Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms

Chief Scientist's Group report

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SC210016

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Dr Jo Nettleton
Chief Scientist

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Executive summary

The appearance and spread of antimicrobial resistant (AMR) microorganisms and their genes in the environment are a major concern. While little is known about these microorganisms within the atmosphere, recent studies report of their presence in the air covering the UK. This report aims at summarizing sampling options for airborne microorganisms including assessing their potential for containing antimicrobial resistance genes and whether the microorganisms possess the capability for transmission through the atmosphere to other parts of the environment.

The review extends previous works on antimicrobial resistant microorganisms in the atmosphere by

- Assessing the composition of the atmospheric microbiome, where AMR organisms occur.
- Determining the specification for bioaerosol samples suitable for analysis for AMR.
- Reviewing methods available for bioaerosol sampling and compare them with the sample specification.

The work was used to identify the most suitable approach for identifying antimicrobial resistant microorganisms in the UK atmosphere and finds the following:

- Airborne fungal spores and bacteria with the potential to contain antimicrobial resistant genes may be present all year round, but the highest concentrations should be expected in the summer and autumn.
- Sources of antimicrobial resistant microorganisms are expected to be mainly anthropogenic. Some sources (e.g., crop fields) will peak in summer or early autumn, while other sources (e.g., agricultural buildings or waste sites) will be linked to activities and can be more or less constant throughout the year.
- It is not known if antimicrobial resistant microorganisms have spread to the wider environment and if the atmosphere contains a non-trivial, expectedly low, concentration of these harmful microorganisms.
- There are two main analytical approaches to quantify biodiversity and antimicrobial resistant microorganisms. One approach is based on culturing and a second is based on molecular methods. Both have advantages and disadvantages, and it is recommended to use both approaches in campaigns and long-term monitoring.
- There is no superior device for the collection of antimicrobial resistant microorganisms and the type of device depends on the objectives of the study. Many available instruments have been developed for one specific purpose. The best sampling strategy is often to combine at least two types of instruments: One type that samples directly onto growing media such as a cascade impactor and a second type that uses a set of filters such as a high-volume cascade sampler. In some cases, a cost-effective solution for long term campaigns or monitoring can be the application of semi-automatic mini cyclones.

- Guidelines for storing and processing of fungal spores and bacteria have been produced based on general knowledge on fungal spores, bacteria and how to handle genetic material. It is important to apply a common set of protocols, partly to allow for robust intercomparison of studies and partly to protect the samples against loss of material during transport, storage, or handling.
- A decision tree and a set of questions that typically need addressing for developing a campaign has been produced, where the aim is the detection of airborne microorganisms, suspected to contain antimicrobial resistant genes. This is supported by two practical examples on how to develop a campaign at several locations addressing fungal spores or a single site campaign addressing both fungal spores and bacteria.
- A number of data sets as well as models are needed for further understanding and potential mitigation. Basic atmospheric models from air quality studies are already available, while more advanced models handling viability and potential transmission have not yet been developed. The most import data sets are meteorological data supported by specific vegetation variables with land cover and land use data. Activity data around anthropogenic activities such as harvesting, handling of waste sites or animal productivity may also be important.

Until now, it has not been possible to identify studies on antimicrobial resistant microorganisms covering the UK atmosphere. Consequently, it is not possible to assess the extent of the problem and whether this causes a significant risk to humans, animals, or the environment. Neither is it known if there is a trend such as increased concentrations of specific harmful microorganisms or if there is an overall increase in biodiversity of microorganisms with antimicrobial resistant genes.

1. Introduction

Antimicrobials are compounds that are used to treat microorganisms, for example antibiotic use to treat bacterial infections, or antifungal pesticides to treat fungal infection in crop fields (Jørgensen and Heick, 2021). In agriculture the most widely used fungicides are the antimicrobials belonging to the group of azoles with a market share of 20%-25%, this group is of particular interest as it is the only antifungal class used in both agriculture and healthcare. Microorganisms exposed to azoles and other antimicrobials can become resistant to these compounds leading to the ability to survive and thrive in their presence, termed antimicrobial resistance (AMR).

AMR bacteria and fungi are of particular interest to the Environment Agency as it is thought that resistant organisms could disrupt the natural metabolic processes in ecosystems, as well as being a potential route of exposure to AMR microorganisms causing difficult-to-treat disease in humans, plants (including crops), and animals (United Nations, 2022). It is therefore important to obtain knowledge about key sources of AMR and factors that are driving these sources, and if possible and deemed necessary look into measures to mitigate the occurrence and impact of environmental AMR.

Very little is known about AMR in the atmospheric microbiome (Jones, 2020). A review on AMR in the environment from 2016 (Singer et al., 2016) had a specific section on air transmission. This review suggests that air transmission of AMR is very likely, and that there is robust evidence of AMR in potential sources of airborne microorganisms. These sources include agricultural soil, slurry from animals, and waste sites (Singer et al., 2016). The review also identified studies on increased prevalence of AMR caused by airborne spores of the fungus *Aspergillus fumigatus,* which is associated with decomposition processes such as those which occur at biowaste sites, (Verweij et al., 2009; Snelders et al., 2008) but could only identify one relevant study on atmospheric transmission of AMR, here a range of bacteria originating from cattle feed areas (McEachran et al., 2015). A summary on Environmental Dimensions of Antimicrobial Resistance, published by the United Nations (United Nations, 2022), lists multiple possible sources for AMR organisms and pathways for their transport in the environment. The summary lists eight areas with major knowledge gaps; most of these involve the spread and transmission of AMR in the environment. Importantly, the summary clearly recommends monitoring of AMR in surface water, waste, and airborne particulate matter (United Nations, 2022), which in the case of air will require systematic monitoring of bioaerosols. Consequently, due to the lack of knowledge, designing a strategy for how to conduct research and monitoring for AMR in the air most efficiently will have to rely on existing knowledge of bioaerosols in general, particularly airborne fungal spores and airborne bacteria.

In this review we

- Consider the composition of the atmospheric microbiome within which AMR organisms occur and must be detected.
- Determine the specification for bioaerosol samples suitable for analysis for AMR.

- Review methods available for bioaerosol sampling and compare them with the sample specification.

2. Bioaerosols in the environment and their sources

Bioaerosols are a subset of the atmospheric aerosol, estimated to represent about 25% of the total aerosol mass on global scale (Després et al., 2012), although huge geographical variations in overall mass and concentrations exist. Bioaerosols are a heterogeneous group of particles (Jones and Harrison, 2004) consisting of pollen (Crouzy et al., 2016; O Connor et al., 2014; Oteros et al., 2015), fungal spores (Brown and Hovmøller, 2002; Sesartic and Dallafior, 2011), bacteria (Burrows et al., 2009), viruses (Arzt et al., 2011; Stenfeldt et al., 2016), lichen/ microalgae (Tesson et al., 2016; Moffett et al., 2015), fragments of plants, animal material or other biological components (Després et al., 2012). The definition excludes large biological components with high settling velocity, and non-passive components such as insects (Després et al., 2012). One definition can be solid airborne particles derived from biological organisms, including microorganisms and fragments of biological materials such as plant debris and animal dander (Després et al., 2012). Primary biological aerosols (PBA), or bioaerosols, are directly released into the atmosphere from both aquatic (Wilson et al., 2015; DeMott et al., 2016) and terrestrial ecosystems (Sadyś et al., 2014). Anthropogenic activities such as farming (Apangu et al., 2020; Skjøth et al., 2012), composting (Robertson et al., 2019), and other biowaste treatment processes (Xu et al., 2020) are known to release large quantities of bioaerosols. Sources not directly impacted by anthropogenic activities also release bioaerosols, sometimes in large quantities. These include water courses, woodlands, and natural grass lands. Such sources are often directly impacted by meteorological conditions, particularly wind, which influence release and subsequent dispersal of bioaerosols (Grewling et al., 2019). Some sources are heavily impacted by regular, nearly circadian rhythms, releasing spores either at night, in the early morning or midday (Oneto et al., 2020). It should be noted that some sources related to anthropogenic activities will also respond to meteorological conditions. A good example is croplands. They will often release bioaerosols through most of the year and certainly outside the main harvesting period. A consequence is that comprehensive assessment of the abundance and biodiversity of bioaerosols within their main season require 24h monitoring, particularly if the local source(s) releases a larger number of different bioaerosols over time. Anthropogenic activities can affect emission rates significantly, and so sampling should take place during all activities at a suspected source site. Furthermore, as a result of long-distance transport, bioaerosols may be detected outside their ordinary season locally and far away from their origin (Rousseau et al., 2003; Brown and Hovmøller, 2002). A consequence is that early warning systems, aimed at detecting harmful bioaerosols in the ambient atmosphere at a regional scale, should be active throughout most of the season and involve careful consideration of spatial design.

2.1. Concentrations of fungal spores and bacteria in the atmosphere, amount and seasonality focusing on the UK

Seasons of fungal spores and bacteria vary significantly from location to location. A European-wide study on the fungus *Alternaria* (Skjøth et al., 2016) showed that the annual

variations at one location are much smaller than the variations between locations, probably driven by the overall number of sources in the environment. A new systematic review (Anees-Hill et al., 2021) showed that for most of Europe the concentrations of the majority of fungal spores in the atmosphere peak during summertime (normally June—August). The exception is warm areas in Southern Europe where there can be decreased concentrations in the middle of the summer (Anees-Hill et al., 2021; Skjøth et al., 2016), probably driven by very high temperatures. In the UK it was found that the peak concentrations of *Alternaria spp*. were in July and August (Skjøth et al., 2016) and a recent study using metabarcoding showed that the appearance of a larger number of crop pathogens usually associated with specific hosts were detected in the air coinciding with the time of the harvesting of these specific crops (Hanson et al., 2022). Furthermore, it has been found that in some regions a few fungal species such as *Aspergillus/ Penicillium* can have a more uniform distribution throughout the year (Sadyś et al., 2016a) in contrast to spores often associated with agricultural activities, such as *Alternaria* (Skjøth et al., 2012).

The systematic review by Anees-Hill et al., 2021 found that most seasonal investigations were made using volumetric traps of the Hirst design (Hirst, 1952) and optical microscopy for detection and numeration of the fungal spore concentrations (Grinn-Gofroń et al., 2019a; Martínez-Bracero et al., 2022). Only a few studies used either culturing or molecular methods for detection, e.g., by using the Illumina® MiSeq platform (Núñez et al., 2021). Furthermore, the majority of European studies focus on the detection of a single fungal species, e.g., *Alternaria* (Fernández-Rodríguez et al., 2015; Rodriguez-Rajo et al., 2005; Escuredo et al., 2011) and/or *Cladosporium* (Grinn-Gofroń et al., 2016a; Sadys et al., 2015), while a few studies aim to identify a much larger number of taxa with the purpose of creating a fungal spore calendar (Sousa et al., 2016; Sadyś et al., 2016a; Martínez-Bracero et al., 2022). Generally, the highest spore concentrations are found to be *Cladosporium spp.*, which in extreme cases have daily concentrations exceeding 100,000 spores/m^3 (Grinn-Gofroń et al., 2016a), while monthly mean concentrations are much lower as illustrated in the spore calendar for Worcester (Fig 1). These concentrations and the associated seasonality are expected to be typical for large areas of England because the other operational site for fungal spores shows similar concentrations for investigated fungal spores (Apangu et al., 2020). Areas with a very different type of land use, landcover and vegetation compared to most of England such as parts of Wales or Scotland can be expected to have very different spore concentrations. It is suspected that long-term changes in land use within the regions of Cardiff and Derby is responsible for either halving or doubling the observed concentrations of *Alternaria* (Corden et al., 2003). Similarly, it must be expected that Southern England is more exposed to import of fungal spores from especially France, similar to what has been seen for investigated fungal spores (Sadyś et al., 2014) or allergenic pollen (Skjøth et al., 2009).

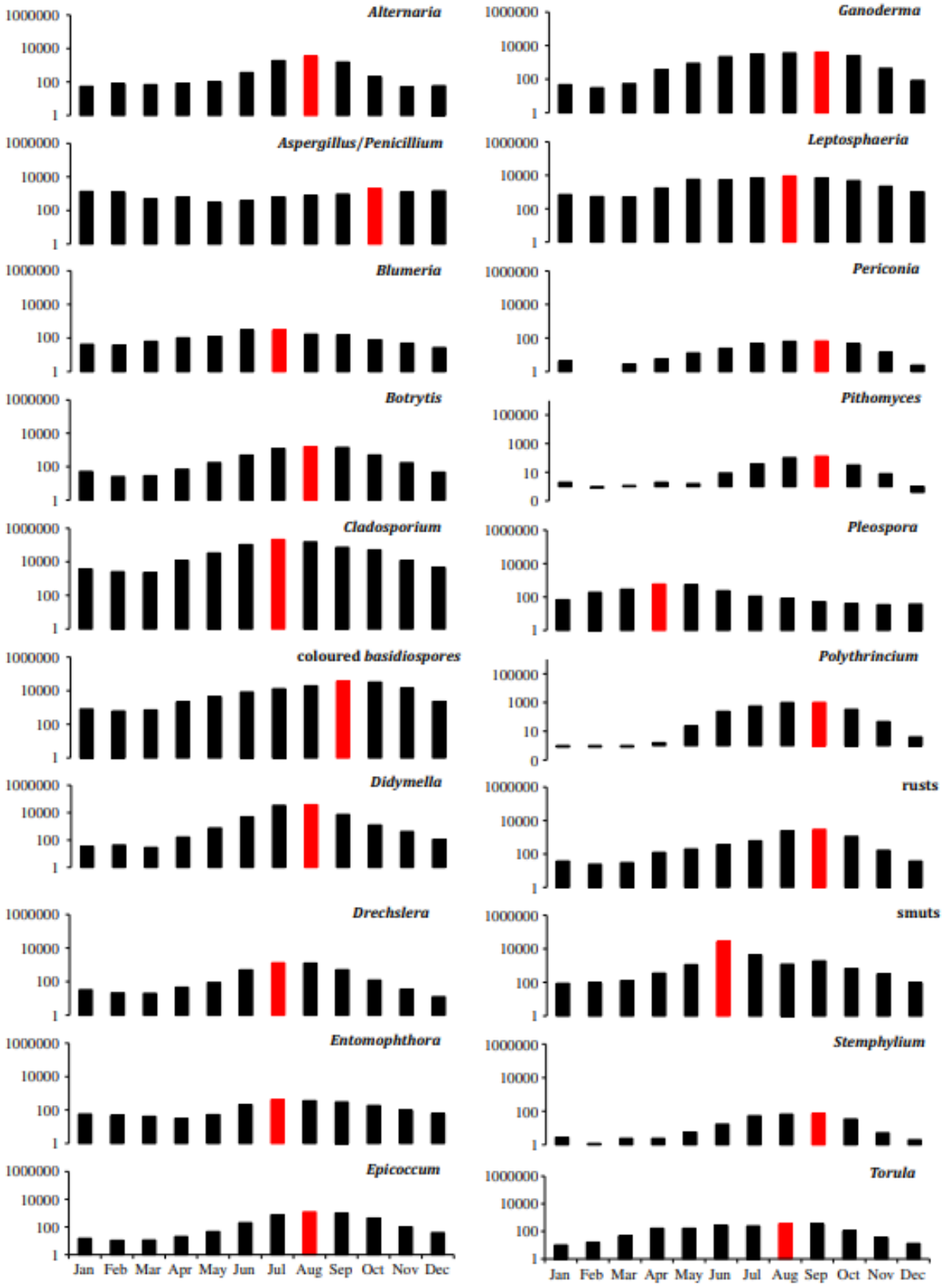

Figure 1. 5-year mean distribution of examined fungal spore types presented on a logarithmic scale. Peak months for each taxon are highlighted in red (Sadyś et al., 2016b).

Commonly for all the studies using optical microscopy there does not appear to be either a lower or an upper limit of how many spores are identified and the studies do not include a group of unidentified spores, thereby illustrating that these studies involve a subset of all available spores (Fernández-Rodríguez et al., 2018a). A comparison between the real-time laser instrument WIBS (Wideband Integrated Bioaerosol Sensor) and manual counts using optical microscopes (Fernández-Rodríguez et al., 2018a) showed that the WIBS provided a spore concentration that is typically twice the concentration obtained with the optical approach (Fernández-Rodríguez et al., 2018a) and that most of this higher concentration is found in the spore fraction at the size of *Cladosporium* (6-22 x 3-8 µm) or below. The reason for this difference may be caused by limitations with the optical microscope as the counting approach may impact overall concentrations (Apangu et al., 2020). More likely, the impaction approach, used by the Hirst sampler for collection of fungal spores, will have decreased sampling efficiency for small spores compared to large spores, but experimental verification for most common spores remains the be produced. This suggests that the current upper limit of 100,000 spores/m^3 is an underestimation and could be much higher. There is very little information about seasonality of fungal spores based on molecular methods that can analyse spores to the species level (Hanson et al., 2022). A previous study from Leicester analysed one season and separated the spores into dry and wet days and used an optical microscope to quantify the main season (Pashley et al., 2012). Results from this study found that the period with high concentrations are in the three summer months June, July, and August. This study did not make any detailed assessment of individual species. A study covering five sites in Northern and central Italy for one year (Banchi et al., 2020) using Hirst traps and molecular approaches. It found that the three species *Alternaria, Cladosporium,* and *Epoiccocom* typically accounted for 40%-60% of all fungal spores during spring, summer, and autumn, but did not make any further detailed assessment in the genera. They found that the seasonality did not vary that much between the 5 sites in Italy. A related study, also using a Burkard trap and molecular approaches, covering Rothamsted in the UK, Slagelse in Denmark, and Waageningen in the Netherlands found a similar seasonality at all three locations (Nicolaisen et al., 2017), suggestion that seasonality is a large-scale phenomenon relating to prevailing climatic conditions. As with the Italian study they also focused on genera. A more detailed study also from the UK Midlands, confirmed *Alternaria* and *Cladosporium* as dominant genera. This study used cyclones to capture the fungal spores, covered two types of land cover, three spore seasons combined with atmospheric modelling, harvesting data and remote sensing (Hanson et al., 2022). This study also assessed individual species and found that the seasonality of many crop pathogens belonging to the most frequently found genera was very short and that this was related to when the hosts of the species were being harvested (Hanson et al., 2022). This study also assessed the interannual variability and found marked differences in the seasonality between different years. It concluded that some crop pathogens could almost disappear during specific years, depending on the environmental conditions, while they could flourish in the following year.

The atmospheric microbiome is generally the least explored microbiome. The overall understanding appears to be highest for pollen, followed by fungal spores, supported by a well-established monitoring network (Buters et al., 2018). A recent review on bacteria found that many factors, including weather impacts, are not understood with respect to the

bacterial community within the air environment. The review further highlighted that the natural (outdoor) environment is much less studied compared with indoor environments (Ruiz-Gil et al., 2020). Bacteria are emitted from a wide range of sources such as among others coastal and marine environments, urban zones (Gandolfi et al., 2013), including compositing facilities (Robertson et al., 2019), forests, agricultural land (Zweifel et al., 2012), or deserts (Stern et al., 2021; Maki et al., 2015, 2014).

Atmospheric concentrations of bacteria usually outnumber the concentrations of fungal spores by several orders of magnitude, involving a very high biodiversity (Tanaka et al., 2019). Bacterial microorganisms can either be attached to larger particles or be found in clusters as the majority of particle mass associated with bacteria is often found in particles fractions larger than 2.5 µm (Bowers et al., 2013; Stern et al., 2021). However, there are also reports of concentrations of bacteria exceeding 10^4 cell/m^3 (Wei et al., 2019) and 10^7 cell/m^3 (Michaud et al., 2018). The fraction existing as isolated cells has a very long residence time in the atmosphere enabling them to penetrate the atmospheric boundary layer (Zweifel et al., 2012) and undergo long distance transport (Stern et al., 2021; Murata and Zhang, 2014) regularly reaching remote regions such as the Arctic region (Cuthbertson et al., 2017). A recent review has identified the following five groups as the most prominent bacteria groups: *Pseudomonadales, Burkholderiales, Rhizobiales, Rhodospirillales,* and *Sphingomonadales* (Ruiz-Gil et al., 2020). *Bacillales, Lactobacillales, Corynebacteriales, Micrococcales,* and *Bacteroidetes* are also frequently found within the air environment, but there will be large variations between sampling areas (Ruiz-Gil et al., 2020), a common phenomenon for most bioaerosols and typically related to the local source distribution (Innocente et al., 2017) and the prevailing meteorological conditions. As an example, the hazy conditions in China had a significant impact on observed bacteria concentrations (Dong et al., 2016) and more than 1000 CFU/m^3 have been detected in the respiratory range in China (Li et al., 2017). However polluted air may negatively impact the fraction of viable bacteria (Gao et al., 2015), which in the region of Beijing have been shown to reach 3000-4000 CFU/m^3. Polluted air is frequently found in urban areas, but large-scale air pollution episodes are also frequently observed in the UK (Vieno et al., 2016). Such episodes transport a range of different bioaerosols northwards from remote regions (Skjøth et al., 2021; Grewling et al., 2019) and may both import AMR from these remote regions and impact viability of bioaerosols already present in the UK both in the rural and urban zones.

Sources of airborne bacteria can be separated into natural and anthropogenic sources. Natural sources can be dust from deserts and other arid regions, volcanic ash, leaf surfaces, pollen, soil, and sea spray (Ruiz-Gil et al., 2020). Generally, very little is known about most of these natural sources, where dust episodes from desert regions seem to be among the most commonly studied phenomena, while studies that relate atmospheric bacteria with vegetation are very limited, contrasted with a larger amount of studies investigation sea spray (Ruiz-Gil et al., 2020). There is a distinct difference between rural and urban zones, where the distribution of bacteria often reflects local activities, such as handling of animal faeces from husbandry (Bowers et al., 2011) or wastewater treatment (Genitsaris et al., 2017; Wéry, 2014) and estimations of bacterial fluxes in the atmosphere are significantly lower in naturally undisturbed areas compared to managed agricultural systems (Després et al., 2012). The seasonal variation of bacteria varies from place to place. A study in

Thessaloniki, Greece found highest concentrations during summer but overall, no statistically significant difference throughout the seasons (Genitsaris et al., 2017), probably because the atmospheric concentrations of many bioaerosols are often reflected by the nearby sources and their release pattern, which in this case are anthropogenic with similar activities and release patterns throughout the year. Individual sources that are impacted by anthropogenic activities such as waste sites, bare agricultural soil and crop areas will usually have vastly increased emissions of bacteria when they are managed (e.g., harvesting of crops, ploughing, managing the soil during sowing, turning of waste), while release from natural sources will depend on both abundance of the bacteria and the mechanical release from wind (e.g., breaking of waves or passing of storms). Importantly, the optimal growing conditions for bacteria will vary between species. This means that their concentrations of individual species can be expected to vary throughout the seasons similar to what has been observed for airborne pollen and fungal spores (Figure 1). Calendars are produced for some pollen and fungal spores, illustrating their seasonality, but a similar tool is not available for atmospheric bacteria. A recent review summarizing both the seasonality of bacteria and their atmospheric dependencies illustrates the large knowledge gaps by making a crude assessment of the seasonality of bacteria at the Phylum level, grouped into Proteobacteria (winter, spring, summer, autumn), Firmicutes (winter, spring, summer, autumn), *Deinococcus-Thermus* (spring), Bacteriocutes (winter, summer, autumn), and Actinobacteria (winter, summer, and autumn). The same review identifies only three studies exploring AMR in atmospheric bacteria (Ruiz-Gil et al., 2020). One of these three studies involved the collection of filters from air conditioning units from cars in a global survey involving 19 large cities and found a strong and statistical linear correlation between local drug consumption and the presence of AMR in airborne bacteria (Li et al., 2018).

2.2 Aerodynamic properties of bioaerosols

Bioaerosols consist of cells, reproductive units or fragments of plants and other organisms (Jones and Harrison, 2004), a fraction of which are viable microorganisms, including bacteria, viruses, and fungi. Their sizes range from a few nanometres (virus) up to about 100 µm. This means they span the entire range of the atmospheric aerosol (Pöschl and Shiraiwa, 2015) as illustrated by Figure 2. The upper limit is restricted by gravitational settling, while the lower limit is defined by the size of molecular clusters or macromolecules (Pöschl and Shiraiwa, 2015). Of particular interest in this report are bacteria (typically 100nm – 4 µm) and fungal spores (typically 2 µm – 20 µm, but some spores are much larger).

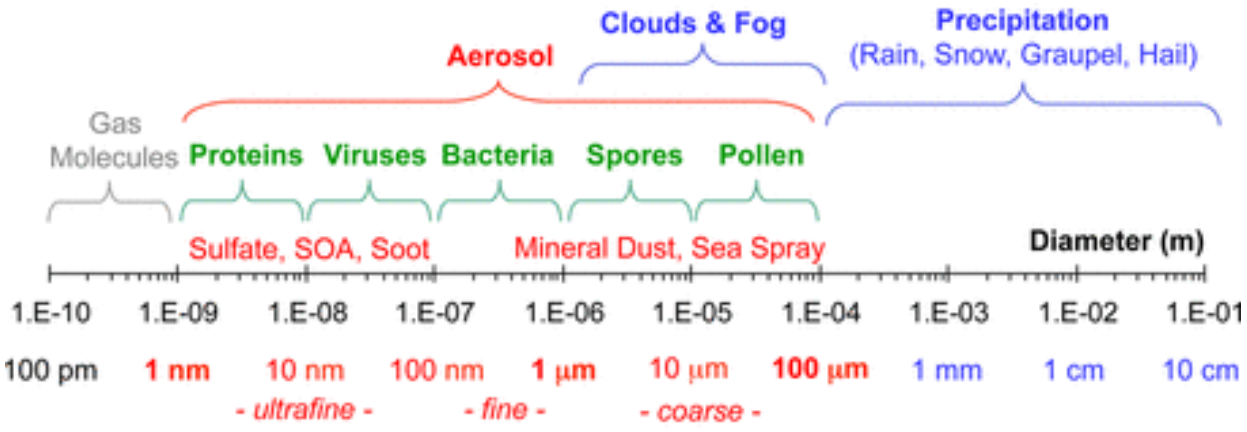

Figure 2. Size ranges of different biological particles in the atmosphere (Fröhlich-Nowoisky et al., 2016).

Fungal spores can be either near-spherical, elongated or club-shaped and the shape, the size along with the density affect the settling speed and hence their lifetime within in the atmosphere. Bacteria tend to be coccus (near-spherical), bacillus (rod shaped), or spiral (filament or spiral like), whilst some bacteria have been reported to have densities of 1.1 g/cm^3 -1.3 g/cm^3 (Tamir and Gilvarg, 1966), which is heavier than water, but lighter than mineral aerosols. A general overview of the density of airborne bacteria and fungal spores is missing and unclear. There are reports of densities of ~1.2 g/cm^3 for *Lycopodium* (McCartney et al., 1993), which is a classical bioaerosol used for testing sampling instruments. Settling speeds for spores have been reported to vary by more than a factor of 10 (McCartney et al., 1993), but importantly observations of the settling speed for individual species (e.g., *Alternaria* ranging from 0.3 cm/sec-0.55 cm/sec) suggest that the spores may have a density either below or above 1g/cm^3, with an overall assumption that 1 g/cm^3 is suitable for generalised calculations (McCartney et al., 1993). This physical behaviour is supported with reports of floating spores found in liquid sampling media suggesting that some spores have a density below 1 g/cm^3. Nevertheless, the reported sizes and settling speeds ensure that they are all in the range for efficient dispersion in the atmosphere, while their densities may be important for some sampling methods. Many bioaerosols, such as pollen (Tang et al., 2019), fungal spores (Reponen et al., 1996) and bacteria (Lazaridis, 2019), have been reported to have hygroscopic properties where the water uptake varies between species and with environmental conditions (Reponen et al., 1996; Tang et al., 2019; Katifori et al., 2010). A consequence is that settling speeds and densities of many bioaerosols will vary depending on the environmental conditions which again impacts physical properties such as their gravitational settling speeds in the atmosphere, likelihood of inertial impaction or their ability to float on water surfaces including liquid sampling media. Bacteria and fungal spores have the potential for long distance transport if transported further up in the atmosphere and captured by the air currents (Mayol et al., 2017), mainly caused by their limited settling speed. However, it should be noted that some bioaerosols can settle onto larger particles (Stern et al., 2021) or are released as agglomerates, where these larger particles or agglomerates will have much higher settling speeds than individual particles and therefore a limited tendency for long distance transport.

2.3 Biological and physical properties of bioaerosols

Bioaerosols are known to have an important impact on climate, ecosystems, and human health (Fröhlich-Nowoisky et al., 2016). One of the most highly studied microorganisms is the group of bacteria belonging to the genus *Pseudomonas*. Most species in the group of *Pseudomonas* have an outer membrane of surface proteins (Hulin et al., 2018) enabling them to act as ice nuclei (IN) or cloud condensation nuclei (CCN) in the atmosphere (Šantl-Temkiv et al., 2015; Konstantinidis, 2014). This process is known to affect viability, although it is unclear (de Araujo et al., 2019) whether bacteria need to be viable in order to affect CCN (Konstantinidis, 2014). IN/CCN are very important processes for meteorology and climate (Tanarhte et al., 2019; Cziczo et al., 2013). Other bacteria also have these properties, but *Pseudomonas* is currently considered the most important genus. Despite this there are no mechanistic descriptions in climate models, partly driven by lack of observational data. This lack of observational data at the species level is widespread for all bacteria, which is contrasted by their impact in other areas. As an example, the *Pseudomonas* group are important plant pathogens worldwide (Xin et al., 2018) considered by the UK Agricultural and Horticultural Development Board to be among key airborne bacterial pathogens along with *Xanthomonas spp.* and *Pectobacterium*. Damage to UK agriculture caused by bacterial pathogens is substantial and, for example, for bulb onions have been estimated in the range £3.5m to £15.1m or more than 10% of the economic value of the harvest (Roberts and Elphinstone, 2017). Similar numbers are found for other key crops such as potatoes and stone fruit. Some fungal spores belonging to the genus *Aspergillus, Penicillium, Byssochlamys,* and *Fusarium* are known to produce mycotoxins such as aflatoxins and fumonisins. They are known to be present in crop fields such as cereals and can be accumulated in production facilities that handle the crops (Abdel Hameed et al., 2012). Fungal spores are also known, under certain conditions (Oneto et al., 2020), to maintain their viability during atmospheric transport (Chamecki et al., 2012; Isard et al., 2007). Good examples are rusts known to impact wheat or legumes and often they can successfully settle if temperatures are high combined with leaf wetness over a period of time, e.g., caused by rain events (Isard et al., 2007). There is hardly any knowledge on airborne bacteria and fungal spores with AMR properties and their transmission (Singer et al., 2016), but it is reasonable to assume that bacteria and fungal spores with AMR properties have identical physical and respond in a similar way to the environment as those bacteria and fungal spores without AMR.

3. Analytical methods to quantify AMR in bioaerosols

The analytical methods in relation to bioaerosols sampling can be split into two groups: a) methods that rely on sampling and growing microorganisms, then identifying and enumerating, such as the number of colony-forming units and b) molecular methods that extract DNA, proteins or other biological material and assess their properties, abundance, or diversity, such as next generation sequencing (e.g., metagenomics or metabarcoding) or quantitative Polymerase Chain Reaction (qPCR). A recent review on methods to assess bioaerosols from waste sites in relation to human health concludes that methods such as culturing, qPCR, and next generation sequencing all have major limitations (Franchitti et al., 2020). This review finds that the use of molecular methods provides a much better characterisation of the bioaerosols than the culture-based approaches and also that culture dependent methods are essential for fungi (Franchitti et al., 2020). This suggest that both method approaches are needed for a comprehensive analysis of airborne fungal spores and bacteria. While in principle, the conclusion can also cover AMR, then the practical application is not straightforward as in-depth gene data bases do not cover both bacteria and fungi. This means that actual sampling of AMR in the air, combined with extension of existing gene databases is needed to verify this. Furthermore, it is important to note that there can be a difference in the focus, when comparing regulation of waste sites and detecting AMR. In regulation, abundance of a specific microorganism is often important. For very harmful species, including AMR, then presence/absence can have a higher priority. Such difference in objectives can determine the choice of the applied techniques and over time their relative importance can change.

With respect to fungal AMR the molecular information is scarce, whilst there are a number of fungal gene databases, they mainly cover ITS1 and ITS2 regions (internal transcribed spacer) used to assign taxonomy, such as GlobalFungi, the PROTAX (PRObabilistic TAXonomic placement) databases used by the Global Spore Sampling Project (Ovaskainen et al., 2020). The challenge with these databases is they are using conserved regions in the DNA that are suitable for species identification, but it is highly unlikely that these regions contain genetic information about AMR. The only database that focuses on AMR genes in fungi is MARDy (Mycology Antifungal Resistance Database) (Nash et al., 2018), although the initial version is far from complete suggesting that more research is needed to make such databases mature in relation to AMR and antifungal genes, particularly of environmental origin. The current understanding is that horizontal gene transfer in fungi is very rare (Fitzpatrick, 2012). Because of these factors when measuring AMR in fungi it is important to focus on culture-based techniques, as molecular techniques (for now) could underestimate the levels and diversity of AMR fungi. Conversely, AMR in bacteria is complicated by their ability to horizontally transfer genes by mobile genetic elements (Schlüter et al., 2007; Lopatkin et al., 2017), however due to most AMR research being focused on antibacterial resistance there are large number of AMR focused genetic databases detailing AMR genes, and which classes of compounds they are likely to provide resistance to, such as ResFinder (Bortolaia et al., 2020), CARD (Comprehensive Antibiotic

Resistance Database) (McArthur et al., 2013; Alcock et al., 2020), and ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) (Gupta et al., 2014). Because of these factors both culturable bacteria and eDNA could be useful depending on the question being asked. A comprehensive analysis on bacteria and AMR will most likely require both methods.

3.1. Culturing bioaerosols, including antimicrobial resistance (AMR)

AMR can be tested for phenotype by growing cultures of microorganisms with antimicrobial agents. Culturing of bioaerosols, including those for AMR testing, usually involves collection of the bioaerosols directly on a culture medium (Urbano et al., 2011) or collection of the bioaerosol in other media, such as a liquid (Angen et al., 2021) or onto a filter (Hwang et al., 2016; Möritz et al., 2001), and then transfer to a growth medium, where it should be noted environmental conditions such as humidity will impact both the capturability and viability of microorganisms during the sampling with filters. Depending on the sample collection approach both fungal spores and bacteria can be enumerated as either colony forming units (CFU) or colony forming units per m^3 (Galán et al., 2017). Only a fraction of the viable microorganisms is culturable (King and McFarland, 2012), which means that the number of culturable fungal spores/ bacteria represents only a subset of all viable fungal spores/ bacteria (Galán et al., 2017). Furthermore, the sampling approach impacts the fraction of microorganisms that can be cultured (Hubad and Lapanje, 2013). This means that the reported number of culturable fungal spores/ bacteria is only a fraction of the culturable microorganisms that are present at the time of sampling and each design of either an isolated study or a monitoring network should take this into account within the planning and, ideally, estimate the fraction of sampled viable/culturable microorganisms that have not been successfully grown.

Whilst cultures can be grown on many media the most cost-effective approach is considered to be culturing in circular petri dishes using a nutrient rich medium. Often these are commercial products such as malt extract agar as recommended by the American Conference of Governmental Industrial Hygienists (Wu et al., 2000) or dichloran-glycerol agar (Viegas et al., 2020). Malt extract agar is used for growing a broad spectrum of fungi (Wu et al., 2000) and has been found to outperform dichloran-glycerol agar (Viegas et al., 2020) for *Aspergillus* spp., but other media may also be used, depending on the application. A mini review found that Sabouraud dextrose agar generally produced the best results for growing airborne fungi and that the application of bacterial antibiotics into the growth media to avoid bacterial growth was effective (Black, 2020). This investigation did not find significant improvements in growth by adding growth enhancing factors such as mineral supplements and therefore recommended the use of Sabouraud dextrose agar with or without antibiotics. Similarly, it has been shown that adding fungicides can prohibit fungal growth when the target is bacteria (Murinda et al., 2006). Importantly it was shown that some of these anti-fungal agents were not effective against fungi with AMR properties (Murinda et al., 2006). This suggests that a combination of antibacterial and antifungal agents in the growth media can be used to promote the growth of those bioaerosols with AMR properties, whilst supressing those without. The choice of agents used to suppress growth however clearly depends on the application and the species under investigation. The large variety of culturable bioaerosols has a wide range of optimal growing conditions. As an example, some

species thrive with culture temperatures from 5-55°C, whilst others need temperatures above 50°C (Ghosh et al., 2015). As a consequence, the optimal growing media and growing conditions may be species specific, while a wider group of species will grow more or less abundantly. Furthermore, as the growing media will favour growth of all viable microbes this will in some cases cause problems. As an example: if the collection is carried out in an environment with a very large number of colony-forming fungal spores and limited numbers of colony forming bacteria, then the abundance of the fungal colonies can cause problems in the counting of the colony-forming bacteria. Similarly, when colonies overlap it becomes very difficult to enumerate the number of colonies (Chang et al., 1995). One solution could be to reduce sampling time in order to balance the expected number of colonies as this number ultimately depend on atmospheric concentration, air flow rate of the sampler, collection time and the sample surface area. An alternative is to sample into a liquid and then dilute the liquid appropriately in order to get a suitable number of colony-forming units. However, both reducing sampling time or dilution of a liquid has the problem that if the target species is much less abundant compared to other colony forming species, then this approach reduces the likelihood of sampling the target species. A better approach is to use a growth medium that promotes a specific type of bioaerosol or to add growth suppressant, which prevents growth of unwanted bioaerosols (Chang et al., 1995), where the use of combinations of growth suppressants can be used to either highlight or prevent AMR (Murinda et al., 2006).

Overall, the culture approach has the advantage that it is cost effective and much cheaper than some molecular approaches. Additionally, cultures of fungi can often be identified to specific taxa (Ghosh et al., 2015), although this is considered more difficult for bacteria without further assay. Furthermore, the culture approach allows for simultaneous detection of a large number of different species. The downside is that the approach is often associated with substantial uncertainty and that the approach only detects the culturable part of the bioaerosol. The culturable part of the bioaerosol is impacted by the sampling method as some approaches cause stress on the bioaerosols, thereby reducing the fraction which is culturable. A comparison of impingers, impactors and filter-based approaches found that impactors directly onto the growing media provided the highest diversity of culturable bacteria (Li, 2011). Also, some culture methods can become labour intensive when large numbers of samples are involved, and specialist culture media can be expensive. Overall, this suggests that sampling onto a well-prepared culturable medium (e.g., impactor with a growth medium containing a suppressant) should be the preferred method when the focus is on culturing.

3.2 Molecular methods for analysing AMR in bioaerosols using qPCR and metagenomics

There are many methods for extracting and amplifying DNA from environmental samples. A review covering different media such as water, ice, soil, and litter generally suggested the use of kits for extracting DNA, in most cases specific products, to enhance effective comparisons of data collected by different groups (Lear et al., 2018). The same study also found that there was a large number of manual (non-commercialised) methods that were applied on all sampling media (Lear et al., 2018). However, this review did not make

recommendations for air samples. The methods used to extract fungal spores and bacteria from air vary substantially between studies. One study used the DNAeasy plant kit from Qiagen (Pashley et al., 2012), another used its own manual approach without using a commercial kit (Abrego et al., 2018; Ovaskainen et al., 2020; Serrano-Silva and Calderón-Ezquerro, 2018), a third used the Fast DNA Spin Kit from MP Biomedicals (Hanson et al., 2022), a fourth used ZR Fungal/Bacterial DNA MicroPrep kit (Banchi et al., 2020) and a fifth used PowerSoil DNA Isolation kit (Stern et al., 2021). The Powersoil and the Fast DNA Spin kit from MP Biomedicals were both listed in the review as commonly used on bacteria. A comparison of several protocols and kits found that the Fast DNA Spin Kit yielded the highest amount of DNA (Ettenauer et al., 2012), and subsequently this kit has been used by others for extraction of DNA from airborne fungal spore samples (Chen et al., 2020; Degois et al., 2019). As such this kit is considered a suitable candidate for extracting DNA in relation to AMR relating to airborne bacteria and fungal spores. Other kits can be considered as most studies appear to be using commercialised kits. Extending from the review on DNA extraction, the use of existing commercialised kits and their associated protocols can be recommended, ideally kits that have previously been used on fungal spores and bacteria or kits that have been compared against previous kits for effective comparison of data collected in different studies or with different samplers. Such kits have previously been used to assess AMR in the environment followed by qPCR or shotgun metagenomics, as covered in a review on the challenges and opportunities of airborne metagenomics (Behzad et al., 2015). Common for all the kits is that there are specific instructions that need to be followed. Extra steps may need to be taken, e.g. to account for a filter substrate (Stern et al., 2021) or to further purify a sample. Furthermore, steps in relation to sampling methods may also be considered. As an example, it is known that the presence of wax in a sample can reduce DNA extraction (Oliver et al., 2021) and other products such as oil, grease, and vaseline are all suspected of limiting DNA extraction. These potential risks are associated with a number of common sampling methods such as impactors of the Hirst type and some applications of impingers. Due to this, the PollerGEN group decided to use dry cyclones to collect airborne DNA throughout the UK (Rowney et al., 2021).

Once the eDNA has been extracted from a sample it can be treated as any other sample from a different matrix. However, a specific issue with bioaerosols is that the amount of DNA is often small. Studies involving metagenomics (Serrano-Silva and Calderón-Ezquerro, 2018) or metabarcoding (Hanson et al., 2022) often require a substantial amount of DNA. Exceeding the minimum requirement may require the pooling of samples in order to secure enough DNA for the analysis (Hanson et al., 2022). This amount of DNA depends on the type of analysis that needs to be conducted, whether it is for next generation sequencing or PCR-based analysis. As an example, one commercial provider requires 1 ng/µl in a sample using a purified PCR product when delivering 150-300 base pairs, which is contrasted by 20 ng/µl using an unpurified PCR product when delivering 1000-3000 base pairs. It should be noted that AMR in fungal species is under-characterised, particularly in the environment (Nash et al., 2018), and so any existing genomic assays will focus on AMR in bacteria, although these assays are likely to be developed in the future for fungal AMR. The inclusion of negative PCR controls is essential in any PCR-based study. Negative controls reveal the presence of contaminating DNA in the laboratory reagents (Lear et al., 2018), which can be complemented with positive controls such as a mock community with a selection of specific

microorganism in well-defined quantities. Similarly negative controls must be used for mixed samples of bioaerosols (Hanson et al., 2022; Brennan et al., 2019), irrespectively of the method (e.g. metagenomics, metabarcoding, etc) and ideally also positive controls (Hanson et al., 2022). However, there is currently no wider consensus on mock communities in relation to fungal spores or bacteria and hence not for fungal spores or bacteria with AMR properties.

3.3 Storing and handling of bioaerosols

Collection and handling airborne bacteria and spores that are likely to be viable and with AMR potential should be undertaken with great care. The collection in the field will typically require standard operating protocols developed for a specific species or a group of species in order to minimize exposure to staff but also to avoid further spread of AMR in the environment. This may involve the use of personal protective equipment, use of double bagging of harmful material and cleaning of instruments after a site has been visited. Within the laboratory the handling of AMR material should generally follow national guideline requirements under the Control of Substances Hazardous to Health Regulations 2002 (Advisory Committee on Dangerous Pathogens, 2018). In relation to AMR it may be considered whether laboratories should have to work under specific containment levels, most often containment level 2 (CL2) or containment level 3 (CL3). In many cases working with fungal spores and bacteria with AMR potential according to CL2 or CL3 can be relaxed when the microorganisms have been deactivated, e.g. by extracting the DNA. An exception is those microorganisms known to develop mycotoxins as these mycotoxins can be active from both viable and non-viable microorganisms as well as subsets of these microorganisms. Degradation procedures vary between the toxins and may involve irradiation, heat, or treatment with strong acids or bases or oxidizing agents.

After the collection of bioaerosols, samples that need to be cultured need quick processing in order to avoid degradation of the culturable material or to avoid growth, e.g. if collected into a liquid. Following the guidelines from a review on sampling DNA (Lear et al., 2018) it is suggested that sampled material should be stored at 4°C before leaving the sampling site, a similar recommendation found in UK regulations. If material is brought to a laboratory for processing some recommendations set a time limit from the executing of the sampling to the start of the culturing, which for the UK is 12h in the evaluation of compost sites. However, a recent review on bacterial viability states that many species of bacteria when under stress may enter a dormant state to survive and do not grow on culture media but retain metabolic activity and may return to their virulent state in the presence of nutrition or after the removal of stress (Kumar and Ghosh, 2019). This suggests that the capability of viability varies between species and that a comprehensive assessment of viability should involve direct collection onto cultural media in order to avoid stressors which will lead to a bias in the sampling towards species that are less affected by stressors. It has not been possible to find robust investigations on how growing of airborne bacteria or fungi is impacted by a delay in culturing when using sampling into a liquid. This suggests that sampling into a liquid or onto filters should be avoided, despite the fact that the filter or liquid-based methods in some cases may be easier to implement.

After the collection of bioaerosols, samples that need processing using molecular approaches should be cool as soon as possible in order to limit DNA degradation. Storing material at -80°C (Pashley et al., 2012) has successfully been used for airborne material that needed storage for a full season, whilst in a study that involved only one day of sampling (Li et al., 2016) the samples were stored at -20°C. Drying or freeze drying should generally be avoided but specific methods may be needed for individual taxa (Lear et al., 2018). The review recommends immediate cooling to 4°C after the collection, whilst still in the field. For some sampling designs, this is not possible. As an example, the UK funded PollerGEN project (Brennan et al., 2019) and the Global Spore Sampling Project (Ovaskainen et al., 2020) both use cyclones and generally dry samples of bioaerosols collected in tubes. These samples are collected continuously over a long period at many different locations and frozen at -20°C locally before they are shipped to a central location either in the UK or Finland using ordinary mail. This suggests that a relaxation of the recommendations is possible, depending on the taxa, the collection method, and the analytical method. The review recommends that cooled samples are frozen to -20°C within 48 hours and later to -80°C for storage periods extending several months. Experience from a BBSRC funded project on collecting and analysing airborne spores (Hanson et al., 2022) found very good DNA extraction after four years of storage at -80°C. The same study (C. Skjoth, personal communication) also found that about 9 months of storage at -20°C resulted in much lower DNA extraction rates, suggesting that long-term storing of spores at -20°C is not a feasible solution. A range of other methods have also been reviewed such as the use of chemicals or liquid nitrogen for extreme long-term storage (Nagy, 2010), but these methods are generally not applied to bioaerosols. Overall, it can be recommended that storage of bioaerosols beyond a few months, including those with AMR potential, should be at -80°C, while shorter periods can be at -20°C.

3.4 Other methods such as counting and real-time

There are other ways to enumerate bioaerosols. One common approach to collect airborne fungal spores is to use impactors and count them under microscope. Often this approach can identify groups to the genus level such as *Alternaria spp., Aspergillus spp., or Penicillium spp.* (Sadyś et al., 2016b; Martínez-Bracero et al., 2022). Another approach is to use real-time devices, which may be able to separate airborne fungal spores and bacteria (Huffman et al., 2020), although reliable recognition at the genus level is not yet possible. These methods can therefore not identify AMR, but they may be used as supplementary sources of information such as estimation of the total concentration of viable bioaerosols (Li et al., 2016) e.g. in relation to quantifying total exposure to AMR, to assist in the selection of time periods where grab sampling will collect most material or in the selection of sampling period or sampling method, when the choice of approaches is affected by the overall concentration of bioaerosols.

4. Sampling of bioaerosols

4.1 Review on the sampling methods of bioaerosols, focusing on fungal spores and bacteria

The following section reviews the main methods to sample bioaerosols in the field complemented with perspectives in real-time detection. The three most common methods are air filtration, liquid impingement, and impaction on a physical surface (Ferguson et al., 2019), which are given specific sections below. Furthermore, it is important to separate between high and low-volume sampling, which is also given a specific section. There is a larger number of customized instruments in the literature, illustrating the need for specific instrumental properties, and also that the technical development in the collection of bioaerosols is at a less developed and harmonized stage when compared to atmospheric chemistry and the collection of gasses and aerosols. This larger variety compared with a very low number of intercomparisons makes it very difficult to make large scale assessments using data from many studies. Importantly, a recent review on bacterial viability states that many species of bacteria when under stress may enter a dormant state to survive and do not grow on culture media but retain metabolic activity and may return to their virulent state in the presence of nutrition or after the removal of stress (Kumar and Ghosh, 2019). This suggests that the capacity of viability varies between species and that a comprehensive assessment of viability, a vital element in detecting potential transmission of AMR, should involve direct collection onto the cultural media in order to minimize stressors as they will lead to a bias in the sampling towards species that are less affected by these stressors. This impact from stressors is supported by a number of studies that find a higher biodiversity of culturable microorganism when using plate-based methods compared to other methods such as cyclone approaches or filter-based approaches (Li, 2011). A consequence is that sampling into a liquid or onto filters should be avoided when the focus is viability and culturing for AMR, despite the fact that filter or liquid-based methods may in some cases be easier to implement.

4.2 High and low volume sampling, definitions

High and low volume samplers have different designs and possibilities for the sampling of bioaerosols. There is some variation in the naming of samplers and whether they are termed high volume samplers or low volume samplers, when referring to their technical documentation and their names given by the manufacturers. Similarly, there seems to be some variation in the scientific literature. Some authors term samplers that collect 100 l/min – 180 l/min as high-volume samplers (He and Yao, 2011; Xu et al., 2013), whilst an extensive list of samplers for bioaerosols produced by the US Department of Health has not termed the samplers in that sampling volume range as high-volume samplers. In the related field of air quality monitoring of particulate matter, there is generally good agreement among agencies. The Department for Environment, Food, and Rural Affairs (Defra) (e.g. 68 m^3/hour) and the Australian government (62.5 m^3/hour) use slightly different definitions of high-volume sampling, which again is related to recommendations and directives such as

the EU First Air Quality Daughter Directive (1999/30/EC). The definitions both equate to a sampling rate of about 1 m^3/min. Similarly, the values for low volume sampling rates can be converted to 38 l/min or 16 l/min. In the following, high-volume and low-volume samplers will use the definitions by Defra, irrespectively of names given to specific samplers within or outside these sampling regimes. High or low volume sampling rate is important as this determines the diversity of bioaerosols that can be captured, especially with the less abundant bioaerosols. A good example is a 3-year NERC funded monitoring campaign combining molecular approaches with low volume sampling using cyclones (Brennan et al., 2019; Rowney et al., 2021). They found that allergenic pollen from specific plants was not detected when the first plants started to release their pollen, but about 10 days later (Brennan et al., 2019). Nevertheless, there was a good agreement with the appearance and abundance of pollen in the samples with the overall progression of the central part of the flowering season (Brennan et al., 2019). Similarly, they found that it was not the most abundant species that had the largest health impact (Rowney et al., 2021). The limited correlation at the beginning of the season was caused by dilution of the bioaerosols decreasing the chance for collecting the relevant bioaerosols, when they were present in very low quantities. A better sampling approach would therefore have been to use high volume samplers, which however often comes at a much higher capital cost, a more complicated and time-consuming installation procedure, and also higher labour costs. Overall, such findings are transferable to all bioaerosols such as fungal spores and bacteria, including those harbouring AMR. When the overall atmospheric concentration is high in more remote locations (here termed background locations) then low volume samplers will be suitable. Similarly, if the sampler is near to a specific source releasing fungal spores and bacteria or suspected to release AMR, then a low volume sampler will be suitable. However, if concentrations are expected to be low, then a high-volume sampler is needed in order to increase the likelihood of the sampler collecting sufficient material for downstream analysis in a laboratory. The downside of this is that a priori information about concentrations of specific bioaerosols is required before a cost-efficient sampling design can be implemented.

4.3 Use of impactors in literature

The two most widely used impactors in relation to bioaerosols are arguably the volumetric pollen and spore sampler of the Hirst design (Hirst, 1952; Buters et al., 2018) and the Andersen sampler, either as single stage or the commonly used 6-stage cascade impactor (Andersen, 1958). The Hirst trap has mainly been used for optical recognition of pollen and spores but has been applied to molecular analysis such as qPCR (Rogers et al., 2009; Dung et al., 2018; Grinn-Gofroń et al., 2016b). However, as the typical sampling medium on the Hirst trap usually involved vaseline and wax that may reduce DNA extraction (Oliver et al., 2021), then this sampler should be used with caution regarding downstream molecular work. The Hirst trap has been evaluated in a pilot study as a tool for collecting spores for culturing and it was found that it was outperformed by plate-based samplers impacting directly onto the culturing media (Dananché et al., 2017) such as the Andersen sampler (King and McFarland, 2012). Similarly, the Andersen sampler has been found to provide much larger biodiversity of culturable microorganism when compared to filtering approaches (Uhrbrand et al., 2017). Such issues with sampling culturable microorganisms are well known and due

to this the Andersen sampler is listed as one of two recommended impactors to be used for monitoring bioaerosols at compost sites (Environment Agency, 2018). In the following sections the principles behind the Andersen sampler as well as sampling media are covered as this sampler offers the best sampling in relation to culturing bacteria and fungal spores with AMR, while at the same time being the recommended sampler for regulatory purposes.

The Andersen 6-stage cascade sampler draws in air at 28.3 l/min with 6 different impactor levels. Each level has 400 small holes, with decreasing size at each level permitting progression of larger particles to the next level. The first level collects particles above 7µm, while the last level collects particles with sizes 1.1-0.65µm. Collection is onto prepared petri dishes with suitable growing media, potentially with added suppressors such as a fungicides or antibiotics, specific antimicrobial compounds that allow for selective growth of AMR resistant bioaerosols. A recent review reports the sampling efficiency of the 6-stage Andersen sampler to be 40% (Kumar et al., 2021) confirming other studies that found that the 28.3 l/min Andersen sampler collected fewer fungal spores compared with a 16.5 l/min multi-vial cyclone sampler, sampling directly into Eppendorf tubes (Parker et al., 2013). The problem with low efficiency is well known, often caused by bioaerosol particles bouncing off the agar in the petri dishes, hence it has been recommended to use cyclones using the impingement method (Kumar et al., 2021). However, a study comparing the recommended agar plating technique with plates enhanced with mineral oil showed a statistically significant improvement in both sampling efficiency and the fraction of culturable microorganisms (Xu et al., 2013). The study showed an improvement in sampling efficiency of aerosolized *B. subtilis*, *E. coli*, and other environmental aerosols between 50% and 100%. While the study was limited to a few species under controlled environments and a few outdoor experiments, the improvements are so substantial that this calls for further experiments. One hypothesis that can be tested is that the relatively high airflow over the agar surface will dry out the surface making them less sticky and that this impact will vary depending on the environmental conditions. These studies should include AMR, focus on varies types of sampling media and outdoor sampling with the purpose of adjusting the recommended sampling media from standard plates to improved plates such as the combined used of agar and mineral oil and eventually also making recommendations on the optimal environmental conditions for sampling onto growing media.

4.4 Use of impingers in literature

Impinger and impactors are similar in their design. The main difference is that unlike impactors which trap collected onto a solid surface, e.g. a sticky compound partly consisting of vaseline or agar, in impingers the collection medium is a liquid. This liquid can either be a buffer, an isotonic solution, a mineral oil-based product, or a buffer with additives, depending on the purpose of the study (Kesavan et al., 2010; Šantl-Temkiv et al., 2018). A common instrument is the AGI-30 Impinger (Kesavan et al., 2010). Impingement, by using the AGI-30 Impinger has shown to recover 80% of viable aerosolized yeast compared to less than 20% for filtration methods (Lin and Li, 1999). Impingers often have a big advantage in that there is limited risk of overloading the sampling medium and it has been demonstrated that they work in challenging environments such as the Arctic (Šantl-Temkiv et al., 2017). Furthermore, it is possible to dilute the liquid in the laboratory for efficient culturing on plates

without the risk of overloading the plate medium with too many culturable microorganisms. Finally, it is often possible to add suppressors or other chemicals to the liquid in impingement systems, e.g., for fixation of RNA within microorganisms in order to analyse their reactivity (Šantl-Temkiv et al., 2018). A recent review states that impingers in general have a low efficiency (Kumar et al., 2021) particularly in comparison with cyclone-based approaches and liquid impingers have also been reported to be less robust compared to impactors and filter-based samplers (Hubad and Lapanje, 2013). A recent review reported the impinger sampling technique to be less efficient compared with the cyclone sampling technique (Kumar et al., 2021). Liquid impingers have the problem that many liquids quickly evaporate, which may be solved by using higher density liquids such as mineral oil or glycerol. However, these may have the effect that light density microorganisms such as spores float on the surface and escape the sampling medium. In addition, high velocities of air around inlets have been reported to damage or destroy some bacteria (Lin et al., 2010). Furthermore, it is unclear whether oil-based products can disturb the DNA extraction procedure. This illustrates that there are many limitations with impingers. As there are a number of good sampling alternatives for most environmental collections of airborne fungal spores and bacteria, it is recommended to use either impactors (chapter 3.1.3), cyclones (chapter 3.1.4), or filtration methods (chapter 3.1.5) whenever possible with or without AMR as a focus, in relation to collection of airborne fungal spores or bacteria.

4.5 Use of cyclones in literature

The cyclone sampling technique inducts air in a circular motion and uses the centrifugal forces to deposit the bioaerosols on the inner side of the collection vessel. As such these samplers do not offer separation into particle sizes. The cyclone samplers can broadly be separated into two groups: wet cyclone samplers and dry cyclone samplers.

Wet cyclone samplers are mainly used for short sampling periods, typically 10-30 minutes. The sampling is performed into a liquid that provides a vortex for efficient sampling of many bioaerosols. They sample at rates of 100 l/min-300 l/min, such the Coriolis sampler or the SASS2300 produced by Research International (Dybwad et al., 2014), but instruments sampling more than 1 m^3/min have been proposed (McFarland et al., 2010). A new and less widely used instrument is the NIOSH BC 251 instrument for use in hospital settings (Chia et al., 2020), which is an improvement to the commonly used Coriolis sampler from Bertin instruments. The Coriolis sampler is a compact instrument that is useful for easy deployment both indoor and outdoor sites with an operating time up to 6h. It has often been used to detect both fungal spores and bacteria in the air (Watt et al., 2020; Carvalho et al., 2008), but an evaluation of the sampling efficiency found that it was around 40% for bacteria (Langer et al., 2012) and according to a recent review also for virus (Kumar et al., 2021). Furthermore, it has been observed that the sampler, similarly to impingers, sometimes has floating fungal spores, leading to the suspicion that fungal spores may, under some conditions, be lost from the sampler. It has been shown a number of times that a high fraction of the microorganisms collected with this and other wet samplers can be cultured, where this fraction is substantially higher than bioaerosols captured with dry methods such as filters (Dybwad et al., 2014). However, when compared with impactors, it has been found that impactors of the Andersen design have an even higher recovery rate for culturing,

particularly with respect to biodiversity (Bellin and Schillinger, 2001; King and McFarland, 2012), suggesting that this approach is currently the best approach to minimize stresses that affect the viability of some microorganisms. This suggests that samplers of the Coriolis design have a niche for sampling bioaerosols, where speedy action is of very high priority (e.g. in case of accidents) due to their easy deployment and relatively high sampling rate. It also suggests that filter-based samplers should be preferred when accurate concentrations or high volumes of air require to be collected. Finally, it suggests that impactors of the Andersen design should be preferred when it is of importance to determine the biodiversity of culturable microorganisms. The wet wall cyclones can use a number of different liquids as sampling media. Often buffers such as phosphate buffered saline (PBS)-based surfactant are used (Dybwad et al., 2014). However, these types of media may evaporate very fast during warm and dry conditions and cannot be used for prolonged sampling in very cold conditions (e.g. below 0°C), depending on the physical properties of the liquid (Wang et al., 2019). Some cyclone samplers can use other liquids than buffer, with lower evaporation potential (Lin et al., 2010). However, it has not been possible to identify studies as to how their performance and efficiency change under different environmental conditions. As such, these types of samplers cannot be recommended as first choice for outdoor conditions, but can show great potential in those cases where rapid reaction is needed or within constrained areas with stable environmental conditions (e.g. indoor or within production buildings).

Dry cyclone samplers have often been used in air quality studies but have until recently rarely been used for bioaerosols compared with other instruments, partly because they are considered less useful when the focus is culturing. A generation of miniature cyclones or multi-vial cyclone samplers was developed to simplify the handling of the sample by sampling directly into an Eppendorf tube (West and Kimber, 2015). This allows for easy application of immunological or DNA-based diagnostic methods rather than microscopy, as demonstrated with a fungal spore study from Leicester (Pashley et al., 2012). This type of sampler belongs to a group of low volume samplers drawing in air at 16.5 l/min and initially used in crop monitoring programmes (West and Kimber, 2015). A further development was the multi-vial cyclone with 8 Eppendorf tubes on a carousel, which could be programmed to automatically replace used tubes with fresh tubes. This enabled a cheap deployment of semi-automatic instruments that efficiently collected time-dependent continuous samples of bioaerosols. They have been used successfully in large scale molecular studies both in the UK (Brennan et al., 2019; Rowney et al., 2021) and internationally (Ovaskainen et al., 2020) as well as collecting data over a number of years (Hanson et al., 2022). A disadvantage with this approach is that the samples are not size dependent. Secondly, the manufacturers information states that they are efficient in collecting pollen, fungal spores, and bacteria, but the efficiency with respect to bacteria has been questioned in a review (West and Kimber, 2015). Thirdly, the collection method is not well suited for collecting microorganisms that need culturing. However, this limitation can be outweighed by a limited capital cost (C.Skjoth, pers comm) compared with other instruments, the very low price of sampling media (Eppendorf tubes) and that 7 daily samples can be acquired in less than 15 minutes of work as demonstrated by the UK PollerGEN group (Brennan et al., 2019). If the samplers are placed in very moist environments (e.g. a dense woodland), then the Eppendorf tubes may collect water during extended periods with 100% humidity which over time can lead to fungal growth. This is a problem most types of samplers will experience when used over

long periods. The standard approach with the mini cyclones is to coat the tubes with fungicides to avoid further fungal growth. It has been claimed (Griffith and Petch, personal communication) that this coating will enhance sampling efficiency of smaller bioaerosols such as bacteria. While this is probably due to the similarity with the more efficient wet-walled cyclones, it has not been possible to identify confirming studies within the literature that coating enhance sampling efficiency. Due to this experience, although in literature unconfirmed, these mini cyclones are likely to be a preferred instrument for networks where the focus is fungal spores and molecular analysis such as the Global Spore Sampling Project (Ovaskainen et al., 2020). The reasons include limited capital costs and the low cost of the sampling material, combined with the semi-automatic data collection. The use of these mini cyclones for bacteria should be avoided until their efficiency on these microorganisms has been tested.

4.6 Use of filtering in literature, focusing on high volume sampling

Filter based samplers can be grouped into single filter samplers (Wang et al., 2015) and cascade samplers (Grewling et al., 2020; Stern et al., 2021). The instruments are often robust, where the cheapest instruments are usually those using just one filter, which can then then be analysed for bioaerosols. It has been reported that gelatin filters, polytetrafluoroethylene filters and polycarbonate filters often provide the relatively highest amount of culturable bacteria, whilst a specific comparison ranked the polycarbonate filters as the best option (Wang et al., 2015). However, a disadvantage with respect to AMR is that filter-based collection has a large impact on viability, and it has repeatedly been found that filter-based samplers provide a much smaller fraction of culturable bioaerosols compared with impaction methods (Dybwad et al., 2014; Lin and Li, 1999). A second problem, particularly with the single-filter samplers is that the filter, although in rare cases, can contain large amounts of bioaerosols complemented by mineral dust and air pollutants. This may impact the flow rate, but it has been shown that it is possible to extract DNA from filters that are heavily polluted such as car cabin filters (Hurley et al., 2019). A newer generation of high-volume cascade samplers, often developed for simultaneous monitoring of several particle fractions for traditional particulate matter (e.g. PM10, PM2.5, PM1) has been used successfully for analysis of bioaerosols (Stern et al., 2021) using molecular approaches. These high-volume samplers are very robust and have been demonstrated to work in challenging environments such as deserts (Tawabini et al., 2017) or the Arctic (Wex et al., 2019) and it has been demonstrated that it is possible to operate such samplers in large national or international networks (Buters et al., 2012) for continuous sampling over long periods. A disadvantage is that it will be more time consuming to extract DNA and analyse multiple filters instead of one filter combined with very often high capital costs. Some instrument providers provide options which automatically replace filters for the most expensive sampling systems, but it has not been possible to verify whether these instruments have been used with success to sample bioaerosols. A number of samplers, often those that are very robust, can be very heavy and require substantial electric power and are therefore often less mobile. A huge advantage is that the samplers, when used with approved filters, have a very high sampling efficiency for both fungal spores and bacteria and that it is possible to deploy samplers with sampling characteristics that comply with

recommendations from authorities like Defra for the sampling of particulate matter and international directives such as the EU First Air Quality Daughter Directive (1999/30/EC). These high-volume samplers provide the best option for providing accurate atmospheric concentrations of bioaerosols in selected size fractions, which may be further processed to species level using molecular approaches such as qPCR, metabarcoding or metagenomics. Furthermore, such high-volume samplers have a much higher chance of collecting bioaerosols with less common characteristics, such as bacteria or fungal spores with AMR. These high-volume samplers should therefore be the first choice, when viability is not an issue, but where it is important to collect large amounts of air over an extended period of time or when it is important to get accurate assessments of atmospheric concentrations, e.g. in the regulation of AMR.

4.7 Common list of instruments used for the sampling bioaerosols

The following Table 1 contains a list of common instruments used for sampling of bioaerosols. They are sorted into groups according to their sampling principle. The list focuses on main types of samplers and does not include all brands from all companies as instruments such as filter samplers exist in many different versions from many different instrument providers.

Table 2. List of typical instruments used for sampling bioaerosols

Cascade samplers						
Name	Details	Producer	Fungal spores	Bacteria	Other	References
Compact Cascade Impactor	5 particle fractions from >10 µm to < 0.5 µm	Custom built, University of Harvard		X	X	(Stern et al., 2021)
6 stage Andersson sampler	Indoor study, PCR and agar plates focusing on CFU		X	X		(Xu and Yao, 2013)
ChemVol cascade impactor	Originally a two-stage, but with an extension covering particles down to ~ 0.5 µm	Butrago	X			(Grewling et al., 2020)
Filter samplers						
No-name, custom for study	Particles on filter >0.2 µm from Fisher scientific	Unique designed sampling system	X	X		(Kellogg et al., 2004)

		using pipe, pump, and a filter.				
SASS 3100	Dry electrostatic filter sampler, 92% efficiency at 5.0 µm to 0.5 µm, 120 l/min	Research International, Monroe, WA, USA		X		(Mbareche et al., 2018)
High volume filter sampler	Coarse and fine mode	Not specified	X			(Fröhlich-Nowoisky et al., 2009)
Dichotomous aerosol sampler	Single stage 2.5 µm to 10 µm approx. 1000 l/min	Not specified	X	X	X	(Finn et al., 2021)
Low volume samplers, cyclones						
Single cyclone or multi-vial cyclone	Low volume cyclone sampler, 16 l/min	Burkard	X			(Ovaskainen et al., 2020)
Multi vial cyclone	Low volume cyclone sampler, 16 l/min	Burkard			X	(Brennan et al., 2019)
Low volume samplers, impactors						
7-day pollen & spore sampler	Low volume, 10 l/min	Burkard	X			(Grinn-Gofroń et al., 2016b)
Hirst Volumetric Spore Sampler	Cut tapes in half for visual inspection and DNA analysis	Burkard Scientific Ltd	X			(de Groot et al., 2021)
Impingers, liquid collection						
Coriolis sampler	a liquid cyclonic impactor with 50% cut-off at 0.5 µm, 300 l/min	Bertin Technologies		X		(Mbareche et al., 2018; Ferguson et al., 2019)
SASS 2300	a liquid impingement/wetted-	Research International,		X		(Mbareche et al., 2018)

	wall sampler, 50% cut-off at 0.9 µm, 300 l/min	Monroe, WA, USA				
Other						
Filter-cup design	Five different membrane filters tested at 100 l/min	Custom design for laboratory experiment in controlled environment		X		(Jeong and Kim, 2021)
Cloud droplet impactor	Collection of cloud samples (no precipitation) at Puy de Dôme	According to Deguillaume (2014) using single stage cloud collector similar to the one described by Kruisz (1993) with a cut-off at 7 µm		X		(Renard et al., 2016)
Wet deposition collection	Collection of rain, snow, or hailstones	Sterile stainless steel funnel				(Šantl-Temkiv et al., 2015)
WIBS	Real-time collection and enumeration of bioaerosols		X	X		(Fernández-Rodríguez et al., 2018b)

4.8 Pros and cons using main sampling methods

Each sampling approach has both advantages and disadvantages, as a grand unified approach that determines everything with 100% accuracy is unlikely to be developed. Table 2 therefore provides an overview of the pros and cons in relation to main sampling methods in relation to bioaerosols.

Table 2. List of pros and cons in relation to main sampling methods in relation to bioaerosols

Cascade samplers		
Sampling methods	**Pros**	**Cons**

Impingers	• Efficient in capturing a large size range covering virus, bacteria, and fungal spores. • Maybe better for keeping certain biological properties of the bioaerosols, such as viability compared to filter-based approaches. • Strong responsive company behind some samplers. • Can capture very large amounts of bioaerosols. • Possibilities for using sampling liquid that keeps or restricts further biological activity. • Often high-volume samplers providing large amounts of material, good for rare species investigations. • Expenses for sampling limited.	• Bioaerosols may be lost from liquid again. • Sampling efficiency in bacteria range below 50%. • Liquid can be lost very fast during dry hot periods. • Known samplers limited to short operating time. • Robust network capability not demonstrated.
Impactors	• Probably the most widely used method worldwide on larger bioaerosols, such as pollen & fungal spores. • Network capability demonstrated. • Can collect bioaerosols on sticky surfaces for optical recognition using microscopes or on growing medium. • Expenses for sampling limited.	• Some sampling instruments limited to larger bioaerosols, such as fungal spores. • Sampling efficiency impacted by weather, in particular to wind speed. • Methods usually restricted to low volume samplers, best for common bioaerosols.
Rain collectors	• Captures very large amounts of bioaerosols by scavenging entire air column for nearly all bioaerosols. • Expenses for sampling limited.	• Data collection only during rain episodes causes gaps in time series. • Bias expected in sampling concentrations due to dependence on rain events. • Material may stay in compartment and liquid for a long time.
Filter based approaches	• Easy to use. • Sterilization straightforward. • Analytical method can provide volumetric concentrations. • Often high-volume samplers providing large amounts of	• Requires laboratory work to extract material, which may be time consuming.

	material, good for rare species investigations.	
Cascade samplers	• Network capability demonstrated. • Sampling efficiency can be high for both large and small particles. • Sterilization straightforward. • Limited costs in sampling media. • Often high-volume samplers providing large amounts of material, good for rare species investigations. • May be time consuming.	• Instrument expenses high, especially for high volume samplers. • Requires additional laboratory time, which may be very high due to need to analyse the entire cascade.
Real-time devices	• Provides data in real-time, separation into size bins and some separation into groups of bioaerosols. • Network capability demonstrated. • Species separation very limited.	• Instrument expenses very high.
Dry cyclones	• Very easy to use, also by non-scientific and technical staff. • Efficient in capturing a range of bioaerosols, such as pollen spores and bacteria according to manufacturer. • Network capability demonstrated. • Sterilization straightforward. • Easy service, such as cleaning of cyclone. • Expenses for sampling limited.	• Loss of material from compartment not investigated. • Efficiency may be low at sizes below larger fungal spores for some instruments, but lack of published material on efficiency.

5. Equipment used to sample bioaerosols, technical details

The following section contain the information from producers and resellers and feedback from networks.

5.1 Instrument details from providers

The following section contains technical details from instrument providers and suppliers. A supplementary table (S1) contains additional information and with some information split into columns. It should be noted that some suppliers state that an external pump is needed, and experience suggests that these pumps can be the most expensive component in the system, consume substantial amounts of power and be heavy (+50kg). In some cases, the instrument providers describe sampling range of their instrument, which in some published studies seems to be translated to sampling efficiency. Some authors recommend that 50% sampling efficiency for a specific microorganism should be regarded as a threshold or a cut-off value (West and Kimber, 2015), but it has not been clear if this cut-off value is generally accepted by the scientific community. Generally, 50% cut-off values are not provided from suppliers. There are a number of 50% cut-off values from a review (West and Kimber, 2015), but the source to the values in that review cannot be identified. It has been possible to find a few independent experiments of sampling efficiencies covering specific instruments, but the obtained results did not agree with the values from the review or the information from the instrument providers. It is therefore recommended to be very careful when using values for sampling ranges, unless there are accessible independent results that verify the values in relation to 50% cut-off values.

Table 3. List of instruments and the technical details that have been obtained. Generally, 50% cut-off values are not provided from suppliers and therefore listed as N/A. In cases where both a N/A and a value is listed, this reflects the 50% cut-off values provided by the review of West and Kimber (2015), although no information of the origin of these numbers was available and the values provided within the review do not correspond to the factory information.

Name & producer, supplier	Price of instrument and costs for collection	Sampling volume, if applicable	Sampling Size range	50% cut-off (µm)	Other (e.g. weight and power requirements)
Cascade samplers					
Andersen 6-stage viable sampler, Westech Scientific Instruments	Quote not provided	28.3 l/min	N/A	N/A >0.43	~5.25kg, mains

IMP6-BIO, TCR Tecora	Quote not provided	28.3 l/min	N/A	N/A >0.43	N/A, mains
Andersen 6-stage ambient viable sampler Tisch Environmental	£4595 (6), £2862 (2), £2281 (1)	28.3 l/min	N/A	N/A >0.43	N/A, mains
Chemvol, Butraco	£10000, plus cost for pump required	~1000 l/min	N/A	N/A Coarse 9 - 4 Fine 4 - 1	5kg (head only), mains Pump system may be ~70 kg. Extension possible sampling below 1 µm
Biostage, SKC Ltd.	£373 plus cost for pump £815	28.3 l/min	N/A	N/A	7.25kg, mains
Buck Bioculture, A.P. Buck	£1095	30-120 l/min	N/A	N/A	1.25kg, mains
Bioculture, Zefon International	£1077	30-120 l/min	N/A	N/A	1.25kg, mains
Filter samplers					
SASS 3100	£7530	50 - 300 l/min	N/A	N/A	3 kg, mains, battery
Digitel DHA-80	£22000	100 - 1000 l/min	N/A	N/A	60 / 30 kg, mains
Digitel DH-77	£12000	100 -1000 l/min	N/A	N/A	46 kg, mains
Digitel DPA14	£14000	5 - 50l/min	N/A	N/A	45 / 32kg, mains
Low volume cyclone samplers and other cyclones					
Single Cyclone	Quote not provided	16.5 l/min	N/A	N/A	11 kg, mains, battery

Multi Vial Cyclone	Quote not provided	16.5 l/min	N/A	N/A	10-24 kg, mains, battery
High vol. Cyclone Air Sampler	Quote not provided	270 l/min	N/A	N/A	15 kg, mains
LFD & DNA Auto Spore Trap	Quote not provided	300 l/min	N/A	N/A	46 kg, mains
Aerosol Sense	Quote not provided	200 l/min	N/A	N/A	12 kg, mains
Coriolis Compact	£8023	50 l/min	N/A	N/A	1.2 kg, mains-charged battery
Low volume impactors and other cyclones					
7-day Volumetric Spore Trap	Quote not provided	10 l/min	N/A	N/A	11 kg, mains, battery
VPPS 2010	Quote not provided	N/A	N/A	N/A	9 kg, mains or battery
MTIST	Quote not provided	16.5 l/min	N/A	N/A	12 kg, mains or battery
SAS100 Microbial Air Sampler	£4705	100 l/min	N/A	N/A	1.7 kg, mains-charged battery
SAS180 Microbial Air Sampler	£4755	180 l/min	N/A	N/A	1.7 kg, mains-charged battery
SAS Duo 360 Microbial Air Sampler	£6755	360 l/min	N/A	N/A	1.75 kg, mains-charged battery
EM01005 Air Sampler	Quote not provided	100 l/min	N/A	N/A	1.75 kg, battery
Biotest Hycon RCS	£650	100 l/min	N/A	N/A	1.5 kg, mains, battery
VPPS 2010	Quote not provided	N/A	N/A	N/A	9 kg, mains or battery

Impingers, liquid collection					
Coriolis Micro	£8420	100 – 300 l/min	N/A	N/A	4.3 kg, mains-charged battery
Coriolis Recon		600 l/min	N/A	N/A	
SASS 2300	£12768	300 l/min	N/A	N/A	5.7 kg, mains, mains-charged battery
SpinCon II	£21950	450 l/min	N/A	N/A	23.6 kg, mains
SKC BioSampler	£516	~12.5 l/min	N/A	<0.2	7.25 kg, mains
AGI-30	£85	12-13 l/min	N/A	N/A	Mains
Other					
Rotorod Model 40	£1470	Not applicable	N/A	10 - 20	1.5 kg, mains
Air-O-Cell	£572	15 l/min recommended	N/A	<1	0.7 kg, mains
Air-O-Cell	Cassettes 10 @ £38	15 l/min recommended	N/A	<1	0.7 kg, mains

6. Drivers behind AMR concentrations in the atmosphere and how to quantify them

6.1 Supplementary data needed to quantify drivers

This section focuses on model tools used to quantify source areas to detected bioaerosols and source strength using numerical models.

6.2 Weather data

Many places globally experience large variation in weather conditions and meteorological factors throughout the year. This impacts the abundance and biodiversity of bioaerosols where the concentrations tend to have a seasonal behaviour (Zhong et al., 2016; Bragoszewska et al., 2017). Often it is found that the importance of specific weather variables varies throughout the year (Zhong et al., 2016; Bragoszewska et al., 2017) and when comparing different geographical locations, the peak can be found in the summer(Bragoszewska et al., 2017) at one location while it can be a minimum during summer at another location (Qi et al., 2014). Most likely this is related to prevailing emission patterns for bioaerosols which often increase with increased temperatures up to a certain threshold. Once that threshold has been reached, emission rates tend to decrease. Many weather variables have been found to correlate with fungal spore and bacterial concentrations. Humidity and temperature are often linked to abundance whilst other variables, like UV light, have been found to be related to viability (Bragoszewska et al., 2017). Typical variables that have been found to be correlated with the concentration of fungal spores or bacteria include temperature (Grinn-Gofroń et al., 2019b; Skjøth et al., 2016), humidity(Grinn-Gofroń and Strzelczak, 2013), wind speed, and wind direction (Fernández-Rodríguez et al., 2015; Grinn-Gofroń et al., 2016b). They also have a strong dependency on precipitation and sometimes grouped into dry and wet fungal spores due to the presence under these conditions (Pashley et al., 2012). Recent findings from the UK have shown that whilst relative humidity or precipitation and other atmospheric variables may have a considerable correlation with fungal spore abundance or diversity, the related variable leaf wetness may be much more important (Hanson et al., 2022). While leaf wetness is directly related to growth conditions of fungal spores and the conditions on the host, then this is not a standard variable from traditional weather stations and rarely used by the scientific community. However, a sensor for leaf wetness is relatively simple and straightforward to implement on most research grade weather stations that use data loggers.

6.3 Land cover, land use and activity data

Abundance and diversity of both fungal spores and bacteria are influenced by land use (Apangu et al., 2020; Grinn-Gofroń et al., 2016b), land cover (Sadys et al., 2015; Sadyś et al., 2015), point sources (Xu et al., 2020), and timing of specific activities, such as harvesting (Olsen et al., 2019). Furthermore, there is a distinct difference in the atmospheric microbiome between rural and urban landscapes (Hanson et al., 2022) and substantial

variations between years, depending on the prevailing meteorological conditions. Relevant supplementary data to quantify drivers is therefore detailed land cover data (e.g. UKCEH land cover+) sets coupled with so called activity data (e.g. harvesting data from the Agricultural and Horticultural Development Board) as illustrated for the UK (Apangu et al., 2020). A similar data set to the land cover+ is the commercially available CROME data set, which can also be used under Open Governmental Licence. This approach has a high similarity to how ammonia emission from agriculture is envisioned to be quantified in a new generation of models (Sutton et al., 2013). Further detail, particularly in relation to the status of the vegetation, may be obtained by using remote sensing, where in particular the Sentinel-2 satellites are useful due to their high geographical detail combined with a high revisit time, which enables monitoring of both plant relevant processes such as stress detection (Segarra et al., 2020) and anthropogenic activities within the agricultural landscape (Ottosen et al., 2019) .

6.4 Use of numerical models

This section focuses on model tools used to quantify source areas to detected bioaerosols and source strength using numerical models. It focuses on so-called receptor models which are typically used in relation to a single source or if the source is unknown and therefore excludes the group of source models, that require detailed emission inventories, typically Eulerian models.

6.5 Back trajectory and particle dispersion models

Back trajectory models are a common tool to analyse atmospheric transport of bioaerosols. They are computationally easy to use and there are even on-line tools such as HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory model) (Stein et al., 2015) with graphical easy to use front-end or web-based platforms or the UK Met Office's Numerical Atmospheric-dispersion Modelling Environment (NAME). A numerically more advanced tool is backwards dispersion models that take into account additional processes such as gravitational settling and rain-out, so-called wet deposition. The advantage with this type of models is that they do not need a priori knowledge about sources (Tesson et al., 2016) and they are therefore good tools to analyse the potential source region for captured material. These tools have been used with success to analyse both fungal spores and bacteria and can be used on data analysed with microscopes (Fernández-Rodríguez et al., 2015), molecular approaches (Grinn-Gofroń et al., 2016b) or culturing techniques (Urbano et al., 2011), covering both viable and non-viable material. As such this technique is readily available in relation to AMR, when the question involves atmospheric transport. Typically, this type of tool is suitable for geographical scale beyond 20km (Smith et al., 2013) and in some cases on a scale between 2km and 20km (Frisk et al., 2022; Smith et al., 2013). The results from these types of models are sensitive to the quality and detail of their input data (Hernández-Ceballos et al., 2014). They used gridded meteorological data and it has often been reported (Bilińska et al., 2017; Hernández-Ceballos et al., 2014) that geographical resolutions of 10km or higher make a large improvement in the results.

6.6 Local scale models

Gaussian dispersion modelling is a tool that is applicable on a geographical scale of 20km or below. Gaussian models typically require *a priori* knowledge about locations of sources and their release patterns over long periods (Spijkerboer et al., 2002) and have recently been applied on the AMR resistant *Staphylococcus aureus* (Angen et al., 2021) demonstrating strong correspondence with observations. One of the limitations with the Gaussian models is that it is difficult to handle gravitational settling and some versions of common Gaussian models can have problems with handling the conditions in the atmospheric surface layer, an aspect that can be important for spore release from fields (Skelsey et al., 2008). Furthermore, in most cases the Gaussian models often make substantial simplifications in relation to changes in the biological properties of bioaerosols over time such as how viability will change during the lifetime in the atmosphere.

6.7 Other models

There are two other model types that can be applied on bioaerosols. One type is the grid-based Eulerian model or related models such as semi-Lagrangian models. They are technically more difficult to operate and computationally more demanding than the trajectory models or the Gaussian models. The advantage is that they can handle explicit processes such as viability, gravitational settling, rain-out (wet deposition) and atmospheric transport and they are able to operate on many geographical scales. They often require a substantial amount of knowledge about sources therefore they are rarely applied on less studied bioaerosols (Tesson et al., 2016) due to lack of information. Another group of models are the statistical models that can be very efficient when atmospheric dispersion can be neglected, e.g. to assess emissions profiles or make source attributions within an emission source such as a compositing facility. Source attributions or source-apportionment approaches are rarely used on bioaerosols and AMR but are commonly used in air quality planning on aerosols (Thunis et al., 2019) and these models may therefore be suitable tools for specific bioaerosols including AMR.

7. Practical options and decision support tree for bioaerosol sampling

Designing a sampling campaign at a single site or several sites is often challenging as there are many things to consider. Furthermore, if the campaign is supposed to run over a long time, sometimes years, then there can be and extra set of challenges to consider such as redundancy and reliability. Many of the questions that will be faced can be put into decision tree which can aid in the network design so that samples that are obtained from the network can be analysed and deliver data that will satisfy the main aim of the campaign.

7.1 Decision tree for sampling and guidance to design bioaerosol sampling campaigns.

The sampling design or the approach will usually require that the expenses within a project and/ or the feasibility of the study is balanced out so that it matches the available resources, without compromising the objectives of the study. Therefore, using a decision tree just once is often not sufficient. Often the approach must be revisited a number of times. During this process as many as possible of the important questions must be addressed already during the planning process of the campaign. This is important in order to ensure that when a campaign has been initiated, then the setup of the network or the campaign does actually deliver data that can be used to address the main objectives. Below the decision support tree there are two examples on how to design a campaign for either a short or longer period aiming at one or several groups of AMR in the atmosphere.

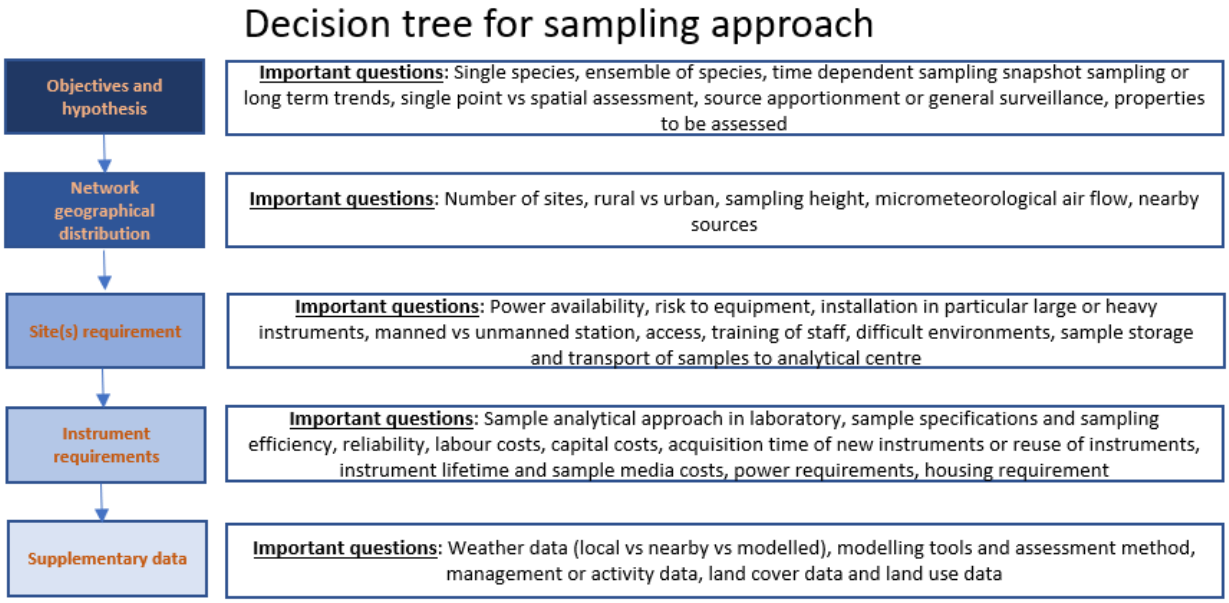

Figure 3. Requirements and feasibilities to be considered in relation to the actual implementation of an isolated campaign or temporary network of samplers starting with a hypothesis or objectives of the study. It is recommended that a scientist trained in the collection of airborne material is visiting each sampling location before a final decision is taken, concerning suitability.

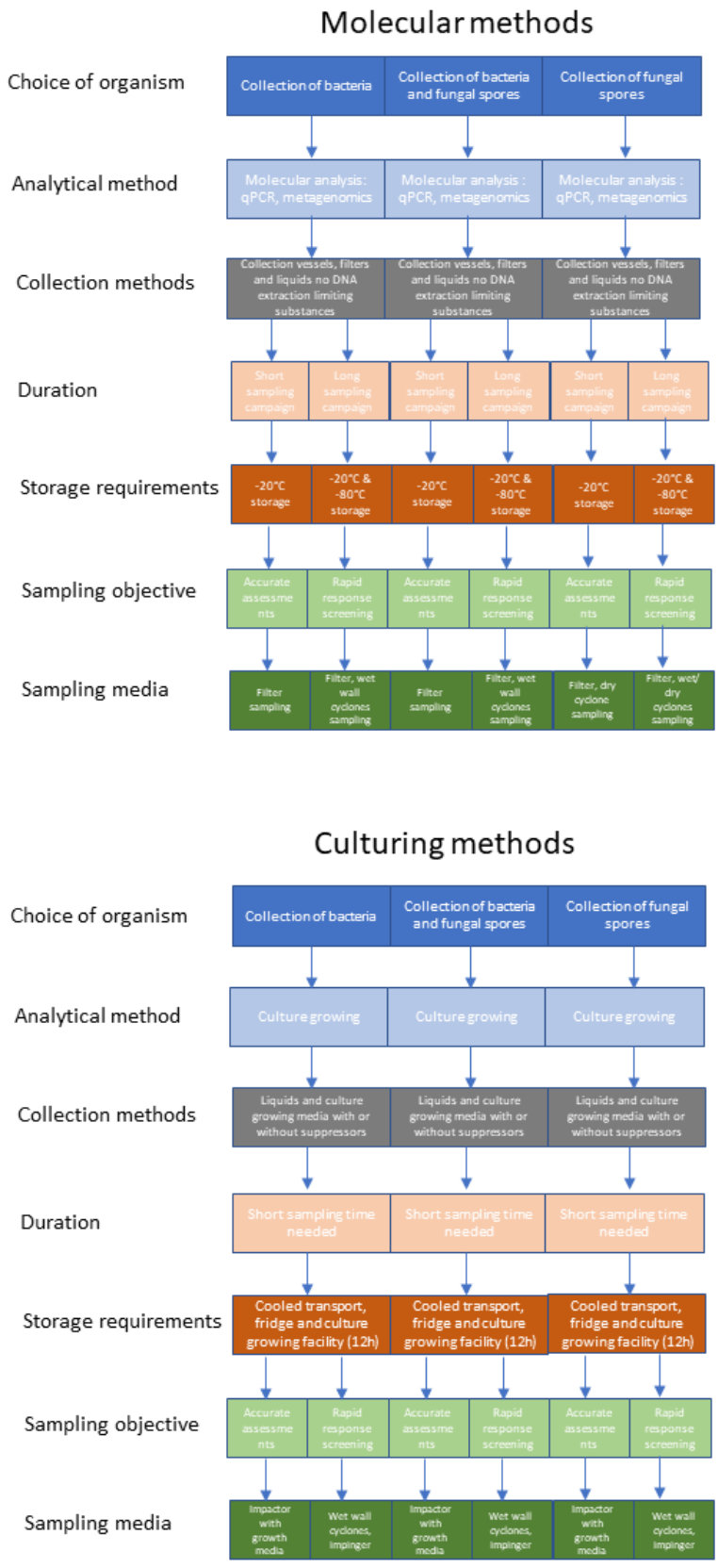

Figure 4 Decision tree for either molecular methods or culturing methods illustrating the need for an integrated approach covering all aspects from choice of microorganism to be collected to type of media to use. To be used together with figure 3.

7.2 Example 1: A single site combined short-term & long-term campaign focusing on bacteria and fungal spores

Example: A single site combined short-term & long-term campaign is designed to test this hypothesis: *There is no difference in the seasonal biodiversity of airborne key bacteria and fungal spores in the Midlands, UK and a fraction of these microorganisms will, under certain weather conditions, maintain their viability during atmospheric transport*.

The objective will then be to quantify the difference in biodiversity using molecular approaches along with a culturing approach

Design: The approach will require accurate assessment of collections and concentrations of both fungal spores and bacteria using partly molecular approaches, such as metagenomics, and partly using culturing approaches. Going down the top figure and the decision tree then the recommended sampling approach will be a filter-based solution, ideally with a high volume sampler. This sampler will provide material for the molecular work. The sampler should be a cascade sampler and be able to efficiently sample large volumes of air and filtrate particles below 1 µm efficiently and automatically, ideally for a full year. The best samplers will be cascade samplers with automatic filter replacement, but cheaper solutions can be used on a manned site as long as filters are not required to be replaced every day. It should be noted that long-time filter collections should be avoided in order to avoid overloading of the filters. Filters need to be stored at -20°C during the daily operation but should be moved to a -80°C freezer for long term storage. Filters can be separated into sections, one for DNA extraction and analysis of bacteria and another for fungal spores using guidelines on DNA extraction kits. At least one spare section should be kept safe, in case procedures need to be repeated. The long-term sampler campaign will then be complemented with short term sampling periods with an Andersen-type cascade sampler for culturing. In this case there is a requirement for local collection of weather variables, such as temperature, humidity, wind speed and radiation that according to literature have been shown to impact viability and capturability of both fungal spores and bacteria. The sampling locations should be at least 400m away from known sources that are expected to be strong, and the samplers should be placed at an elevated position, ideally 10m-20m from the surface. Furthermore, it should be placed at locations without high pollution, thereby excluding city centres and places with a high density of potential pollution sources including residential heating. Suburbs or rural locations are therefore likely to be the best locations.

7.3. Example 2: A multi-site long term campaign focusing on fungal spores

Example: A multi-site long term campaign is designed to test this hypothesis: *There is no difference in the abundance (geographical and temporal) of two key fungal spores with AMR potential, relevant to human health and agriculture (Aspergillus spp and Alternaria spp), within the UK,* here excluding impact individual sources known to be strong sources to fungal spores with AMR.

The objective will then be to quantify the difference throughout the country in the so-called seasonal fungal spore integral.

Design: The approach will require accurate assessment of concentrations and the use of molecular approaches such as qPCR, assuming there will be available primers suitable for qPCR analysis. Going down the top figure and the decision tree then the recommended sampling approach will be either a filter based or a dry cyclone sampling approach, while impactors, impingers or wet cyclones will be either less ideal and should be avoided. Sampling using low volume mini dry cyclones into prepared Eppendorf tubes will be substantial cheaper compared to filter based sampling using high volume sampling. This impacts the amount of air that is collected and reducing the chance to collecting rare bioaerosols. However, daily samples may be pooled to 7 day or monthly accumulated samples, thereby reducing the risk dramatically for not having enough DNA from rare species in order to answer the hypothesis. A cost-effective solution is so called multi-vial sampling designs operates semi-automatically, thereby reducing the number a site needs to be visited by staff to acquire the samples. It is possible to train local staff to carry out the exchange and store the samples securely if there is a -20°C freezer available. The samples need to be transferred to a central laboratory for storage at -80°C for long term storage. Ideally, the samples need to be transferred in a frozen state, but there is good experience with using ordinary mail within large distributed networks, although it may questioned if this approach impacts the DNA. A distributed network will most likely require a minimum of 8-10 sampling sites providing a near complete data series. As temporal failures increased with the length of the campaign and the number of sites, it is recommended to aim for 12-14 sites. If samplers are less expensive it is recommended to have at least 1 extra sampler, “a hot spare”, used to replace an instrument that fails to ensure service of an instrument without major interruptions of the sampling campaign. The sampling locations should be at least 400m away from known sources that are expected to be strong and the samplers should be placed at an elevated position, ideally 10m-20m from the surface.

7.4 Supporting videos

The report is supported by two supporting videos illustrating the usage and installation of instruments used in example 6.2.1 and 6.2.2, made at the supersite located at University of Worcester.

8. Conclusions

Very little is known about AMR in the atmosphere and there are less than a handful of studies worldwide focusing on the atmosphere and AMR. As such the atmosphere is far less studied compared to the aquatic or terrestrial environment. Abundance and seasonality of AMR is therefore mainly assumed, using existing knowledge on airborne bacteria, and fungal spores.

Sources of AMR can be both related to anthropogenic or natural activities such as wastewater treatment, waste disposal sites, agriculture, or natural environments. Atmospheric AMR has been found worldwide and there is an indication of a positive correlation of AMR and the usage of antibiotic drugs in large urban environments. A similar connection has not been investigated for the rural zone and usage of pesticides. Currently there is no information available for the UK. Nevertheless, the atmosphere is expected to be a relevant pathway for transmission of AMR. Abundance and seasonality of AMR are largely expected to follow activities in potential sources. AMR from agriculture is therefore expected to follow crop growth and in particular harvesting, hence in the UK peaking late summer and in the autumn. In contrast, AMR from wastewater facilities or compositing sites is expected to have a more uniform pattern throughout the year with vastly increased emissions when the sources (e.g. water or composting material) are disturbed. As such AMR must be expected to be present in the atmosphere throughout the year. While there is a distinct long-term seasonality, then it must be expected that concentrations of AMR will show huge daily variations, similar to observations of airborne bacteria and fungal spores. More than ten groups of fungal spores and ten groups of bacteria are abundant in the atmosphere, but a much larger number are frequently found. The most abundant fungal spores are the three groups of *Alternaria, Cladosporium,* and *Epoiccocom,* while the most abundant bacteria are expected to be *Pseudomonadales, Burkholderiales, Rhizobiales, Rhodospirillales,* and *Sphingomonadales.*
In the UK it must be expected that there will be import of AMR from other countries, particularly in the most southern parts of UK. However, this import is expected to be episodic, similar to what has been observed for other airborne particles. It must also be expected that the fraction of imported AMR will be largest for the smallest fungal spores and bacteria, while the majority of larger fungal spores will have a short lifetime in the atmosphere. It is expected that viability of resistant microorganisms will decrease during atmospheric transport but so far there are no studies quantifying this aspect.

Analysing for AMR in the atmosphere is very challenging and it is recommended to use a combination of molecular approaches and culturing approaches to quantify abundance of AMR and its fraction in relation to similar species without AMR capability. The greatest potential for using both approaches simultaneously is for bacteria, whilst for fungal spores it is limited. The reason is that comprehensive genetic libraries covering fungal spores are not yet available. It is therefore important that whenever AMR is discovered, particularly in fungal spores, that the species are sequenced in order to expand the currently limited libraries. Furthermore, it is important that the handling and storing of collected bacteria or fungal

spores follow strict protocols such as cooling or freezing in order to ensure that these microorganisms can be either cultured or sequenced.

A considerable number of instruments have been presented as bioaerosol samplers. Most of the samplers have not been used in intercomparisons and it has been near impossible to identify studies of the instruments that test their sampling efficiencies covering a range of different bioaerosols and environmental conditions. A number of the most common collection systems have been reviewed both with respect to their efficiency to capture culturable resistant microorganism and for sequencing these microorganisms as well as their capital and running costs. Impaction directly onto growing media has been found to provide the highest survival rate for culturing, while high volume cascade samplers using filters has been found to provide the best sampling for sequencing. Furthermore, high volume sampling over days and weeks may be needed to capture AMR that are less abundant in the atmosphere. The most cost-effective solution for long term sampling campaigns, in particular for distributed campaigns, has been found to be the use of semi-automatic micro cyclones. The mini cyclones however, may be limited to fungal spores as their efficiency on sampling bacteria is currently unknown. The most expensive solution is semi-automatic high-volume samplers which are often less mobile compared to many other samplers such as mini-cyclones, some impactors and impingers.

A number of different supporting data sets are needed for further understanding of AMR in the atmosphere. A traditional weather station using a data logger and extended with a sensor for leaf wetness seems to be the best option. A number of different land cover and/or land use data sets along with activity data from either agriculture or waste sites are very useful. This may be complemented with remote sensing data from publicly available sources such as the Sentinel satellites. The modelling of atmospheric transport is currently estimated to be best done using receptor-based models. There are several model tools available, which have been developed and used extensively by the air quality community. The most suitable models are Gaussian dispersion models operating on geographical scales of 10km or less, whilst Lagrangian particle dispersion models are best suited for geographical scales beyond 10km. Models that are capable of simultaneously assessing the impact of the governing atmospheric processes on atmospheric transport and deposition as well as viability of fungal spores or bacteria have not yet been developed.

Overall, the rationale for collecting fungal spores or bacteria in the air must be clearly articulated as well as which analysis needs to be conducted (for both laboratory and modelling) before any campaign is planned. Decision support trees can be used to assist the design of a campaign, where both hypothesis and sampler design must be evaluated simultaneously. This will ensure that the sampling provides information for the laboratory processing as well as the modelling, often split into specific objectives. Here the design needs to ensure that both laboratory work and the modelling are provided with specific and sufficient data in order to answer the main hypothesis or central aim of the campaign.

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Appendix: sensitive information

Fifteen different companies were contacted regarding 28 samplers. More samplers are available, but in some cases the same type of sampler was made by different companies, sometimes samplers were specific to one company and in other cases a number of different samplers were made by one company. Eleven companies responded providing 9 quotes. Further two passed the enquiries to an UK agents, which didn't respond. Prices for six samplers given in catalogues. The samplers from the 6 companies without a response are marked in the supplementary information table S1 as "No Quote".

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