independent AMR focused downstream analysis by UKHSA.

The pilot study aimed at field test the effectiveness of a suite of sampling methods involving filtration, impingement and cyclone for longer and short durations testing collection efficiencies under varied flow rates to identify suitable methods for field trials.

Objectives

The aim of the pilot study was to field test a suite of sampling methods involving filtration, impingement and cyclone for longer and short durations testing collection efficiencies under varied flow rates to identify suitable methods for field trials. The pilot study will aim to identify the optimal sampling methodologies to allow culture based and culture independent AMR focused downstream analysis.

Sample design

Sampling was undertaken at the Cranfield sewage treatment works facilities located at the Cranfield University site. Cranfield WWTP received water from Chicheley brook which is a watercourse that runs through a predominantly rural catchment including the villages of Cranfield, Hardmead, Chicheley, North Crawley and Newport Pagnell. It is a tributary of the River Great Ouse and has a number of smaller streams and brooks discharging into it. Its national grid reference is SP94SW and its water body ID is GB105033038040. The river flow is largely dependent on rainfall. Land use in the catchment is predominantly small-scale arable, horticultural and livestock farm holdings including one dairy farm, two beef farms, two sheep farms, three grass-keeping farms and two large arable farms. The site is surrounded by agricultural fields and woodland.

Sample location and position

The objective of the sampling design was to compare 4 different samplers within one sample location, in order to reduce the number of variables to allow a robust inter-comparison exercise. The two sample locations (Figure C1) were chosen on the following basis:

1. At source immediately adjacent to the trickle filter beds to maximise possible biological load uptake.
2. Upwind of the source, to represent ambient air, reflecting different sources in comparison to wastewater treatment.

Figure C1 - Sampling locations at Cranfield sewage treatment works

Instrumentation

The three methods selected in the Sampling Plan for the Pilot Study were filtration (IOM, Leckel with Bio-inlet), impingement (SKC BioSampler) and cyclone (Coriolis Compact (dry cyclone). These methods were selected to provide a range of post-collection analysis options for both point source and ambient atmospheric AMR in Bioaerosols. A summary of the sampling methods used is presented in Table C1 below.

Table C1 Summary of selected sampling methods.

Sampling method	Sampler	Collection medium	Flow rate
Filtration	Institute of Occupational Medicine (IOM) sampler	Polycarbonate filters (25mm)	2
	Sven Leckel sampler with Bio inlet. (Sampling of fungi)	Gelatine filters (80mm)	50

Impingement	SKC Bio Sampler	Phosphate-buffered saline (PBS)	12.5
Cyclone	Coriolis® Compact (dry cyclonic air sampler)	Particles collected in a cone	50

Duration and extent

Three repeated measurements were carried out for short (1) and long (2 hours) durations at the source and ambient location. Four samplers were deployed concurrently during each measurement.

Sampling was undertaken as dictated by weather conditions during the sampling period with an extended cold period with snow stopping monitoring on consecutive days.

The original sampling plan had set out 1 hour and 3-hour sampling durations. However, following total viable bacterial count results from 1 hour sampling a 3-hour sample was deemed too long, with decreased sample viability and loss of buffer solution to evaporation on the impingement methods. Therefore, the plan was amended to have a maximum 2-hour sampling duration.

Sample records

Across the sampling campaign, a total of 48 samples were collected, excluding travel blanks. This was as per the Sampling plan. A record of all samples collected is presented in Table C2 below.

Table C2 – Pilot Study Sample plan

Day	Sampler	Sampling site	Duration	Number of measurements	Number of samples
Day 1 (7/12/2022)	1.IOM sampler 2.SKC Bio Sampler 3.Coriolis® Compact 4. Sven Leckel	At source Ambient (Upwind)	1 hour	2 (at source) 1 (Ambient)	12
Day 2 (04/01/2023)	1.IOM sampler 2.SKC Bio Sampler	At source Ambient (Upwind)	1 hour	2 (at source) 1 (Ambient)	12

	3.Coriolis® Compact 4. Sven Leckel				
Day 3 (18/01/2023))	1.IOM sampler 2.SKC Bio Sampler 3.Coriolis® Compact 4. Sven Leckel	At source Ambient (Upwind)	2 hour	1 (at source) 1(Ambient)	8
Day 4 (24/01/2023)	1.IOM sampler 2.SKC Bio Sampler 3.Coriolis® Compact 4. Sven Leckel	At source Ambient (Upwind)	2 hour	1 (at source) 1(Ambient)	8
Day 5 (31/01/2023)	1.IOM sampler 2.SKC Bio Sampler 3.Coriolis® Compact 4. Sven Leckel	At source Ambient (Upwind)	2 hour	1 (at source) 1(Ambient)	8
				Total number of samples	48

Preservation and dispatch of samples for analysis

The collected samples were contained, labelled and preserved in fridge overnight and shipped next day to the UKHSA Laboratories (Porton Down) for analysis as per their requirements. The transportation matrix of the collected samples (filters, liquid and cyclone cones) are outlined in the Table C3 below.

Table C3 - Summary of sample preservation and dispatch methods

Sampler	Sampler device/collection medium	Sample post collection preservation	Post Collection shipping
Filtration	Sven Leckel sampler with Bio inlet / Gelatine filters (80mm)	Filter dissolved in 20 ml of PBS in a sealed sterile Centrifuge Tube (20ml), chilled <4oC	Sealed and chilled samples collected by within 24hrs by courier
Filtration	IOM sampler/ Polycarbonate filters (25mm)	Filter suspended in 15 ml of PBS in a sealed sterile Centrifuge Tube (15ml), chilled <4oC	Sealed and chilled samples collected by within 24hrs by courier
Impingement	SKC Biosampler / Phosphate-buffered saline (PBS)	Collection liquid (PBS) from field and rinse the vessel with PBS in a sealed sterile Centrifuge Tube (25ml), chilled <4oC	Sealed and chilled samples collected by within 24hrs by courier
Cyclone	Coriolis compact / Particles collected in cone (dry)	Collection cones rinsed with 10 ml PBS in a sealed sterile Centrifuge Tube (10ml), chilled <4oC	Sealed and chilled samples collected by within 24hrs by courier

Field sampling set-up

During field sampling, instruments were set-up in close proximity to maximise sampling representative across all four samplers (Figure 3-1 & Figure 3-2). Instruments were powered using a continuous electrical power source available on-site.

Figure 3-1 - Sampling setup upwind

Figure 3-2 - Sampling setup at source

Collection of supporting metadata

During the pilot study, temperature and humidity records were taken as well as wider meteorological conditions. These are presented in Table 3-1 below.

Sample Survey	Sampling dates	Temperature (°C)			Humidity (%)			Wind Speed (m/s)		Principal Wind direction
		Average	Max	Min	Average	Max	Min	Max	Average	
1	07/12/2022	2.7	4.4	1.5	92.9	99.2	80.2	12.8	10.47	W
2	04/01/2023	12.4	12.9	11.8	79.9	84.8	75.8	49.6	41.46	WSW
3	18/01/2023	4	6.3	-1.3	75.1	90.2	57	22.4	17.53	W
4	24/01/2023	6.3	13.7	-2.1	63.5	94	34.7	11.2	2.47	W
5	31/01/2023	11.4	14.3	8.7	62	82.7	52.2	12.8	10.47	W

Results

Sample analysis undertaken

The samples were dispatched by courier to a UKHSA laboratory. Samples collected by the four samplers, were to be tested for a range of culture based and culture independent downstream analysis options for AMR in bioaerosols surveillance strategy. However, only culture dependent analysis was carried out for both total viable bacteria counts and fungi. The concentration of total viable bacteria was calculated as Colony Forming Units per cubic metre (CFU/m3) for all four samplers.

Analysis for Colony Forming Units

Below (Figure 4-1) are our findings of viable bacterial counts, which reflects the comparative overview of bacterial counts from different sampling methods at source and ambient location during pilot study at Cranfield sewage treatment works.

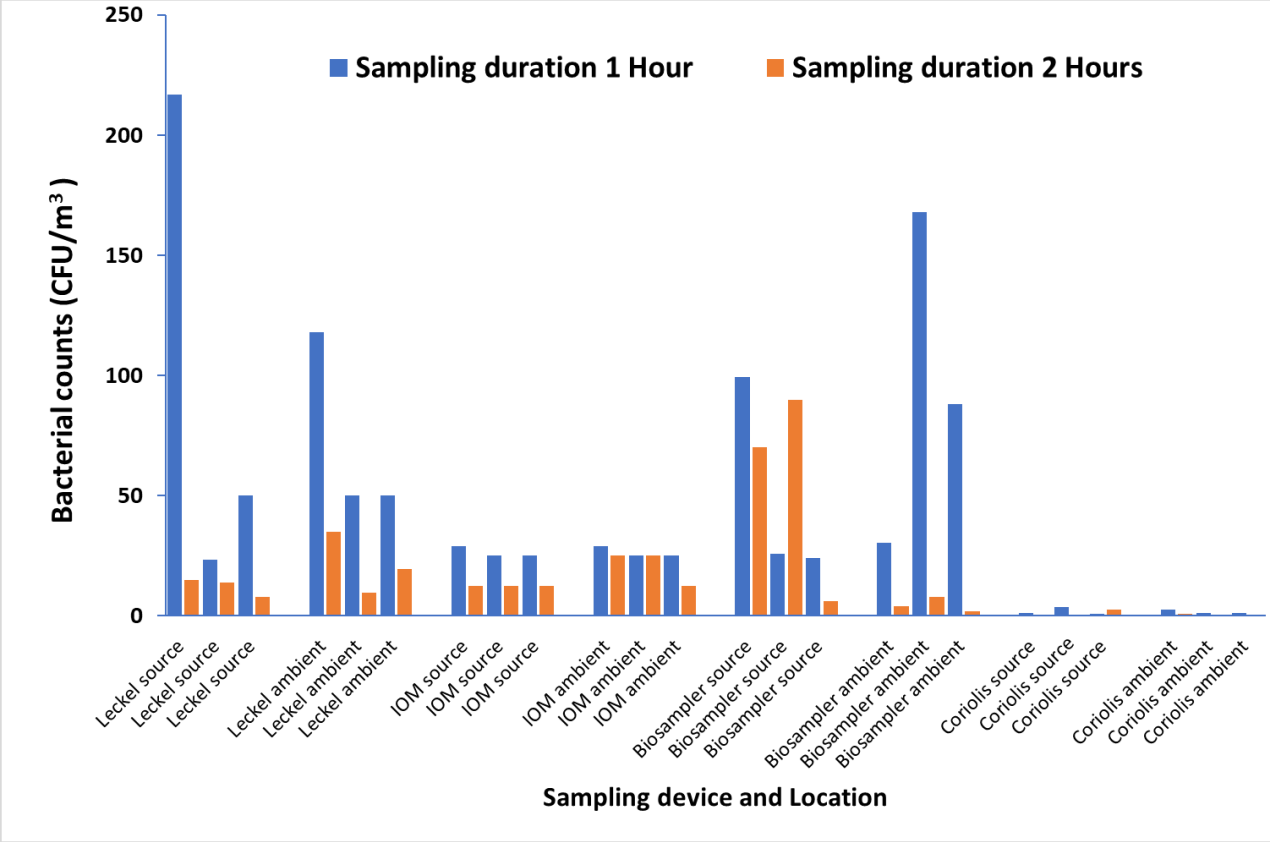

Figure 4-1 - Bacterial counts for different methods

Overall, of the samplers the Leckel using gelatine filters was found to result in the highest concentration of viable bacteria. This was then followed by the Biosampler, using impingement, then the IOM, using filtration and the Coriolis, using a cyclone collection system.

The low viable counts of the Coriolis cyclone sampler is likely due sample desiccation, and the shear sample stream force. It was estimated that this dry cyclonic collection method would be an appropriate collection method for culture independent analysis

Generally, across all four samplers, higher CFU/m3 concentrations were recorded the shorter sampling periods (1 hour sampling) in comparison to the longer (2-hour sampling) duration. This suggests that there may be loss of cell viability during longer sampling periods.

Pilot study results indicated that there was a good preservation level of culturable bacteria onto gelatine filters over the 1-hour sample duration at a sample rate of 50 l/min, though loss of viable sample becomes a risk, as sampling volumes and sampling duration increase.

The Biosampler impingement method provided a high viable CFU collection rate, (second to Leckel), however, there are likely to be challenges where samples are collected over 30 mins due to loss of sample impingement collection fluid to evaporation. However, this method could be valuable where a short source specific sampling duration is required.

It is recommended that filtration (gelatine filters) and cyclone collection methods be promoted to deploy in the sample Field Trials.

Analysis for Azole Resistance

All samples were screened for their azole resistance and were found to be susceptible to both itraconazole and voriconazole.

Practical considerations

Of the four sampling systems tested, the following practical considerations were noted.

1. IOM filter method.
    - With samples being taken at a rate of 2 l/min, the volume of sample is considerably reduced in comparison with the other samplers.
    - Extending the sampling duration leads to loss of bio efficiency due to desiccation

2. SKC Bio-Sampler

- Capable at collecting higher sample volumes due to higher sampling rate.
- Longer sampling duration leads to loss of collection solution from the sampler through evaporation.
- General fragility of sampler does not lead itself for easy repeat measurements in the field
- Requirement for a sterile sample train for each separate sample collected, prevents repeated field use of a single Biosampler without undertaking a sterilisation process.

3. Coriolis compact

- High sample volume
- Sheer forces as a result of the cyclone collection method makes sample not suitable for culture-based analysis approach
- Best for collection of genetic materials

4. Sven Leckel with Bio-inlet

- High sample volume, sample is on gelatine filter and therefore allows options for culture based and culture independent analysis
- A customised sampling train can be developed comprising, air sample pump, flow meter and filter cassette holder, resulting in simplification of the sampling system and cost savings from the method tested

Conclusions

The pilot study aimed to test a range of potential bioaerosol sampling methods allowing culture-dependent and culture-independent downstream options for characterising AMR in both source specific sites and ambient air. These sampling methods involved filtration, impingement and cyclone for long and short durations, testing collection efficiencies under varied flow rates.

The results of the pilot study of source and ambient air sampling suggest that using filtration (gelatine filters) and liquid impingement were the best for capturing sufficient amounts of viable bacteria. However, liquid impingement was subject to losses through evaporation and the impingement instruments were found to be highly fragile in the field. The results also indicated a decline in viable bacteria counts from 1 hour sampling duration to 2 hours sampling duration, highlighting the loss of viability as sampling duration increases. Based on the pilot study, filtration (gelatine filters) and cyclone were selected for field trials.

Appendix D – AMR in bioaerosols field trial sampling report

Introduction

Building upon the findings and outcome from the pilot study, the field trails were planned across different locations and environmental settings.

Objectives

The objective of the field trails was to test suitability and viability of a short-listed set of candidate sampling methods from pilot study to allow culture based and culture independent AMR focused downstream analysis The field trial aimed optimise shortlisted candidate sampling methods across multiple sampling locations and differing sample settings across England.

Sampling approach

The sampling design involved utilising Leckel (Filtration - gelatine filters) and Coriolis (cyclone – dry cone) in six locations to assess the sampling effectiveness across different sample settings. The final sample sites were all agreed and approved with the Environment Agency prior to sampling taking place.

Three measurements were made during daytime at each site using both sampling methods concurrently. The following settings were sampled:

1. Rural background
2. Urban background/ riverside
3. Rural - Composting facility
4. Rural - Beef farm
5. Urban Coastal site
6. Rural – Dairy farm

Sampling locations

Sampling was undertaken in each setting, where there was a potential bioaerosol source was present sampling was undertaken adjacent to the source. Figure 2-1 shows the locations of the candidate sites across England.

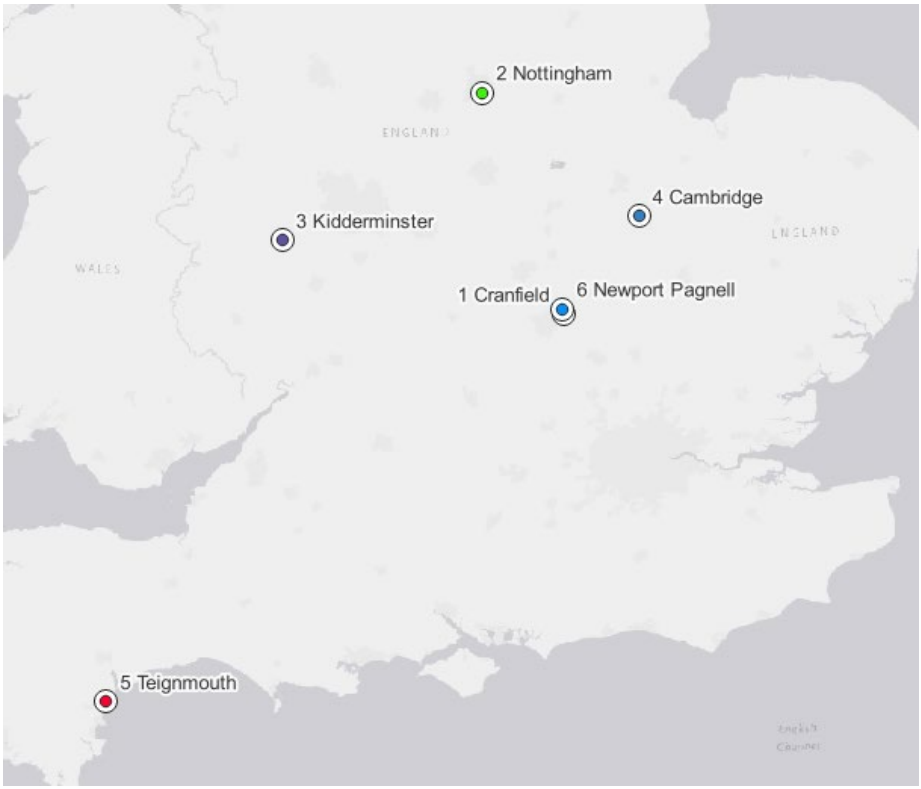

Figure 2-1 - Overview of sampling sites

Instrumentation

From the pilot study two chosen candidate sampling methods were selected, these were the Leckel with a Bio inlet (filtration method), and Coriolis Compact (dry cyclone method). Whilst impingement stood second to filtration for number of viable (culturable) bacteria counts, there are a number of logistical challenges, including issues for sampling over 30 mins due to loss of collection fluid to evaporation. Therefore filtration (gelatine filters) and cyclone were selected to use in field trials as the appropriate sampling methods which can offer culture based and culture independent AMR focused downstream analysis for bacteria and fungi. A summary of the sampling methods used is presented in Table 2-1 below.

Table 2-1 - Summary of selected sampling methods

Sampling method	Sampler	Collection medium	Flow rate (l/min)
Filtration	Sven Leckel sampler with Bio inlet.	Gelatine filters (80mm)	50
Cyclone	Coriolis® Compact (dry cyclonic air sampler)	Particles collected in a cone	50

Duration and extent

During the pilot study, higher concentrations of viable (culturable) bacteria were recorded for 1-hour sampling in comparison to 2-hour sampling duration. Therefore, a sampling duration of 1 hour was chosen in field trials.

Sampling was undertaken as dictated by weather conditions during the sampling period with an aim at sampling up to 1 hour on each round. Sampling could not be undertaken on days when there was precipitation. Three sample rounds were undertaken each day for each method.

Post collection handling and shipping

The collected samples were contained, labelled and preserved in fridge overnight and shipped next day. Filters were shipped to UKHSA, while samples from Coriolis were frozen at -20 °C for analysis at a later stage. The transportation matrix of the collected samples (filters, cyclone cones) is in Table 2.2 below.

Table 2-2 – Post collection and shipping of samples

Sampling method	Sampler device/collection medium	Post collection handling
Filtration	Sven Leckel sampler with Bio inlet / Gelatine filters (80mm)	Filter dissolved in 20 ml of PBS in a sealed sterile Centrifuge Tube (20ml)
Cyclone	Coriolis compact / Particles collected in cone (dry)	Collection cones rinsed with 10 ml PBS in a sealed sterile Centrifuge Tube (10ml)

Sample records

A total of 36 samples were collected. A record of the samples taken is presented in Table 2-3 below

Table 2-3 – Field Trial Sample plan

Date	Site Location	Site Setting	Sampling device	Sampling method	Flow rate	Sample Duration	Collection medium (On site sampling matrix)	Number of samples
23/02/2023	Cranfield University	Rural Background Site	Coriolis® Compact	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3
			Sven Leckel	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
28/02/2023	Nottingham Trentside	Urban Background/ Riverside	Coriolis® Compact	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3
			Sven Leckel	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
02/03/2023	Ramsay	Rural Composting facility	Coriolis® Compact	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3
			Sven Leckel	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
07/03/2023	Kiddermin ster	Rural Site - Beef	Coriolis® Compact	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3

			Sven Leckel	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
14/03/2023	Teignmouth	Coastal Site	Coriolis® Compact	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
			Sven Leckel	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3
21/03/2023	Newport Pagnell	Rural Site - Dairy Farm	Coriolis® Compact	Cyclone	50 l/min	1 hour	Particles collected in cone (dry)	3
			Sven Leckel	Filtration	50 l/min	1 hour	Gelatine filters (80mm)	3

Field records

At each field trial sampling site meteorological conditions such as temperature and humidity were recorded as well as wider meteorological conditions. Locations where data could not be recorded, meteorological data was extracted from local met stations. A summary of recorded conditions along with details for each site is presented below.
In addition to meteorological data, particulate matter was also recorded at four of the six sampling sites. Data was recorded using a AQMonitors DM11 for PM10 and PM2.5. A summary of results is presented in Table 2-3 below.

Table 2-4 – Summary of particulate matter concentrations recorded during the field trials.

Date	Locations	$PM_{2.5}$ (µg/m3)			PM_{10} (µg/m3)		
		Average	Max	Min	Average	Max	Min
23/02/2023	Cranfield*	-	-	-	-	-	-
28/02/2023	Nottingham	1.44	6.80	0.50	2.64	22.00	0.50

02/03/2023	Cambridge	2.70	19.4	1.3	5.43	68.7	1.5
07/03/2023	Kidderminster	0.87	9.2	0.3	2.18	34	0.4
14/03/2023	Teignmouth	1.49	10.3	0.9	2.61	39.3	1.1
21/03/2023	Newport Pagnell**	-	-	-	-	-	-
*** Mobilisation of monitoring equipment in progress so not available at the time** **Particulate monitoring data invalid							

Survey 1 – Cranfield (23/02/2023)

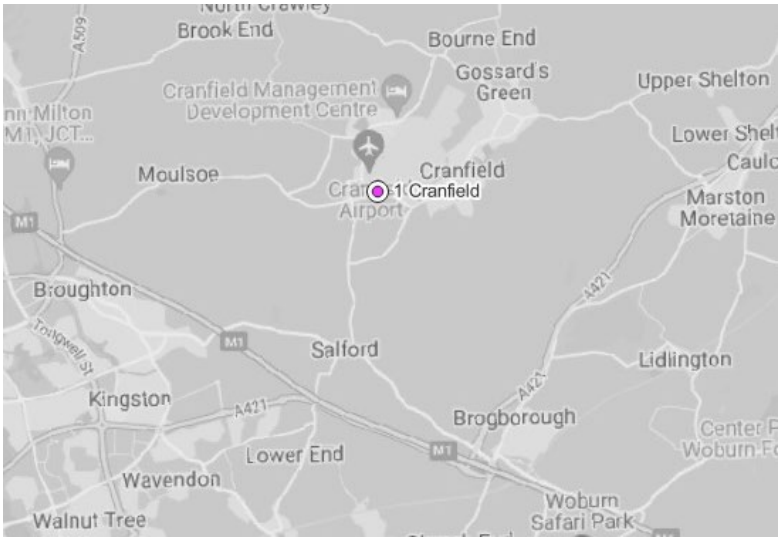

Figure 2-2 - Location of Survey 1

Figure 2-3 – Field setup at Survey location 1

Data Recorded	Leckel with Coriolis Bio-Inlet		Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 1	X	X	X	-	-

Setting: Rural background South-west corner of Cranfield University campus.

Meteorological conditions: Wind North north-easterly, Temperature: 6ºC with humidity dropping through the day from 96% to around 87%. Barometric pressure is steady at

1036mbar. Meteorological data taken from nearby meteorological stations available from (customweather.com)

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min

No Dust results as equipment not available

Survey 2 – Nottingham Trentside (28/02/2023)

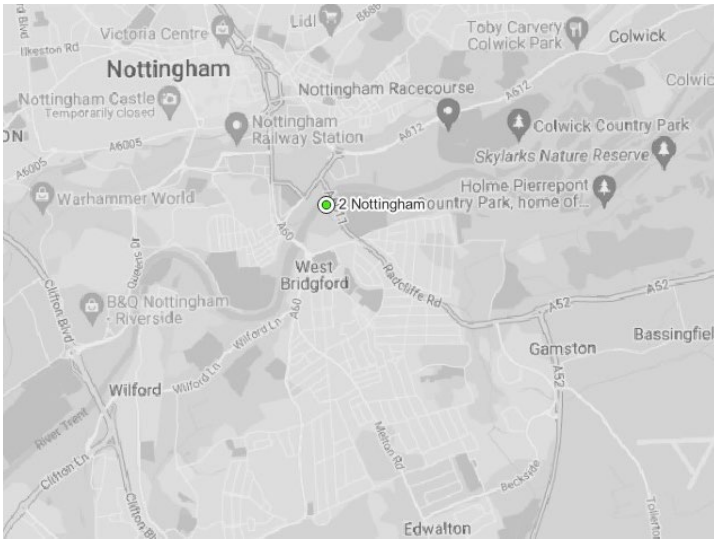

Figure 2-4 - Location of Survey 2

Figure 2-5 – Field setup at Survey location 2

Data Recorded	Leckel with Bio-Inlet	Coriolis	Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 2	X	X	X	X	X

Setting: Urban background/Riverside - Environment Agency Trentside offices in the centre of Nottingham.

Meteorological conditions: Wind North north-easterly, Temperature: 8ºC with humidity dropping through the day from 87% to around 66%. Barometric pressure is steady at 1020mbar. Meteorological data taken from nearby meteorological stations available from (customweather.com)

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min

Average PM_{10} – 2.64 μgm^{-3} (**Max** – 22.0 μgm^{-3})

Average $PM_{2.5}$ – 1.44 μgm^{-3} (**Max** – 6.8 μgm^{-3})

Survey 3 – Cambridge (02/03/2023)

Figure 2-6 - Field setup at Survey location 3

Data Recorded	Leckel with Bio-Inlet	Coriolis	Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 3	X	X	X	X	X

Setting: Rural – Composting facility. Cambridgeshire.

Meteorological conditions: Wind North north-easterly, Temperature: 7ºC with humidity dropping through the day from 98% to around 76%. Barometric pressure is dropping from 1032 to 1027mbar.

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min

Average PM_{10} – 5.43 μgm^{-3} (**Max** – 68.7 μgm^{-3})

Average $PM_{2.5}$ – 2.7 μgm^{-3}(**Max** – 19.4 μgm^{-3})

Survey 4 – Kidderminster (07/03/2023)

Figure 2-7 – Field setup at Survey location 4

Data Recorded	Leckel with Bio-Inlet	Coriolis	Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 4	X	X	X	X	X

Setting: Rural – Beef Farm. Bewdley.

Meteorological conditions: Wind west north-westerly, Temperature: 6ºC with humidity staying high at 96% to around 90%. Barometric pressure is steady 1013mbar.

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min

Average PM_{10} – 2.18 μgm^{-3} (**Max** – 34 μgm^{-3})

Average $PM_{2.5}$ – 0.87 μgm^{-3} (**Max** – 9.2 μgm^{-3})

Survey 5 – Teignmouth (14/03/2023)

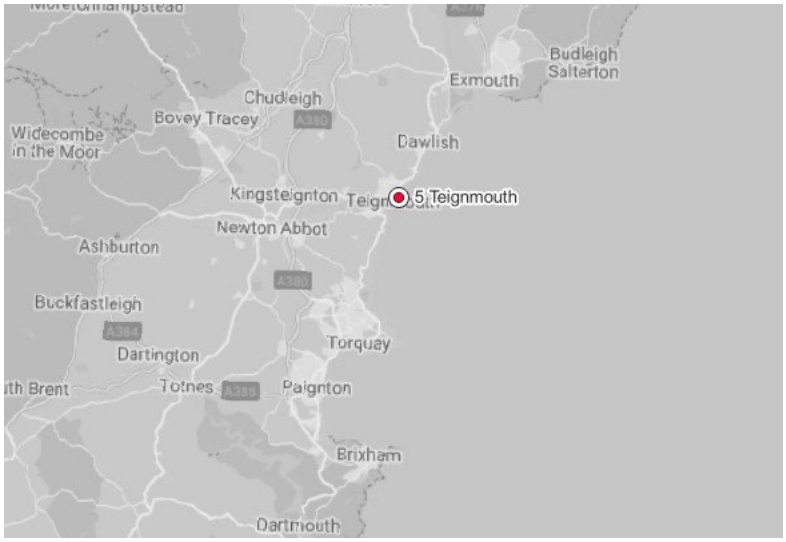

Figure 2-8 - Location of Survey 5

Figure 2-9 – Field setup at Survey location 5

Data Recorded	Leckel with Bio-Inlet	Coriolis	Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 2	X	X	X	X	X

Setting: Urban - Coastal

Meteorological conditions: Wind West north-westerly, Temperature: 10ºC with humidity averaging around 58%. Barometric pressure is steady 1011mbar.

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min.

Average PM_{10} – 2.61 μgm^{-3} (**Max** – 39.3 μgm^{-3})

Average $PM_{2.5}$ – 1.49 μgm^{-3} (**Max** – 10.3 μgm^{-3})

Survey 6 – Newport Pagnell (21/03/2023)

Figure 2-10 – Local setup at Survey location

Data Recorded	Leckel with Bio-Inlet	Coriolis	Temperature and Humidity	Particulate Matter	Wind Speed and Wind Direction
Survey Location 2	X	X	X	-	-

Setting: Rural – Dairy Farm

Meteorological conditions: Wind South south-westerly, Temperature: 6C with humidity around 94% dropping to 77%. Barometric pressure is steady 1008mbar.

Samples taken: 3 x Leckel with Bio-Inlet at 50l/min on Gelatine filters. 3 x Coriolis in dry cones at 50l/min.

No PM data due to equipment malfunction

Results

Samples were sent to UKHSA labs and no results are available at the time of publishing.

Appendix E - Costs and other Considerations for culture-dependent approaches

Indicative costs for culture-based microbiological methods are listed below according to product and laboratory service costs at the tie of writing this report. Costs within this section do not including staff time.

To target the three sentinel pathogens:

Culturing from field samples:

- **£0.34 per air sample for culturing *A. fumigatus* from field samples**. This is based on buying 500G Sabouraud Dextrose Agar at £143.00[21] + £27.20 streptomycin (400mg/L needed to suppress bacterial growth) +30.90 for 25g penicillin at 200mg/ml= £170.20, which makes approximately 15L of agar (65g per litre), enough for 500-600 90mm petri dishes, so this is enough for 500 plates. One plate is needed per air sample (assuming no technical replicates): £170.2/500 = £0.34.
- **£0.36 per sample for isolating azole-resistant *A. fumigatus***. This is based on the above costs for agar to isolate *A. fumigatus* supplemented with itraconazole (buying 200mg of itraconazole at £183.00[22], which is enough for 10,000 assays.
- **£0.57 per air sample for culturing *S. aureus* from field samples**. This is based on buying 500g Mannitol Salt agar at a cost of £84.60[23], which makes approximately 4.5L of agar (111g per litre). enough for 150 plates. 84.60/150= ~£0.57
- **£0.59 per sample for isolating MRSA isolation.** This is based on the above costs for agar for isolated *S. aureus* and supplementing with oxacillin. Buying 300mg of oxacillin at £177.00[24] is enough for 7500 air samples if using 2 mg/L per plate.
- **£0.73 per air sample for culturing *E. coli* from field samples**. This is based on buying 500g TBX agar at a cost of £312.00[25], which makes approximately 13L of agar (36.6g per litre) enough for 430 plates or air samples (assuming no technical replicates). 312/430 = ~£0.73

[21]https://www.fishersci.co.uk/shop/products/sabouraud-dextrose-agar-dehydrated/10086012
[22] https://www.sigmaaldrich.com/GB/en/product/sial/phr1834
[23]https://www.sigmaaldrich.com/GB/en/product/sial/63567?gclid=CjwKCAjw_MqgBhAGEiwAnYOAep6jzng9V18BEY1WW8DivIlzx4fL_guAl1KsMrEaE4pfXUS8_xU_4RoCKCcQAvD_BwE&gclsrc=aw.ds
[24] https://www.sigmaaldrich.com/GB/en/product/sial/phr2488
[25]https://www.sigmaaldrich.com/GB/en/product/sial/92435?gclid=Cj0KCQjw2cWgBhDYARIsALggUhpo8JtY3nQ5ExuDliEx_F9kXO6nktnxCLTrAsVrhgB7uAbqiDFIz8QaAggYEALw_wcB&gclsrc=aw.ds

- **£0.75 per sample for isolated cefotaxime-resistant *E. coli*.** This is based on the above costs for TBX agar supplemented with cefotaxime. Buying 1g of cefotaxime at £133.00[26], will be enough for 8300 assays if using 4 mg/L cefotaxime.
- **£4.98 per air sample for sterile Petri dishes.** This is based on buying500 90mm Petri dishes for £414 (£0.83 each), and using two for selective and non-selective culturing each of *A. fumigatus, S. aureus* and *E. coli* (assuming no technical replicates).0.83*6 = £4.98
- **TOTAL per air sample: £8.32** per air sample.

This total does not include the cost for quality control, which will include culturing positive and negative control strains per set of assays.

This should be multiplied by the number of technical replicates if technical replicates are desired.

For just azole-resistant A. fumigatus: £2.36 per air sample

For just MRSA: £2.82 per air sample

For just E. coli and cefotaxime-resistant E. coli: £3.14

These costs are indicative only and are likely to change as the scale of sampling increases and the availability of laboratory space, equipment and trained personnel. As well the cost of reagents/consumables for this work, other cost-incurring considerations include:

- **Staff costs:** This work requires staff with microbiological laboratory skills to produce and analyse accurate data, which the institutions conducting surveillance are better placed to estimate. This work also requires staff to accurately label and store samples and keep accurate records of samples received.
- **Speed and space**: Selective culturing for *A. fumigatus* requires approximately 48 hours of incubation time and for *S. aureus* and *E. coli* approximately 24 hours of incubation time.
- **Facilities:** Category II laminar flow hoods are required for this work, as are incubators, and facilities for sterilising equipment and reagents to effectively eliminate sources of contamination.
- **Data management and storage:** The data types produced by these culture-based assays (count of colony forming units or optical density) are not memory intensive and can be easily and cheaply stored in a spreadsheet format. However, long-term storage of isolates will incur additional charges, including -70oC freezers, associated costs of maintaining the temperature of the freezers, along with additional plasticware (cryovial tubes) and reagents (glycerol).

[26] https://www.sigmaaldrich.com/GB/en/product/sial/phr2099

- **Logistics:** These methods require processing upon receipt at the laboratory and cannot be stored in the mid- to long-term before analysis commences. This means everything (reagents, equipment, staff, space) needs to be in place and available for when samples arrive from the field.

Appendix F - Costs and other Considerations for culture-independent approaches

Metagenome sequencing cost varies depending on number of samples processed and sequencing depth but representative costs of shotgun sequencing one DNA library vary from £100 to £400 (for deep shotgun sequencing) and generate data on all known AMR endpoints in the microbial community being analysed. Additional bioinformatic analyses may include microbiome analysis summarising alpha diversity, the most abundant taxonomic groups in each specimen, beta--diversity analysis of taxonomic and functional profiles, with potential to expand into source-apportionment analysis. Commercial providers may offer good value for money, and additional services (including DNA extraction, clean up, bioinformatic analysis) are usually available for an additional fee.

The cost of DNA extraction and deep shotgun metagenomic sequencing (which would permit identification of genes present even at low abundance) is £405 per sample (£25 for DNA extraction; £380 for sequencing). If a conservative 2000 antibiotic resistance genes are identified and quantified, this works out at £0.20 per data point. However, sequencing less deeply is cheaper per sample, and would still allow a good snapshot of highly abundant genes. With DNA extraction, this may work out at £0.09 per data point. Many commercial providers provide bioinformatic analysis services,

High throughput qPCR will cost approximately £2450 for 4 samples of 384 genes. The number of samples and genes per sample can be adapted with cost per sample decreasing with decreasing number of genes included, and discounts available if larger numbers of samples are analysed. Commercial services also provide additional services, such as DNA extraction, statistical analysis, ARG panel customisation and consultation. The indicative cost options below have been provided for the period July 2023 - June 2024.

Number of bioaerosol samples[†]	Number of gene targets per sample	Total price without additional services* (price per sample/ price per data point)	Total price with additional services* (price per sample/price per data point)
4	384	£2450 (£612.50 / £1.60)	£4730 (£1182.50 / £3.08)
6	248	£2450 (408.33 / £1.64)	£4730 (£788.33 / £3.18)
18	96	£2450 (£136.11 / £1.42)	£4730 (£262.78 / £2.74)
128	12	£2450 (£19.1 4/ £1.60)	£4730 (£36.95/ £3.08)

†The ARG analysis fee is based on per-chip analysis (£2450 per chip), each chip can do 4 samples x 384 genes, with flexible configuration allowing high-throughput monitoring of up to 384 genes per sample, or as few as 12 genes over 128 samples.

*Additional services here include DNA extraction (£50 per sample), shipping (£500-600), chip customisation (£360), consultation for choosing genes (£360) and statistical analysis (from £360).

These costs are indicative only, and are likely to change as the scale of sampling increases, since discounts are available with greater numbers of samples:

- ARG analysis chip = 2450 GBP (1 free chip when you order >10 chips)
- DNA extraction = 50 GBP/sample (10-15% off if >100 samples)

As culture-independent methods do not require immediate analysis to preserve the analytic endpoint, unprocessed samples may be stored long term at -70oC to maintain the quality of DNA until ready to send for analysis later as part of a batch. Cost-incurring considerations for culture-independent analyses include:

- **Facilities**: storage of samples requires -70oC freezers, which will incur additional costs of maintaining the temperature of the freezers
- **Staff costs:** Samples will need to be stored upon receipt at the laboratory. This work requires staff to accurately label and store samples and keep accurate records of samples received. If opting to perform DNA extraction in house, additional costs will be incurred through purchasing necessary reagents and equipment.
- **Speed:** HTqPCR produces results in 10 working days. Sequence data may take 4-8 weeks to generate.
- **Data management and storage:** The results of analysis may be stored in a spreadsheet, and on a small scale should not pose an issue with storage. However, sequence data files can be large and data repositories are recommended for storing these files. Storing them for analysis later will allow direct comparisons with future sampling efforts as bioinformatic pipelines continuously update and improve.

Appendix G - Costs of Various Bioaerosol Sampling Techniques

Below are indicative costs for a variety of Bioaerosol Sampling Techniques, calculated for individual sample sites.

Labour Costs

These costs have been assembled on the assumption that an individual sampling technician is required to attend site to collect a Bioaerosol sample from a single site. This task has been predicted as requiring a full working day, including travel and undertaking sampling. Site visit preparation and post site duties will also require up to half a working day.

Indicative sampling equipment investment for equipment used in the pilot study would be:

Sampling Technique	Package considered	Indicative Equipment Purchase cost
SKC BioSampler	BioSampler, BioLite+ pump, ancillaries, 1x additional collection vessel	£1,918
IOM	IOM head and cassette, filters, personal sampling pump	£600
Leckel	Leckel Sampler (including Bio-Inlet) and gelatine Filters	£2,020
Coriolis	Coriolis sampler and ancillaries	£8,050

Ball park ancillary costs have been included, but may offer some saving through bulk purchases such as the Gelatine filters for the Leckel and disposable heads for the Coriolis.

Indicative labour costs (raw): £553

Site Visit Expenses (including travel by car and subsistence): £125

Same day courier fee: £350

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