



# Review of airborne antimicrobial resistance

Chief Scientist's Group Report

February 2020

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Professor Doug Wilson  
**Chief Scientist**

# Executive summary

The spread of antimicrobial-resistant microorganisms and their genes in the environment is of increasing concern, with legislators seeking evidence to advise the regulation of environmental pathways for the spread of resistance. However, most of this evidence has been gathered from aquatic (for example, freshwaters, seawater) and terrestrial (for example, soil) systems, with little concerted effort to establish a knowledge base for airborne antimicrobial resistance.

This report seeks to summarise the available literature on airborne antimicrobial resistance, with a view to establishing what is known, what hypotheses are emerging from this, and what knowledge gaps there are in current scientific work on airborne antimicrobial resistance.

Based on a literature review, this project sought to identify:

- current coverage of the issue
- types of organism studied
- types of antimicrobial studied
- field and laboratory methods used
- environments studied

This information was used to characterise the types and levels of multi-antimicrobial resistance in the different environments that have so far been studied.

- As of August 2018, the literature was dominated by studies focusing exclusively on airborne antibiotic resistance in bacteria. Most of these studies are of Staphylococci (31 out of 88 studies) and Enterobacteriaceae (24 out of 88 studies). Very few studies looked at fungi (9 out of 88 studies).
- No studies or data on airborne antimicrobial resistance for the UK were found.
- Most studies were culture-dependent (that is, they used a laboratory culture medium in agar plates and culturing with antibiotics to measure antimicrobial resistance; 53 out of 88 studies), not culture-independent (that is, using polymerase chain reaction (PCR), quantitative PCR or metagenomics to measure antimicrobial resistance).
- Despite biases towards studying certain classes of antibiotics and differences in the antibiotics for which resistance was tested between studies, it was clear that different environments were associated with different types of levels of antibiotic resistance. For example, tetracycline resistance appeared to be more prevalent in agriculturally associated air.

The literature review suggested that:

- different environments are associated with different types and levels of airborne antimicrobial resistance
- the 2 main conditions driving airborne antimicrobial resistance are:
  - the presence of large accumulations of antimicrobial-resistant organism-laden faecal matter
  - physical processes for the aerosolisation of this faecal matter (for example, animal movement, aeration in WWTPs)

It is hypothesised that livestock-associated environments in the UK will be major hotspots of airborne antimicrobial resistance. However, specific conclusions about which types of environment (for example, CAFOs, WWTPs) are the most problematic source of airborne antimicrobial resistance for the Environment Agency and associated government bodies are difficult because no UK research on airborne antimicrobial resistance was identified by the literature search.

The major knowledge gap in current research is the relative importance of airborne exposure to antimicrobial resistance.

- Is the air a primary route by which antimicrobial resistance spreads to humans and other vulnerable hosts (for example, crops)?
- How does it do so (via microorganism hosts or free-living genes)?
- What are the consequences of this?

More epidemiological evidence is needed in this regard, as are studies on airborne antimicrobial resistance in the UK in the many neglected environments (for example, arable farming, slurry spreading areas), and fungi and other non-bacterial microorganisms.

More research is recommended to close knowledge gaps and test emergent hypotheses. Also needed is a more concerted effort to report and share the results of antimicrobial susceptibility testing in a standardised way, and more careful and standardised collection and reporting of air sampling.

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# 1 Introduction and methods

## 1.1 Background

Antimicrobials – including antibacterial, antifungal and antiviral agents – are an essential component of modern life. For the first time in human history, we are more likely to die of chronic, age-related disease than infection (Surette and Wright 2017). However, there is a growing risk that such gains will be reversed by the ever-growing problem of antimicrobial resistance. Recent predictions indicate that, without policy and regulatory changes, more people will die from infections of multi-drug resistant bacteria than cancer by 2050 (O’Neill 2016). The role of the Environment Agency and its partners in invoking such a policy change may not seem immediately obvious, as antibiotic resistance has conventionally been thought of as a public health issue. However, given that the outdoor environment represents one of the largest reservoirs of antibiotic resistance, environmental stewardship may prove one of the most effective ways to control the spread of antibiotic resistance (Singer et al. 2016).

The outdoor environment is considered a reservoir for antimicrobial resistance due to the close proximity and interactions with indoor and outdoor anthropogenic environments that make heavy use of antimicrobials and the disposal of wastes such as wastewater containing antimicrobials and antimicrobial-resistant microorganisms from water treatment, farming, hospitals and manufacturing into the outdoor environment. This is alongside the host-driven spread of antimicrobial resistance (for example, the direct spread of antimicrobial infections from humans to other humans or animals) and spread within enclosed, high risk environments (for example, hospitals).

It has been suggested that the outdoor environment is the single largest source of antimicrobial resistance (Surette and Wright 2017). Recognising this threat, the Department for Environment, Food and Rural Affairs (Defra) and the Environment Agency have begun to focus efforts on researching, monitoring and limiting the spread of antimicrobial resistance in the outdoor environment. Within the Environment Agency, much of this work has been water-centred since much of the Environment Agency’s work deals with aquatic resource management. However, little is known about the threat of antimicrobial resistance spread via airborne routes and thus little has been done to incorporate antimicrobial resistance into the management of air pollution in the UK.

In this context, this report was commissioned to investigate the current available evidence on airborne antimicrobial resistance, with a view to influencing the direction of future research, pilot studies and eventually the management of the airborne threat if it exists.

## 1.2 Project aims

The overarching goal of this report is to describe what is currently known and not known about airborne antimicrobial resistance. The report was compiled using the scientific literature available as of August 2018, incorporating



information about the scientific content, locations and contexts in which the science was conducted, consensus among studies and the quality of the methods used.

The project had 3 main aims:

1. To establish the degree of knowledge of different organisms, antimicrobials and environments in currently available research on airborne antimicrobial resistance
2. To use the current literature to make a preliminary identification of the environments with the highest risk of producing and/or receiving antibiotic resistant microbes and/or genes via the airborne route
3. To draw out the general patterns emerging from the research as a whole, propose important hypotheses that require further investigation and identify knowledge gaps in the current literature

Aim 2 is perhaps the most subjective and more open to debate given that the interpretation of risk depends on the impacts of antimicrobial resistance being considered. That is, is the spread of antibiotic resistance to agriculturally or pharmaceutically valuable antimicrobials more concerning, or is the interest in the proliferation of multi-antimicrobial resistance in the environment more generally?

Aims 1 and 2 are addressed in Section 2 (Results), which is divided into subsections primarily based on the main environments being studied.

Aim 3 is addressed in Section 3 (Conclusions), which also makes some recommendations for future research and potential management of the problem if it exists.

## 1.3 Methods

A systematic search was made of the titles and abstracts of 3 databases of academic journal papers (Scopus, Web of Science and Entrez) for evidence relating to antimicrobial resistance in the air.

### 1.3.1 Search strategy

The searches, which were all performed on 7 August 2018, took the following form.

**SCOPUS:** TITLE-ABS ({air} OR {airborne} OR {air-borne} OR bioaerosol\*) AND TITLE-ABS (antimicrobial\* OR antibiotic\* OR antibacterial\* OR antifungal\* OR antiviral\* OR antihelm\* OR antipara\* OR antiprot\* OR biocid\* OR \*drug PRE/2 resist\*)

**WOS:** TS=(("air" OR "airborne" OR "air-borne" OR bioaerosol\*) AND (antimicrobial\* OR antibiotic\* OR antibacterial\* OR antifungal\* OR antiviral\* OR antihelm\* OR antipara\* OR antiprot\* OR biocid\* OR \*drug) NEAR/2 (resist\*))

**ENTREZ:**<sup>1</sup> (air[Title/Abstract] OR airborne[Title/Abstract] OR air-borne[Title/Abstract] OR bioaerosol[Title/Abstract]) AND (antimicrobial\*[Title/Abstract] OR antibiotic\*[Title/Abstract] OR antibacterial\*[Title/Abstract] OR antifungal\*[Title/Abstract] OR antiviral\*[Title/Abstract] OR antihelm\*[Title/Abstract] OR antipara\*[Title/Abstract] OR antiprot\*[Title/Abstract] OR biocid\*[Title/Abstract] OR \*drug[Title/Abstract] AND resist\*[Title/Abstract])

The searches were thus very broad and accounted for any mention, alongside air or bioaerosols, of resistance to many types of antimicrobial compounds: antibiotics (bacteria-targeted), antifungals, antihelminthics, antiparasitics, antiprotozoals, biocides and 'multi-drug' resistance (which is sometimes used synonymously to mean broad spectrum antibiotic resistance). Including keywords was found not to influence the results and was not possible for Entrez. Hyphenation (for example, 'anti-biotic') also did not affect results.

There was no explicit intention to compare airborne antimicrobial resistance to antimicrobial resistance in other environmental compartments (for example, soil or water). The focus instead was on developing a thorough evidence base for airborne antimicrobial resistance that could be compared to other environments at a later date if deemed necessary. Nonetheless, discussion of other environmental compartments influencing airborne antimicrobial resistance is inevitable in order to contextualise the topic.

### **1.3.2 Processing of search results**

Full bibliographic information was downloaded from the databases and then processed in R, resulting in 676, 832 and 542 articles from Scopus, Web of Science and Entrez (PubMed) respectively.

#### *Removal of duplicates*

The package 'revtools' (Westgate 2018) was used to remove most duplicates (657 articles removed). This was followed by manual removal of articles with duplicate digital object identifiers (DOIs) (40 articles removed), resulting in 1,353 unique articles.

#### *Classification of identified studies*

Studies testing antimicrobial resistance in airborne microorganisms were defined as either as those sampling the air for microorganisms and testing their antimicrobial susceptibility, or as measuring antimicrobial resistance genes in air samples (using qPCR or sequencing methods) directly. Information about a microorganism's ecology (for example, adaptation to airborne travel/living) was not used in defining it as 'airborne', such that the organisms tested could include both transient and more permanently airborne organisms.

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<sup>1</sup> Only PubMed 'hits' were exported, as relevant (or any) results were only obtained from this database).

Using this definition, the package 'metagear' (Lajeunesse 2017) was used to scan abstracts and classify them into:

- irrelevant articles not measuring airborne antimicrobial resistance in some capacity (929 articles)
- those looking at antimicrobial resistance in a clinical setting such as a hospital or dental surgery (204 articles)
- those looking at airborne antimicrobial resistance in other environments (220 articles)

This last category forms the basis of this report, with a small amount of relevant hospital studies (that is, those concerning numbers of antimicrobial resistance microorganisms or genes in hospital air) mentioned in Section 2.6.3.

### **1.3.3 Retrieval of studies considered most likely to be relevant**

Attempts were made to view and/or download all of the 220 articles identified as most likely to be relevant from the topics and abstracts plus all of the most likely to be relevant hospital articles (13 out of 204). Those articles that were not downloadable via government information services were requested via ResearchGate<sup>2</sup> from the authors, and if no positive response was obtained, excluded from the analysis.

Articles not in English were excluded, with the exception of 2 Polish articles, for which the services of a translator were obtained; none of the other non-English articles were obtainable via information services or ResearchGate anyway.

Careful consideration was given to the introduction of bias alongside these inclusion/exclusion criteria.

For example, older studies were less likely to be obtained from ResearchGate requests as the authors were less likely to be members. However, almost all of the most likely to be relevant studies were recent (176 out of 220 studies were published after 2008, when ResearchGate was launched) and many of those published before 2008 were also on ResearchGate or could be accessed through information services.

The most problematic omitted non-English language studies were several highly relevant reports by the German Federal Institute for Occupational Safety and Health (BAUA) on airborne antimicrobial resistance in concentrated animal feeding operations (CAFOs) and several articles in Chinese, both of which were also often behind paywalls and did not respond to requests. In addition, these papers tended to concern heavily studied environments for which there were plenty of other studies. It is not thought that the inability to include them significantly affected the results. It has been found that, more generally, the omission of non-English papers does not significantly change the results of a systematic review (Morrison et al. 2012).

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<sup>2</sup> A free, professional social networking site for scientists and researchers to share papers, ask and answer questions, and find collaborators.

Any article not directly testing antimicrobial resistance in airborne environments were also excluded at this stage, that is, those that mentioned it in titles or abstracts but did not perform any kind of measurement of it or an organism known to be multi-drug resistant such as methicillin-resistant *Staphylococcus aureus* (MRSA).

These inclusion/exclusion criteria produced the final 88 papers used in the literature review.

### 1.3.4 Quantitative comparison

For quantitative comparison, all those articles reporting the results of culture-based antimicrobial susceptibility testing on strains isolated from the air were identified and their results compiled in a master database. Efforts were made to be as inclusive as possible in doing this, for example, by:

- converting data reported as percentages into raw figures using other information available in the text
- searching for supplementary material
- converting antimicrobial susceptibility testing results reported in unusual formats into a more standard antimicrobial susceptibility table

Once compiled, the master data frame was used to calculate the prevalence of resistance (number of resistant strains as a percentage of total strains tested) to each antibiotic.

For antibiotics tested across multiple studies for a given type of environment and organism (staphylococci or Enterobacteriaceae), the mean prevalence of resistance (percentage of tested strains reported as resistant) and the weighted mean prevalence of resistance were tested. For the latter measure, meta-analyses of proportions (meta R package; see below) were used to calculate the mean prevalence weighted by the number of strains tested, reporting the weighted means of the random effects model within the text. This accounted for differences in the number of strains tested, which varied from 1 to 208 across the data frame.

The meta-analysis of proportions method was also used to compare the multi-antibiotic resistance (MAR) indices for each study (Krumperman 1983) – a simple index adopted from some of the studies (Korzeniewska et al. 2013, Sivri et al. 2016, Zhang et al. 2018) that summarises the number of strains that were resistant to multiple antibiotics. This index is useful because it allows some degree of comparison between studies that tested susceptibility to different types and numbers of antibiotics and numbers of strains, having accounted for variation in the number of antibiotics (using weighting in the meta-analysis) and other sources of variation (in addition models, Appendix B).

A high MAR index indicates an environment dominated by (culturable) antimicrobial-resistant organisms. This can be achieved by the presence of a very abundant strain that has resistance to multiple antibiotics or by having a more even distribution of strains and resistance to the multiple antibiotics tested being shared between them. A MAR value of 0 indicates an environment in

which none of the strains tested is resistant to any of the antimicrobials tested, while a value of 1 represents an environment in which all of the strains tested are resistant to all the antimicrobials tested. The MAR value can be influenced by the antimicrobials tested (for example, it is likely to be lower when lesser used groups of antibiotics are tested) and organisms tested (for example, by the intrinsic resistance of a taxonomic group or its propensity to evolve resistance). Nonetheless, it provides a single index that can be used to compare diverse studies (for example, to ask what environments are most associated with antimicrobial resistance) provided uninteresting variation (that is, that caused by taxonomic or antimicrobial bias, or air sampling methods) is accounted for (Appendix B).

Here the MAR index was used in this way, using meta-analysis of proportions implemented using the `metaprop` function of the `meta` package (Schwarzer 2007) to compare the mean MAR in each of 7 main types of aerial environment for which antimicrobial susceptibility testing data were available. This approach was validated by additionally performing crude analyses to validate the conclusions about the literature (Appendix B). These were implemented using R (R Development Core Team 2017) and associated packages, using a generalised mixed binomial model (Fox et al. 2018), a random forest method (Liaw and Wiener 2002) and a regression tree method (Ripley 2017).

For studies that measured antimicrobial resistance using molecular techniques such as polymerase chain reaction (PCR), quantitative PCR (qPCR) and sequencing, studies were compared qualitatively. This was because differences in methods (for example, specific antibiotic resistance genes covered, sequencing depth) and results reporting (for example, reporting gene copies per m<sup>3</sup> of air versus per amount of DNA/16S copies in a sample, targeted versus metagenomic approaches) made the standardisation and quantitative comparison of results difficult. Nonetheless, there were clear consistencies in results between the quantitative and qualitative analyses.

As well as this report, the following are also available:

- the results of literature searches and code used to process them
- the bibliographical information for the 88 studies used for the literature review
- the master data frame of antimicrobial susceptibility testing from 24 of the studies
- the code used to perform the analysis in this report are publicly available (see Appendix A)

## 1.4 Structure of the report

This report is structured around its main aims.

Section 1 introduces the subject of airborne antimicrobial resistance and the Environment Agency's interest in it, the methods used in this study and the structure of the report.

Section 2 presents the results of the study. It identifies the 3 key environments covered by the current literature and describes in different subsections the background of each environment, the results of antimicrobial susceptibility testing and the results of genetic studies. This is also done for 'Other' environments which, although lesser studies, still provide interesting perspectives on airborne antimicrobial resistance as a whole. Each subsection introduces the environment, describes work on staphylococci and/or MRSA and Enterobacteriaceae and/or extended spectrum beta lactamase (ESBL) resistance (the 2 main target organisms/types of resistance studied) and discusses other research on more general studies looking at multiple organisms (for example, non-selective culture-based approaches and/or molecular approaches looking at antimicrobial resistance genes or using sequencing).

Section 3 highlights the report's 10 main conclusions. It identifies:

- the 3 main things that are known about airborne antimicrobial resistance
- the 3 main hypotheses formulated from information provided in the current literature but for which more information is needed to prove/disprove
- the 4 main knowledge gaps identified in the literature

Recommendations are made for the minimum standards of results reporting by future research (especially aimed at the burgeoning field of UK airborne antimicrobial resistance research) and some final conclusions are made.

## 2 Results

### 2.1 General trends across all studies

This section presents general trends observed from the 88 studies identified in the literature which met the project's criteria for inclusion. It examines:

- the taxonomic coverage of the studies
- whether the studies were culture-based or non-culture
- the antimicrobials covered by the studies

#### 2.1.1 Taxonomic coverage

At the broadest phylogenetic resolution, studies of bacteria dominated the literature. Despite broad search terms covering bacteria, fungi, viruses, helminths, parasites and protozoans, 80 out of 88 of the returned searches focused exclusively on bacteria. Within this group, studies of Staphylococci and particularly *Staphylococcus aureus* dominated (31 out of 88), closely followed by studies of the Enterobacteriaceae family (24 out of 88). The dominance of both groups is primarily driven by concerns about MRSA and related pathogens, ESBL resistance in Enterobacteriaceae pathogens (for example, *Klebsiella pneumoniae* and *Serratia marcescens*) and airborne routes of these pathogens.

The dominance of staphylococci in studies was to some extent reflective of the relative abundance of these organisms in airborne environments; several studies identified these organisms as dominating culturable airborne communities; for example, approximately 92% of all culturable reported by Brooks et al. (2010) and 32% of Enterococci cultured by Chapin et al. (2005) were identified as coagulase-negative staphylococci. However, culture-independent studies suggested a less extreme predominance of staphylococci and even very low abundance in air samples (see, for example, Arfken et al. 2015, Zhang et al. 2018, Li et al. 2018), suggesting staphylococci may only represent the culturable and/or viable part of the microbial community, though this is difficult to determine and the two are not equitable (Li et al. 2014). Arguments have been made that staphylococci are more likely to survive the airborne environment because they are Gram-positive and their thick peptidoglycan cell wall enables them to better withstand stresses associated with aerosolisation, desiccation and irradiation, even long term (Heo et al. 2010, Heederik 2013, McEachran et al. 2015). In contrast, it has been suggested that Enterobacteriaceae and other organisms survive as attached to or embedded within particles, which provide some relief from the stresses of the air environment and a food source (von Salviati et al. 2015, Gao et al. 2017). However, it may be that the dominance of staphylococci-focused studies more reflects academic trends and a clinical bias towards these pathogens rather than ecological reality.

Among other groups, we found 9 out of 88 studies including some measurement of airborne fungi (sometimes alongside bacteria). Only 6 of these

studies tested for antifungal resistance; others looked at bacteria and focused on their antimicrobial resistance. Very few studies were found that looked at airborne antimicrobial resistance in viruses (1 out of 88) or other microbial groups (0 out of 88).

### **2.1.2 Culture/non-culture based**

Given the focus on target organisms, antimicrobial susceptibility testing, price considerations and the longer period during which these methods have been in existence, the literature was dominated by culture-based over culture-independent studies (53 out of 88 versus 18 out of 88 studies). This is not necessarily a problem, especially given that it has still not been established that microbial genotypes easily correlate with their antibiotic resistance phenotype. Nonetheless, given the frequently quoted and partially validated (though not necessarily true) figure that only about 1% of microorganisms are readily culturable (Amann 1911, Ultee et al. 2004), this may provide a skewed picture of airborne antimicrobial resistance – that does not even necessarily equate to the ‘viable’ portion (Li et al. 2014). Recognising the advantages and disadvantages of these 2 methods, both were incorporated when drawing general conclusions.

### **2.1.3 Antimicrobials covered**

The coverage of antimicrobials was broad, and following taxonomic coverage, was mainly dominated by studies based on antibiotic susceptibility testing concerning resistance to different antibiotic (bacterial) substances (47 out of 88). Again following taxonomic trends, antifungals received the second largest share of attention from researchers (6 out of 88). Molecular and sequencing studies followed these trends, with 22 out of 88 studies looking at antibiotic resistance genes and 1 out of 88 studies looking at antifungal resistance genes.

## **2.2 Quantitative comparison**

A total of 24 studies of antimicrobial susceptibility testing identified from the literature review were subjected to quantitative comparison. The sections below consider the general trends observed in these studies in terms of the following 5 features:

- antimicrobials used
- testing methods
- air sampling methods
- time of sampling
- geographical coverage

Section 2.2.6 presents a summary of the findings.



## 2.2.1 Antimicrobials used in susceptibility tests

Within culture-based studies (47 out of 88), a total of 24 studies were identified that presented the results of antibiotic susceptibility testing in a way that was amenable to raw data extraction. Across these 24 studies, 57 antibiotics were studied (ampicillin, penicillin and tetracycline the most frequently) across:

- 28 sub-classes (aminopenicillins and beta lactamase sensitive penicillins predominated)
- 21 sub-sub classes (peptidyl transferases and monobactams predominated)
- 13 modes of action (antifolates and 50S ribosomal subunit-binding antibiotics predominated)

Within studies of antifungals, azoles were the dominant group considered. This was in line with azoles being the dominant group of antifungals in common usage (due to their low toxicity to humans). Itraconazole, fluconazole and voriconazole were the most studied.

Biocide resistance was infrequently considered in its own right, though one study (Zhou and Wang 2013) did consider the *qac* resistance gene of *Staphylococcus*, associated with increased tolerance to disinfecting agents via the efflux pump mechanism. Polymyxins (including polymyxin B and colistin) were also the third largest group studied, and these antibiotics are considered detergents. However, no direct studies were found of biocide resistance, antivirals, antihelminthics, antiparasitics or antiprotozoals – despite including these in the search strategy.

## 2.2.2 Methods of susceptibility testing

As noted above, most studies were culture-based and used standardised antimicrobial susceptibility testing methods to measure antimicrobial resistance. Most studies used the disk diffusion method, in which antimicrobial-soaked disks are placed on a lawn of the bacterial or fungi of interest, and resistance is measured as the width (mm) of zone of inhibition around the disk. Clinical definitions of resistance and susceptibility are calculated using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI) reference standards/thresholds (mm).

The predominance of this method is understandable given the scale of these studies and the greater ease with which large numbers of strains can be tested. The other method used was the broth microdilution technique, in which the focal antibiotic is diluted in a buffer serially to achieve known concentrations and the point at which there is no growth of the tested bacteria or fungi is used to calculate the minimum inhibitory concentration (MIC). Variations on this method use freeze-dried antibiotic solutions and measure colony growth on each concentration. MICs can then be used alongside EUCAST or CLSI reference standards to calculate resistance or susceptibility (that is, is the MIC above the threshold to be considered resistant?).

Given the predominance of the disk diffusion technique and the condensed format in which results are often reported in papers, it was found that authors

rarely reported exact MICs and that the results of antimicrobial susceptibility testing were instead almost always reported as the number of strains resistant or susceptible (and sometimes 'sensitive', a borderline situation) in a table format. For consistency, this study classified all 'sensitive' results as 'resistant' when compiling the results from different papers into one table. Minimum selective concentrations were not addressed by any of the studies.

### **2.2.3 Methods of air sampling**

The studies were dominated by active air sampling approaches, in which air is sampled by suction using one of various devices. These devices can be divided into 3 categories, as detailed in 'Technical Guidance Note (Monitoring) M9' (Environment Agency 2018).

#### *Filtration*

This method captures the total suspended particulate in a sample by drawing all particles 1–100µm into the device and capturing them on a filter disk. It was used in one of the 24 quantitatively compared studies. Examples include:

- use of a gelatine filter (1 study)
- use of a glass filter
- use of a quartz microfibre filter

#### *Impaction*

Impaction consists of drawing air into a device and relying on inertial forces to 'impact' bioaerosols and other particles onto a sampling surface. In bioaerosol studies, this surface is usually a Petri dish filled with an agar-set culture medium, though a solid surface and molecular biology methods can also be used for a culture-independent approach. An important variation on this method is the Andersen cascade sampler in which air is drawn through various stages of the device through a series of nozzles or jets. This method allows the separation of different particle sizes, working on the different inertial sources of different sized particles. Despite using this method though, it was common for studies to only use it for measurements of the total culturable airborne microorganisms and/or aggregate particle size bands of bioaerosols and did not report size-resolved antimicrobial susceptibility testing. Impaction was used for 18 out of the 24 quantitatively compared studies. Examples include:

- Andersen two-stage (6 studies)
- Andersen six-stage (2 studies)
- MAS-100® (8 studies)
- BioStage® 400 (1 study)
- SAMP'AIR™ (1 study)

## *Impingement*

This method is similar to impaction, except that instead of hitting a solid surface, particles are 'impinged' into a liquid. This method is intended to result in greater preservation of live cells, because impaction can kill cells. It was used in 1 out of the 24 quantitatively compared studies. Examples include:

- AGI-30 glass impinger (1 study)

## *Other types of sampling*

Passive types of sampling also featured in the literature, though this type of sampling was less established. It included the following.

### **Petri dish sampling**

This is the most primitive form of air sampling and involves leaving a Petri dish exposed to the air in a location of interest. Sometimes collection is directly onto an antibiotic-supplemented culture medium in agar. The method was used in 8 out of the 24 quantitatively compared studies.

### **Dust sampling**

Dust may provide a longer term, more viable reservoir (but distinct from air) of microorganisms in both indoor and outdoor environments. Sampling is either by manually collecting dust into sterile containers (4 out of 88 studies) or using electrostatic dust samplers (cloths, 3 out of 88 studies). Dust 'archives' may be more representative of the environment than shorter term samples. None of the 24 quantitatively compared studies used this method.

### **Car air conditioning filters**

This method of sampling emerged in the past few years and uses the filters from the air conditioning units of motor vehicles as a record of outdoor bioaerosols (Li et al. 2018), although its original purpose was intended to reflect the indoor vehicle environment (Viegas et al. 2018). However, this method is still in its early stages. It assumes that the indoor environment of the car represents the outdoor environment and does not correct for differences in the types of environment in which cars travel. It has also not yet been used in a controlled manner normalising for time/miles of sampling, for example. Nonetheless, a recent global study using this method produced interesting and sensible results (Li et al. 2018). None of the 24 quantitatively compared studies used this method.

## **2.2.4 Time of sampling**

Samples were taken at many different times of year.

Given the limited data, this project did not seek to quantify:

- seasonal changes in airborne antimicrobial profiles
- the different climates and environments of the locations studied

- other complicating factors (for example, air conditioning in indoor environments)

Future work, however, may prove fruitful for understanding airborne antimicrobial resistance dynamics over time and in assessing seasonal risk.

### **2.2.5 Geographical coverage**

The literature was dominated by studies from the USA, China and Poland.

Particular regions were associated with particular topics – the USA and China dominated research on the most prevalent topics (CAFOs and urban environments). Germany also contributed heavily to the literature on CAFOs, though it was not possible within the project's resources to access most of these studies.

Poland dominated the literature on wastewater treatment plants.

There were also a few global studies of antibiotic resistance in the air of cities worldwide that represented large international collaborations between North American, Chinese and European groups. However, none of the search terms used for this project found any evidence of studies of airborne antimicrobial resistance – in any environment – emerging from the UK.

### **2.2.6 Summary of general trends found in quantitatively compared studies**

- Almost all the studies (~88%) directly concerned with airborne antimicrobial resistance were of bacteria. Fungi, viruses, helminths and protozoans have received little attention. Over half of studies were culture-based.
- Ampicillin, penicillin and tetracycline resistance were the most frequently tested antibiotics. Itraconazole, fluconazole and voriconazole were the most studied antifungals.
- Impaction was the most frequently used method to sample bioaerosols.
- Samples were taken at many different times of year.
- The studies identified came predominantly from the USA, China and Poland. No UK study of airborne antimicrobial resistance was found.

## **2.3 Concentrated animal feeding operations**

CAFOs were by far the most frequent type of environment considered in terms of airborne antimicrobial resistance; 39 out of the 88 studies were concerned with CAFOs, covering pig (21), poultry (12) and to a lesser extent cattle (6) operations.

Antimicrobial-resistant bioaerosol emissions from concentrated livestock rearing operations have been raised as part of a more general public concern over

airborne biological and chemical emissions from such operations, as well as more specific health scares related to them.

A seminal study in this regard is the epidemiological study of Wing and Wolf (2000), which noted increased incidences of headaches, runny nose, sore throat, excessive coughing, diarrhoea and burning eyes among those living near industrial livestock operations in North Carolina in the USA compared with a control group of those not living within 2 miles of such operations. Although this epidemiological study was correlative, it stimulated much research on the airborne transmission of microbial pathogens from livestock operations – a potential route for disease transmission and, of more concern, antimicrobial-resistant microbial pathogens.

CAFOs may provide a fertile breeding ground for antimicrobial resistance due to the high concentrations of antibiotic use in agriculture for growth stimulation and for veterinary purposes, combined with the high concentration of animals exchanging microbiomes and disease. However, the links between antibiotic usage and the number of animals in a facility is not straightforward. Several studies found resistance to antibiotics that were not in common usage on the farms being studied. Analysing the data from one large-scale study, it appears there is no strong correlation between the number of animals in a CAFO and the microbial biomass it produces (Hong et al. 2012).

### **2.3.1 Pig**

Studies featuring pig confinement buildings were dominated by investigations of flagship multi-drug resistant pathogens.

A strong motivation for such studies was the livestock-associated health scares related to MRSA and ESBL-producing Enterobacteriaceae. Among the 21 selected studies, 10 focused on MRSA or the *Staphylococcus* genus, and 10 focused on ESBLs. Four of the studies looked at antibiotic resistance organisms more generally, and one examined antibiotic residues in pig confinement buildings.

Four studies used culture-based techniques and reported the results of antibiotic susceptibility testing in a way that made it possible to compare between studies. Among these studies, erythromycin and tetracycline were the most tested antibiotics, being included in antibiotic susceptibility tests in all studies. Ampicillin, oxytetracycline and penicillin were also a popular choice for susceptibility tests.

#### ***Staphylococci***

Ten of the 21 pig studies focused on this genus.

Three studies (Gibbs et al. 2004, Gibbs et al. 2006, Ferguson et al. 2016) were culture-based and performed antimicrobial susceptibility testing. All 3 reported their results comprehensively enough to allow calculation of multi-antibiotic resistance. Erythromycin (macrolide) and tetracycline (tetracycline) were studied across all 3 studies, all of which tested *S. aureus*. Tetracycline resistance prevalence was high and less variable (always above 50% in all 3 studies), with an average of 79.9% (69.9% weighted mean; metaprop random

effects model) of strains being resistant. Erythromycin resistance was more varied and lower on average, with an average of 36.7% (64.4% weighted mean; metaprop random effects model) of strains being resistant.

None of the remaining *Staphylococcus* studies used molecular techniques purely to look at airborne antibiotic resistance. However, 2 studies did demonstrate the airborne colonisation of pigs and/or farm workers (Schmithausen et al. 2015, Rosen et al. 2018).

Other studies quantified the prevalence of MRSA among farms (Schulz et al. 2012, Schmithausen et al. 2015, Kraemer et al. 2017, Davis et al. 2018). One study (Schulz et al. 2012) determined whether MRSA were present or absent in air and at varying distances from a swine confinement building, but did not test antimicrobial resistance in any way.

### *Enterobacteriaceae*

Eight of the 21 pig studies focused on this genus.

Three studies (Gibbs et al. 2004, Gibbs et al. 2006, Chapin et al. 2005) were culture-based and performed antimicrobial susceptibility testing. All 3 reported their results comprehensively enough to allow calculation of multi-antibiotic resistance. Again, tetracycline and erythromycin were the only antibiotics studied by all these studies, and both were typified by high prevalence of resistance among *Enterobacteriaceae*, with prevalences of 64.6% (64.3% weighted mean; metaprop random effects model) and 68.0% (67.7% weighted mean; metaprop random effects model) respectively.

The prevalence of airborne ESBL among farms appeared to be highly variable, if difficult to quantify because of the different methods used.

- Schmithausen et al. (2015) measured the presence or absence of ESBL in air using the CHROMagarESBL method, and found ESBL-expressing *Enterobacteriaceae* in only 6 out of 35 pig farms in 50% (4 farms) and 100% (1 farm) of isolates.
- Gao et al. (2015) detected ESBL-producing *E. coli* using susceptibility testing (results not fully reported) in air samples from 50% of farms (3 out of 6) in 3, 1 and 2 out of 8 isolates respectively.
- Dohmen et al. (2017) passively sampled air using electrostatic dust collectors and found a low prevalence (<10% of dust samples) of CTX-M-gr1 (ESBL-associated resistance gene) in 10 out of 38 (26%) and 3 out of 36 (8%) farms (2 sampling moments over a 12 month period).
- Von Salviati et al. (2015) found ESBL/AmpC-expressing *E. coli* in 6 out of 63 (9%) indoor air samples and 2 out of 36 outdoor (5%, one 100m upwind, one 50m downwind).

### *Other*

Four out of 21 studies (Létourneau et al. 2010, Hong et al. 2012, Kumari and Choi 2014, Kumari and Choi 2015) used molecular characterisation to quantify

the abundance antibiotic resistance across species in a culture-independent manner. Three of these studies tested only for tetracycline resistance genes; all quantified the relative concentration of genes (using different units) related to the efflux pump mechanism (*tet(B)*, *tet(H)*, *tet(Z)*, *tet(G)*) and genes related to the ribosomal protection mechanism (*tet(O)*, *tet(Q)*, *tet(W)*) using qPCR (Hong et al. 2012, Kumari and Choi 2014, Kumari and Choi 2015). In all these studies, genes related to the ribosomal protection mechanism were, on average, more abundant than those related to the efflux pump mechanism. The remaining study (Létourneau et al. 2010) quantified *tet(G)* only and so it was not possible to compare with other genes. It was difficult to quantify the average concentration of these genes in the air due differences in the methods and units used to report them, but all tetracycline resistance genes were detected in all samples apart from the outgroup office air samples in Hong et al. (2012). This indicated again that pig CAFO aerosols were strongly associated with tetracycline resistance, although the 100% prevalence of these genes across all air samples indicated that resistance gene prevalence was higher than culturable resistant phenotype prevalence. Thus either culture-based methods do not capture the true extent of resistance in bioaerosols, and/or genotype is not straightforwardly linked with phenotype.

Murphy et al. (2007) reported antibiotic concentrations inside a swine feeding operation. They found tylosin concentrations were above the detection limit in 93% of air samples but lincomycin, which had been used at the facility before being discontinued, was only found in 9% of air samples.

### 2.3.2 Poultry

The 12 identified studies of poultry confinement buildings were also clearly motivated by the perceived emergent risk of airborne, multi-drug resistant pathogens emerging from CAFOs. However, more studies examined Enterobacteriaceae and ESBL (6 out of 12) and only 2 focused on MRSA and/or *S. aureus*.

Compared with pig CAFO research, a greater proportion of studies were more general in scope (4 out of 12), and related to this, molecular approaches to characterising antibiotic resistance in aerosols were more common. The highest coverage across antibiotic susceptibility tests across studies was again for tetracycline resistance genes, although only the *tet(W)* (ribosomal protection mechanism) was measured by more than one study (identified in all air samples in both studies), limiting generalisation.

#### *Staphylococci*

Of the studies looking at antibiotic resistance more generally, a single study (1 out of 7; Vela et al. 2012) used culture-based techniques and reported the results of antibiotic susceptibility testing in a way that made it possible to compare between studies. This study of the air in a broiler chicken barn tested *S. xylosus* resistance to ampicillin, erythromycin, penicillin, tetracycline, streptomycin, lincomycin, oxacillin, bacitracin, nalidixic acid and novobiocin resistance. It thus shared many of the 'core' tested antibiotics in pig CAFO studies, but also tested an expanded set of antibiotics (though it was not clear

why these particular antibiotics were chosen). Among the antibiotics tested, the topoisomerase inhibitors/quinolones nalidixic acid and novobiocin, exhibited by far the highest resistance prevalence, with 86% and 85% resistance among *S. xylosus* strains respectively. However, it was difficult to infer general patterns and draw conclusions from this single study of a single target species.

The one molecular-based study of antimicrobial resistance in airborne *S. aureus* (including MRSA) measured tetracycline, erythromycin and gentamicin resistance using 3 genes (Liu et al 2012):

- *tet(M)* (tetracycline resistance) was identified in 80% of all *S. aureus* colonies isolated from 6 farms
- *Erm(C)* (erythromycin resistance; rRNA methylase) was identified in 66% of samples
- *aac(6')-Ie-aph(2'')* (high level gentamicin resistance) was identified in 22% of samples

### *Enterobacteriaceae*

No studies performed and reported antibiotic susceptibility testing in a manner comprehensive enough to permit quantification here.

No studies reported the results of molecular quantification of resistance genes in Enterobacteriaceae specifically. However, Laube et al. (2014) did use mixed methods and detected ESBL/AmpC-expressing *E. coli* in the indoor air of 4 out of 7 (57%) and the outdoor air (50m) of 2 out of 7 broiler chicken farms (28%). Blaak et al. (2015) also used multiple methods and detected ESBL-producing *E.coli* in 2 out of 33 (6%) indoor barn air and 21 out of 35 (60%) indoor barn dust samples, with most positive samples coming from broiler (meat production) hen barns. Li et al. (2013) used antimicrobial susceptibility testing and reported ESBL-expressing *E. coli* in 40% of isolates from a chicken house in China. Thus ESBL prevalence appeared to be quite variable between studies and it was difficult to infer general patterns.

### *Other*

Three studies fully reported the quantification of antibiotic resistance genes in poultry CAFO air more generally, though they reported using different units (gene copies per m<sup>3</sup> of air, gene copies per 16S copies and gene copies per ng of DNA), making cross-study comparison difficult (Hong et al. 2012, Mazar et al. 2016, Gao et al. 2017).

Qualitatively, Hong et al. (2012) and Gao et al. (2017) both considered tetracycline resistance and found a higher prevalence of ribosomal protection tetracycline resistance genes (*tet(W)* in both) than efflux pump resistance genes (*tet(B)*, *tet(H)*, *tet(Z)*). This finding supports the results from pig CAFOs.

Mazar et al. (2016) reported similar levels of the abundance of *int1*, *mecA* and *qnrS* resistance genes between poultry CAFO air and outdoor air on top of a university building 9.18km from the poultry CAFO (though only one sample from the poultry CAFO was taken and results were purely correlative).



### 2.3.3 Cattle

Cattle CAFOs received the least coverage in the literature, with only 6 studies found concerning antimicrobial resistance in bioaerosols of concentrated cow feeding operations. In most of these studies (5 out of 6), tests of antibiotic resistance were highly focused on target pathogens or potential pathogens, namely MRSA and/or *S. aureus* (4 studies) or *E. coli* (1 study). One study (McEachran 2015) was more general in scope and utilised molecular characterisation of resistance genes.

#### *Staphylococci and/or MRSA*

Of the culture-based studies that reported antibiotic susceptibility fully (3 out of 4; Alvarado et al. 2009, 2012, McCreachan et al. 2015), all 3 were from the USA). Only ampicillin susceptibility was tested across all 3 studies of cattle feedyards, the overall resistance prevalence to which was 54.4% across strains. Penicillin and cefaclor were also covered by 2 of the studies by the same US research group working in the south-west USA (Alvarado et al. 2009) and in northern Mexico near the US–Mexico border (Alvarado et al. 2012). Resistance prevalence was found to be 67.0% (69.8% weighted mean; metaprop random effects model) and 27.9% (33.4% weighted mean; metaprop random effects model) respectively.

The other 2 studies are from the same Dutch research group and considered the factors promoting MRSA aerosolisation in veal farms (Graveland et al. 2012, Dorado-García et al. 2015). One noted that MRSA loads rapidly increase once calves are released from individual houses and allowed contact with other calves (Graveland et al. 2012). The other noted that, surprisingly, reduction of antibiotic use combined with jet cleaning on farms can actually promote increase levels of airborne MRSA in the barn (Dorado-García et al. 2015). This may be because antibiotic reduction reduces growth inhibition of MRSA and/or cleaning aerosolises long-accumulated, pathogen-laden dust (though more frequent cleaning programmes may be more effective).

#### *Enterobacteriaceae and/or ESBL*

One study measured *E. coli* in cattle feedyard aerosols in Spain (Navajas-Benito et al. 2017). It tested the susceptibility of isolates to tetracycline (88% resistance prevalence), gentamicin (11%), ciprofloxacin (33%), nalidixic acid (55%), trimethoprim/sulfamethoxazole (77%), tobramycin (11%) and azomicillin/clavulanate (22%). No ESBL-producing *E. coli* were found among the isolates.

#### *Other*

The single molecular-based study (McEachran et al. 2015) in line with pig and chicken CAFO studies examined tetracycline resistance genes (*tet(B)*, *tet(O)*, *tet(Q)*, *tet(W)* and *tet(L)*).

Downwind of the cattle feedyard (~10–20m), the highest prevalence of resistance among bioaerosol samples was found to be associated with

ribosomal protection mechanisms (*tet(Q)*, *tet(O)*, and *tet(W)*), with almost no efflux pump genes (*tet(B)* and *tet(L)*) detected. None of the tetracycline resistance genes were detected upwind of the feedyard, but all were detected downwind.

McEachran et al. (2015) also authors measured actual antibiotic concentrations downwind of the feedyard. Tetracycline antibiotics (tetracycline, chlortetracycline and oxytetracycline) were detected in downwind air samples from 6 out of 10 feedyards sampled, with oxytetracycline detected at all 10. Monensin (a polyether antibiotic) was detected in all air samples both downwind and upwind of the feedyards (below quantitation in the latter) and had the highest downwind concentration. This suggested that the spread of resistance to this antibiotic was also likely, though this was not measured.

### 2.3.4 Summary of general trends in CAFO research

- Across pig, poultry and cow CAFO studies, tetracycline resistance was consistently the most prevalent type of resistance detected among airborne isolates. This finding held even when the bias towards studying this type of resistance was accounted for.
- Erythromycin and nalidixic acid resistance also appear to have relatively high prevalence of resistance, though the prevalence of resistance was more variable.
- Antibiotic resistant organisms have been found in air at least 200m downwind of CAFOs. There is some evidence that this finding also applied to antibiotics themselves.
- MRSA and ESBL prevalence appeared highly variable between studies and it is unclear what drives differences.

## 2.4 Wastewater treatment plants

Wastewater treatment plants (WWTPs) have had a long association with antibiotic resistance. They reduce the overall numbers of microorganisms in the input water, but as sites where many different microbial communities harbouring antimicrobial resistance and antimicrobial residues meet (for example, agricultural and pharmaceutical wastes), they can also be hotspots for the selection and exchange of antimicrobial resistance genes.

In addition, concern has grown about WWTPs because of the mechanical treatment processes they use such as aeration and mixing that explicitly encourage aerosolisation. To meet this concern, a smaller research theme has emerged around antimicrobial resistance in the air of WWTPs. Many of the 9 studies identified came from Poland (5 out of 9) and almost all studies were culture-based (8 out of 9). Again there was a heavy focus on staphylococci (3 out of 9) and Enterobacteriaceae/ESBL resistance (3 out of 9).

## 2.4.1 Findings

### *Staphylococci and/or MRSA*

Of the 3 studies focusing on staphylococci, only one was reported well enough to allow comparison of multi-antimicrobial resistance. This single remaining relevant study investigated resistance to erythromycin, tetracycline, gentamicin, streptomycin, vancomycin, lincomycin, oxacillin, novobiocin and methicillin, and found the highest prevalence of resistance was to novobiocin (66%) and lincomycin (33%). Erythromycin and tetracycline, which were found to have a high resistance prevalence among airborne staphylococci from CAFOs, had resistance prevalences of only 19% and 9% respectively. However, it is difficult to draw general conclusions from this single study.

Of the other 2 studies, Małecka-Adamowicz et al. (2016) only reported percentages and did not state the number of isolates tested. De Luca et al (2001) quantified the numbers of airborne coagulase-negative staphylococci (a type of *Staphylococcus* frequently associated with disease and higher levels of antibiotic resistance) and found that numbers were highest in an enclosed area containing the fine screens.

### *Enterobacteriaceae and/or ESBL*

The 3 studies focusing on airborne Enterobacteriaceae resistance were more easily comparable, as all examined common usage and 'last resort' antibiotics (Korzeniewska and Harnisz 2013, Korzeniewska et al. 2013, Teixeira et al. 2016). All 3 studies tested for resistance to 2 third-generation cephalosporins<sup>3</sup> (beta lactams) – ceftazidime and cefotaxime – and found average resistance prevalences of 37.8% (32.0% weighted mean; metaprop random effects model) and 59.9% (62.0% weighted mean; metaprop random effects model) respectively. This was despite the fact that only one of the studies looked at a WWTP with hospital influence; resistance was 80–100% in the hospital-influenced WWTP (Korzeniewska et al. 2013) and 33–50% in the 2 municipal WWTPs (Korzeniewska and Harnisz 2013, Teixeira et al. 2016) examined.

Two of the studies tested for susceptibility to gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, imipenem and piperacillin (with tazobactam) and the results displayed different resistance profiles for the 2 WWTPs. Erythromycin resistance was not studied in Enterobacteriaceae, and only Teixeira et al. (2016) tested for tetracycline resistance, reporting a mean prevalence of 38%.

The studies by Korzeniewska and Harnisz (2013) and Korzeniewska et al. (2013) also included phenotypic tests for ESBL in the form of (positive) ceftazidime, cefotaxime and cefpodoxime tests (some consider three-way resistance a positive test for EBSL resistance). Korzeniewska et al. (2013) reported ESBL in 27.9% of air samples from a hospital-influenced WWTP, while Korzeniewska and Harnisz (2013) reported it in 23.8% of samples from a non-

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<sup>3</sup> Higher 'generations' of cephalosporins tend to have greater activity against Gram-negative bacteria and less against Gram-positives, with the exception of fourth-generation cephalosporins, which are truly broad spectrum.

hospital influenced WWTP. Plasmid-mediated genes encoding beta lactam resistance (the same *bla* genes were searched for in both studies) were detected in over 38% (*E. coli*) and 32% of (Enterobacteriaceae) isolates respectively.

### *Other*

Only one (Zhang et al. 2018) of the 3 other studies out of the 9 identified was reported well enough to be able to extract antibiotic resistance data. This large-scale study examined resistance to third-generation cephalosporins among 774 diverse Gram-negative bacteria isolated from a Chinese pharmaceutical WWTP. It found the highest resistance prevalence to ampicillin (60%) and cefazolin (64%, first-generation cephalosporin), intermediate levels of resistance to monobactams and second-generation cephalosporins (aztreonam, cefotetan; 40% and 37% respectively) and low levels of resistance to third-generation cephalosporins (ceftazidime) and carbapenems (ertapenem) (10% and 16% respectively). Third-generation cephalosporins are considered to be the most effective against Gram-negative bacteria and the least effective against Gram-positive, so these results are not surprising given that only Gram-negative bacteria were tested.

Of the other 2 studies, antibiotic resistance data could not be extracted in one because only millimetre zones of inhibition was reported in ranges for different species (Kowalski et al. 2017). In the other, the presence of an antibiotic resistance gene was confirmed but not quantified (Li et al. 2016).

## **2.4.2 Summary of general trends in WWTP research**

- Tetracycline and erythromycin resistance at WWTPs appeared to be lower than that found at CAFOs.
- Resistance to cephalosporins was frequently tested and appeared to be high, especially where the WWTP received hospital and/or pharmaceutical waste.
- Little is known about the downwind spread from WWTPs.

## **2.5 Urban areas**

Studies of antimicrobial resistance in urban air (public areas) formed the second most studied environment (14 out of 88 studies). Much of this work was motivated by growing concern from approximately 2016 onwards about people's everyday exposure to antimicrobial resistance and/or air pollution affecting people. For the majority, this takes place in cities.

Much of the earlier pioneering work on this topic was conducted in smaller cities on local scales, looking at target organisms in focal locations such as metro stations or pigeon feeding or roosting sites. More recently, this field has developed into attempts to get a more general overview of antimicrobial resistance in urban air, often using molecular techniques and new sampling strategies to try and achieve this.

Seven of the 14 studies used culture-based techniques to look at target pathogens and/or suspected high risk sites in urban areas; 4 focused on MRSA and/or *Staphylococcus* species, 2 on potentially pathogenic fungi associated with pigeon excrement and one on *E. coli*.

Two of the studies did not attempt to identify the bacteria/fungi isolated or identified them only coarsely (for example, 'rods', 'Enterobacteriaceae', 'mesophilic bacteria', 'mould fungi').

Seven out of the 14 studies were general in scope; all used molecular characterisation of antimicrobial resistance. Within this category, 3 of the studies were large-scale, global studies employing metagenomic (or shotgun) techniques to compare antimicrobial resistance between disparate cities and/or ecosystems. Unlike 16S rRNA amplicon sequencing or qPCR, metagenomic sequencing does not rely on amplifying specific regions (for example, target genes) of DNA and instead sequences genomic material as a whole, giving an overview of the genetic material in a given sample/environment. Combined with global scale studies comparing many cities using the same method, this method has the potential to revolutionise the way antimicrobial resistance in urban air is understood and monitored.

## 2.5.1 Findings

### *Staphylococcus and/or MRSA*

All 4 of the studies that focused on MRSA and/or *Staphylococcus* species tested susceptibility to tetracycline and erythromycin (Chihara and Someya 1989, Zhou and Wang 2013, Yadav et al. 2015, Sivri et al. 2016). The average prevalence of resistance among strains was 20.3% (24.4% weighted mean; metaprop random effects model) and 55.1% (30.5% weighted mean; metaprop random effects model) respectively. The lower resistance to tetracycline indicated that urban air had a different susceptibility profile to agricultural air, in line with the higher use of tetracycline in agricultural settings.

### *Enterobacteriaceae and/or ESBL*

No studies looked at Enterobacteriaceae and/or ESBL in outdoor urban air.

### *Fungi*

The 2 studies that investigated the potential risk of exposure to pathogens associated with pigeon excrement (Soares et al. 2005, Naz et al. 2017) were difficult to interpret and compare due to specific and general methodological issues.

Naz et al. (2017) took air as well as soil samples at pigeon feeding sites in Karachi in Pakistan, but they did not report the proportion found in air versus soil, and in addition, only reported the zone of inhibition (mm) around each of the antibiotic susceptibility disks tested on each fungi. The latter point reflects a more general problem with antifungal susceptibility testing, rather than with the study per se. Not only is the disk diffusion method not validated by EUCAST or

CLSSI, but the broth microdilution technique has only been validated for *Candida* species.

The study by Soares et al. (2005) was also limited by this general problem with fungal susceptibility testing. Although it did report the proportions of *Cryptococcus neoformans*<sup>4</sup> found in air versus excrement, it used EUCAST breakpoints on *Candida* to infer resistance or susceptibility to antifungals in *Cryptococcus*. Using this limited method, resistance was reported only to fluconazole (not 5-flucytosine, itraconazole, voriconazole, amphotericin B) in *C. neoformans* isolated from excrement; *C. neoformans*, however, was not found in the air at any of the 37 pigeon excrement sites tested. These results suggest that antifungal-resistant pathogenic fungi are not easily aerosolised from pigeon excrement and thus do not present a great risk for the spread of antifungal resistance in an urban setting. However, this conclusion is very tentative given the limitations already stated and that this is a single study in which the sites were only sampled for 15 minutes and using a crude method (agar plate exposure).

## General

These 7 studies all used molecular methods to characterise antimicrobial resistance in urban air. Quantitative comparison between these studies was hindered by the fact that each used different methods, studied different genes and/or reported results in different units. Nonetheless, some similarities and differences did emerge.

For example, all the studies on beta lactam resistance (Echeverria-Palencia et al. 2017, Hu et al. 2018, Li et al. 2018), which covered 4 Californian and 2 Chinese cities, identified beta lactam resistance genes as having the highest abundance in air samples relative to other genes. The same studies and one other in Tel-Aviv (Mazar et al. 2016) also identified sulphonamide resistance genes as having a low relative abundance in urban air. Quinolone resistance genes appeared to have a low relative abundance in 2 studies (Hu et al. 2018, Xie et al. 2018), but a higher relative abundance in another (Li et al. 2018).

Tetracycline resistance genes had a moderate relative abundance in the same 3 studies (Hu et al. 2018, Li et al. 2018, Xie et al. 2018).

Resistance to macrolides – including erythromycin – appeared more variable between studies, although the erythromycin resistance gene *erm(B)* was found to be higher in rural areas in one study (Xie et al. 2018).

As demonstrated by Li et al. (2018), these patterns of relative airborne abundance of resistance genes associated with particular antibiotic groups are highly correlated with the global antibiotic usage of each group.

In the one study that did compare resistance mechanisms (not specific to tetracyclines), efflux pump mechanisms appeared to predominate over

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<sup>4</sup> A target pathogen associated with pigeon excrement that can fatally infect immunocompromised people.

ribosomal protection mechanisms, but enzyme inactivation genes were the most abundant.

Two studies quantitatively measured the class 1 integron gene (a gene associated with horizontal transfer of antibiotic resistance) and suggested that it had higher abundance in 'pristine' environments such as rural areas or on 'dust-free' days (compared with urban areas or during dust storms respectively), and was positively correlated with the abundance of antibiotic resistance genes (Mazar et al. 2016, Xie et al. 2018).

Like Li et al. (2018), the remaining 2 studies employed metagenomic techniques to look at antibiotic resistance gene abundance across disparate ecosystems (Fondi et al. 2016, Pal et al. 2016). However, they used others' data, only a small proportion of which were from air samples. Nonetheless, these studies are highly revealing.

Pal et al. (2016) was admittedly limited in its generalisability because air samples were only from one study and this was from the Beijing smog event study (Hu et al. 2018). However, it noted that smog appeared to:

- have a distinct antibiotic resistance profile compared with all other ecosystems considered
- to be an amalgam of all other types of environment considered (including wastewater sludge, pharmaceutically polluted water samples, soil, sediment, water, mine, human and animal gastrointestinal microbiomes, and skin and airway samples)

Briefly, wastewater sludge and pharmaceutically polluted water samples were dominated by sulphonamide resistance genes, soil/sediment/water/mine samples by beta lactam resistance genes, human and animal microbiomes by tetracycline resistance genes, and skin and airway samples by macrolide (includes erythromycin) resistance genes. Beijing smog appeared to be a mix of these with no obvious dominant, suggesting that smog at least (it is difficult to make conclusions about air generally) serves as a mixing medium for antibiotic resistance genes from various terrestrial sources. Interestingly, skin and airway samples had the most similar antibiotic resistance gene (but not compositional) profile to Beijing smog, suggesting that peoples' skin and airway microbiota readily receive antibiotic resistance genes from the air.

These findings are partially validated by Fondi et al. (2016) who used a network approach to look at patterns of putative horizontal gene exchange of antibiotic resistance genes. They found that host, sludge and air ecosystems clustered tightly in the horizontal gene transfer network, suggesting horizontal gene transfer between organisms found in these environmental compartments. Pal et al. (2016) also found the mobile genetic element abundance was highest in air (smog), wastewater sludge and pharmaceutically polluted water samples, suggesting that bioaerosols in urban air represent bacteria that are receiving antibiotic resistance genes from disparate sources.

## 2.5.2 Summary of general trends in urban research

- Resistance to erythromycin appeared more prevalent than resistance to erythromycin across urban settings and the types of method used to study this.
- Urban fungal species associated with pigeon excrement appear to be an emerging area of interest in terms of airborne antimicrobial resistance, but methods remain poorly standardised.
- The antibiotic resistance profile of urban air appears to consist of a range of sources.

## 2.6 Other environments

Other studies (17 out of 88) covered airborne antimicrobial resistance in various environments other than CAFOs, WWTPs and public urban areas. In order of the most coverage, these included:

- indoor spaces – homes, offices, nurseries (9 out of 88)
- arable farming (4 out of 88)
- hospitals (4 out of 88)
- horse stables (2 out of 88)
- other environments covered by one study each (10 out of 88)

Although coverage of these environments was lower than the main research areas, they provided interesting information with which to contextualise results found in the main environments and interesting results in their own right.

### 2.6.1 Homes, offices and nurseries

#### *Staphylococcus and/or MRSA*

##### Homes

The most well-reported studies of antimicrobial resistance in airborne *Staphylococcus* were 3 featuring homes (houses, flats and so on) (Gandara et al. 2006, Lenart-Boroń et al. 2017, Madsen et al. 2018). However, the different studies tested different antibiotics, making direct comparison difficult.

The study by Gandara et al. (2006) was part of the research group's wider efforts to understand human exposure to airborne antimicrobial resistance from cattle CAFOs. They sought to understand whether residential homes in El Paso, Texas, were exposed to airborne *S. aureus* with a similar resistance profile to the CAFOs (ampicillin, penicillin and cefaclor). Resistance profiles were similar to the cattle CAFO studies – prevalences of 49%, 53% and 11% resistance were found to ampicillin, penicillin and cefaclor respectively, suggesting an influence of the Texan agricultural environment on airborne *S. aureus* resistance profiles. More *S. aureus* (183 isolates) with a slightly higher average



prevalence of resistance to these antibiotics were identified inside homes than nearby outside.

Lenart-Boroń et al. (2017) found 63% resistance to erythromycin, 23% resistance to tetracycline and <10% resistance to the other types of antibiotics tested among 55 *Staphylococcus* isolates from flats in Krakow in Poland. This profile was to some extent consistent with the broad picture of the antimicrobial resistance profile of urban air found in other studies. *mphC* and *msrA1* appeared to be the predominant genes responsible for erythromycin resistance.

The remaining study (Madsen et al. 2018) tested only for oxacillin resistance among MRSA stains in the air of homes of Philadelphia in the USA, and found a prevalence of 23%. Although the study did not report on antimicrobial resistance explicitly, it was still noteworthy in that *S. aureus* was isolated only from one indoor environment (including homes and offices) and this was found in a farmhouse and was a clonal lineage typically isolated from pigs. This suggests an influence of the pig farm on the airborne antimicrobial resistance profile of farmhouses, but only 1 of the 6 occupants proved to be MRSA-positive and MRSA was only isolated at one point in time, suggesting the influence (especially on human health) may be minor.

### **Offices**

Two Polish studies investigated staphylococci in offices not associated with CAFOs or WWTPs, though one was not amenable to interpretation given that only growth inhibition zones were reported (Bragoszewska et al. 2018). The remaining study (Wolny-Koładka et al. 2017) identified 33% resistance to tetracycline, 57% resistance to erythromycin, 14% resistance to gentamicin and ≤10% resistance to other antibiotics including cephalosporins and fluoroquinolones.

### **Nurseries**

One Polish study (Kubera et al. 2015) investigated airborne *Staphylococcus* in nurseries and highlighted 20% resistance to tetracycline, 48% resistance to erythromycin, 100% resistance to gentamicin and 60% resistance to rifampicin among 25 airborne isolates. Other antibiotics were also between 16% and 48% resistance prevalence, unlike other studies.

### ***Enterobacteriaceae and/or ESBL***

In the study by Rosas et al. (1997) of *E. coli* in the air of residential homes in Mexico City, 5 serotypes were identified from indoor airborne dust; 4 of these appeared to originate from indoor soil. Two of these serotypes displayed resistance to ampicillin, ticarcillin, piperacillin and tetracycline (22% of tested antibiotics), while the others displayed resistance to these antibiotics plus trimethoprim, cephalothin, gentamicin, tobramycin and chloramphenicol (66% of tested antibiotics). The serotype with the wider ranging resistance was the one not also found in indoor soil, suggesting that the resistance profile of airborne bacteria in homes may be related to the source of the bacteria.

No studies looked at *Enterobacteriaceae* and/or ESBL in offices or nurseries.

## *Fungi*

No studies reported on airborne antifungal resistance in homes.

## *Other*

No studies looked at airborne antimicrobial resistance in microorganisms more generally.

### **2.6.2 Arable farming**

Although there were 4 studies of antimicrobial resistance in arable farming environments (for example, vineyards, rice paddies, orchards), none were reported well enough to permit comparison. These studies showed a greater focus on airborne fungi, driven by vineyard studies and concerns about antifungal resistance among vineyard fungi.

The study by Lago et al. (2014) of Portuguese vineyards was motivated by the fact that Portuguese vineyards consume more pesticides than any other sector in the country; most of the antifungals used are azoles due to their low toxicity to humans. Although MICs for several airborne *Aspergillus* species, penconazole, itraconazole, posaconazole and voriconazole were reported, there is currently insufficient evidence to interpret these values according to EUCAST thresholds. Nonetheless, a few isolates spread across multiple species had extremely high resistance ( $\geq 32$ mg per litre) to penconazole compared with other species and patterns in other antifungals, suggesting this may be an emerging concern in airborne vineyard fungi.

The other vineyard study (Korolev et al. 2011) reported resistance to benzimidazoles and dicarboximides as having the highest prevalence among *Botrytis cinerea* isolates, with the former increasing after 3 years of application of the fungicides in the field trial. Again, it is hard to contextualise these results quantitatively, but nonetheless there is evidence that azole resistance is an emerging problem among vineyard fungi that are airborne.

The study by Heo et al. (2010) of antimicrobial resistance in airborne bacteria from rice field, landfill and waste incineration sites was difficult to interpret because of the non-standardised methods used. However, it but suggested that *Staphylococcus* strains had a higher prevalence (%) of resistance to chloramphenicol and gentamicin than other airborne species (*Micrococcus* and *Microbacterium*).

Feng et al. (2015) reported airborne *E. coli* at higher levels than in irrigation water (but less than in soil, fallen fruit and fresh fruit) in Chinese kiwi orchards. They did not report the results of antibiotic susceptibility testing by each environmental compartment, but reported the highest levels of resistance to tetracycline (35%) and ampicillin (31%) among all isolates.

### **2.6.3 Hospitals**

Several studies of antimicrobial resistance in hospital air exist, but those relevant studies concerning numbers and antimicrobial resistance profile in

hospital air were surprisingly only a small fraction of many hospital studies identified in our initial literature search (5 out of 204).

One of the most interesting studies was by Ling et al. (2013), who measured *tetX*, *tetW* and *int1* genes in pig and cattle CAFOs, hospital clinics and outdoor environments. Tetracycline resistance genes were identified in both CAFOs and clinics, but at much lower levels and more infrequently in clinics and not identified in outdoor environments. *tetW* (ribosomal protection mechanism) was more frequently observed than *tetX* (enzyme inactivation). Class 1 integron genes were only identified in CAFOs.

Three studies looked at antimicrobial resistance among airborne isolates in hospital air with varying degrees of completeness.

Three studies using culture and molecular methods suggested that MRSA-positive isolates are likely to be more abundant than ESBL and/or Enterobacteriaceae positive isolates in hospital air (Malpani and Nanoty 2011, Best et al. 2018, Gao et al. 2018b).

Three studies (Malpani and Nanoty 2011, Solomon et al. 2017, Gao et al. 2018b) suggested that different types of hospital wards (for example, inpatient, outpatient, maternity) are likely to have similar microbiological profiles, while operating theatres have the lowest bacterial counts and waiting rooms have median levels.

Finally, one study (Loeffert 2018) explored whether demolition works at a French hospital were aerosolising azole-resistant fungi and causing patient infections with multi-drug resistant aspergillosis. However, it was reported that both clinical and airborne *A. fumigatus* isolates displayed no increased azole resistance (that is, above EUCAST clinical breakpoints), although there was no significant difference between the resistance profiles of the clinical and airborne isolates, leaving open the possibility that they were the origin of infection.

#### **2.6.4 Horse stables**

Two studies from the same research group reported on airborne antimicrobial resistance in horse riding centres – of concern because horses are frequently treated with the same or similar antibiotics to humans (Wolny-Kołodka 2018, Wolny-Kołodka and Lenart-Boroń 2018).

Wolny-Kołodka (2018) reported on airborne antimicrobial resistance among airborne staphylococci. Erythromycin resistance prevalence averaged 3.6%, tetracycline 20% and gentamicin 18%; other antibiotics had average resistances that fell below 10%. Qualitatively this was similar to the CAFO antibiotic resistance profile. Doxycycline had the highest resistance prevalence at 30%. The presence of the *mecA* (MRSA-positive) gene among airborne isolates was variable between stables, but <20% in 2 stables (one indoor, one free range) and 50% in one stable.

Wolny-Kołodka and Lenart-Boroń (2018) performed a similar analysis on *E. coli* isolates from the same horse stables. As expected given the narrower taxonomic scope, numbers of *E. coli* isolated were lower than staphylococci. Antibiotics studied were broader and different to the other study, but the prevalence of tetracycline and gentamicin resistance (tested in both studies)

was lower (10% and 2% respectively). ESBL positivity among isolates was variable between stables, while *blaTEM* genes were found in the majority of airborne isolates. No *E. coli* was isolated from the air at the free range stable.

### 2.6.5 Other

The remaining 10 studies were more scattered in topic and represented single case studies of different types of environment, making it difficult to make any general conclusions. These studies covered environments such as:

- a lake (Escalante et al. 2014)
- a wet poultry market (Gao et al. 2016)
- a church (Abdulla et al. 2008)
- a space station (Be et al. 2017)

Some of the most interesting studies were those of industrial environments, especially those in which antimicrobial-resistant organisms seemed to be accumulated and aerosolised due to production processes (akin to the WWTPs). For example, a study of French sawmills identified these environments as potential hotspots for the selection and aerosolisation of antifungal-resistant fungi, given the heavy use of azoles in wood treatment and the nature of sawmill production (Jeanvoine et al. 2017). However, this large-scale study searching air (200 samples) and substrate (600) samples from 20 sawmills for azole-resistant *Aspergillus fumigatus* did not find azole-resistant strains in air samples – though this was probably due to small sample size. Nonetheless, the predominant strains in air and substrate samples were the same, and 21 out of 24 substrate-isolated strains were resistant to 4 azoles. Many of these strains carried genes for azole resistance, and the number of resistant strains was significantly elevated in sawmills using fungicides (propiconazole + tebuconazole) and correlated with fungicide use. This leaves open the possibility that sawmills could be a neglected source of airborne antifungal resistance, but more studies with better air sampling and wider in taxonomic and geographical scope are required.

Another study was one of the only studies to look at airborne viruses and antimicrobial resistance, identifying airborne viruses in Italian cheese production plants that carry antibiotic resistance genes (Colombo et al. 2018). This highlighted the fact that airborne viruses can act as airborne vectors for the spread of antibiotic resistance genes.

One study highlighted that it is not only liquid or sewage waste that can be a reservoir of airborne antimicrobial resistance, identifying *Enterobacter cloacae* isolates that were resistant to third-generation cephalosporins in bioaerosols of these plants, with the highest levels in confined waste spaces (as seen in WWTPs) (Casini et al. 2015).

Finally, a study of a Chinese composting plant identified a predominance of *Staphylococcus* species over *E. coli* and high levels of tetracycline, sulphonamide and erythromycin genes in air ( $>10^3$  copies per  $m^3$ ) and suggested livestock manure as the source (Gao et al. 2018a).

## 2.6.6 Summary of general trends in other research

- The air of indoor residential and working environments (including hospitals) appears to have antibiotic resistance profiles that reflect their location. For example, urban indoor air has more erythromycin than tetracycline resistance, and there is some limited evidence that rural indoor air reflects agricultural antibiotic resistance profiles.
- The potential problem of airborne fungal antimicrobial resistance has received some attention in arable farming, hospital and industrial (sawmills) contexts. However, few studies have been able to clearly link airborne antifungal resistance to activities in that environment and not enough is yet known about the clinical relevance of antifungal resistance levels found to interpret these studies properly.
- Other environments provide interesting information to support conclusions about CAFOs, WWTPs and urban areas harbouring particularly high levels of resistance and particular types. However, coverage of these environments is currently too low to say whether they pose a significant risk of airborne antimicrobial resistance.

## 2.7 Comparison across types of air

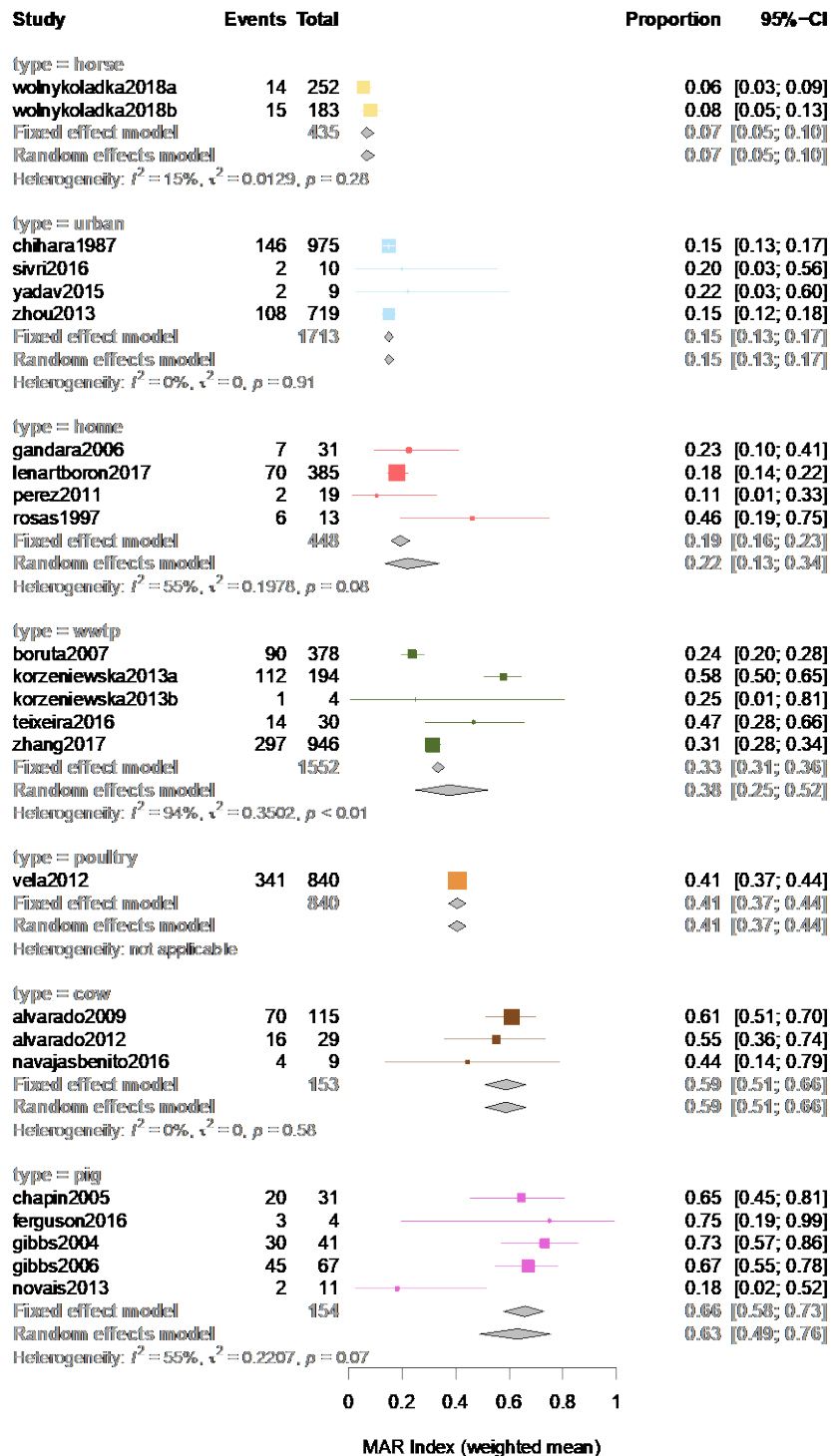
To summarise and contextualise the literature as a whole, an attempt was made to use the available antimicrobial susceptibility testing data to perform a meta-analysis of proportions, comparing total multi-antibiotic resistance between different types/environmental sources of air. To do this, the `metaprop` function of the `meta` package in R (Schwarzer 2007) performed on a per study basis was used. The 'observations' parameter for this was the mean total number of observations of resistance across all antibiotics tested per study (averaged across tests within study for example, across different sites or strains tested). The 'events' parameter was the mean total number of possible observations of resistance across all antibiotics tested per study (averaged across tests within study for example, across different sites or strains tested).

Thus implicitly, the meta-analysis compared the MAR index (Krumperman 1983) between studies and across types/environmental sources of air. The MAR index summarises the number of tested strains that were resistant to multiple antibiotics, allowing a general comparison to be made across studies and qualitative assertions about the literature to be validated. Incorporated within this analysis is a weighting of each MAR index's contribution to the group (in this case type/environmental source of air) by the number of strains tested. This was an important feature given that differences between studies and their reporting of results meant that there was a large range of numbers of strains tested (1 to 208) per row of the antimicrobial susceptibility testing data frame used in the analysis.

The results indicated that there were significant differences both between types/environmental sources of air (random effects model:  $Q = 351.66$ ,  $df = 6$ ,  $P < 0.0001$ ). The MAR index ranged from 0.06 to 0.65 in ascending order from horse riding centre air through that of urban areas (outdoor), indoor environments, WWTPs, poultry CAFOs, cow CAFOs and pig CAFOs (Figure

2.1). This was broadly in line with the qualitative impression of the data. In addition, it revealed a broad transition from a low MAR in outdoor air (horse riding centres, urban outdoor air) to a higher MAR in studies that sampled indoor air as part of their studies (homes, WWTPs, CAFOs). This makes sense given that airborne microorganisms produced by a source (for example, pigs, humans) are likely to be more concentrated in enclosed, more 'trapped' air, while in less stable outdoor air, multi-antibiotic resistant airborne microorganisms are probably frequently diluted by other air.

The meta-analysis was additionally validated by performing additional (but crude) statistical tests on the data. This was to validate that the type/environmental source of air was still important once factors other than the number of strains tested were accounted for (for example, number of antibiotics tested, type of strain(s), antibiotics tested). Three additional separate analyses validated the conclusion that the type/environmental source of air was the most important factor (of those they were data for) in determining multi-antibiotic resistance (Appendix B).



**Figure 2.1 Forest plot comparing MAR indices between types and environmental sources of air (and studies)**

Notes: CI = confidence interval

## 3 Conclusions

### 3.1 What is known

The studies identified from the literature differed in the methods used, study environments, antibiotics studied, target organisms and other factors. Despite this, several widely (but qualitatively) supported conclusions emerged from the literature review. These provide a strong foundation for future research on, and the management of, airborne antimicrobial resistance. These conclusions are discussed here.

#### 3.1.1 Airborne antimicrobial resistance profiles reflect their environment

**This conclusion is strongly supported by current research.**

Across the studies, the dominant antimicrobial resistance phenotypes and genotypes in air were found to be clearly and strongly correlated with land use types.

Although it is possible that airborne microbial communities may be distinct from non-aerial microbial communities due to the tendency for Gram-positive organisms to have greater survival in the desiccated and ultraviolet-exposed environment of the air (Heo et al. 2010, McEachran et al. 2015), strong evidence that airborne antimicrobial resistance profiles were distinct from their non-aerial habitat was not found.

Globally, airborne antimicrobial resistance profiles of cities, for example, are strongly correlated with global antibiotic usage (Li et al. 2018); this should be treated as a null hypothesis for the composition of air. However, the literature review also revealed evidence of finer scale variations associated with specific environments, with culture-based antimicrobial susceptibility testing and genetic approaches telling similar stories.

For example, although there was bias towards testing for tetracycline resistance it was clear that, among CAFOs of all types and bacteria of all types, there was consistently a high prevalence of tetracycline-resistant strains compared with other antibiotics tested. This is in line with the high use of this antibiotic in intensive livestock farming, especially as a growth enhancer in animal feed in the USA, China and India (Granados-Chinchilla and Rodriguez 2017). This was to some extent also true for erythromycin (also used extensively in livestock farming), though this result appeared more variable. In WWTPs, the prevalence of tetracycline resistance was consistently much lower, and there was some evidence from staphylococci that erythromycin resistance was higher (though still lower than CAFOs) but most noticeably, resistance to cephalosporins was higher in WWTPs than in CAFOs (even those not influenced by pharmaceutical pollution). This latter conclusion, however, may be misleading because antimicrobial susceptibility tests with cephalosporins were mainly only conducted in WWTP studies.



Urban areas appeared to have a similar profile to air at WWTPs – in line with greater human influence. They also had a low abundance of tetracycline resistance, a slightly higher but more variable abundance of erythromycin resistance phenotypes/genes, and a dominance of beta lactam resistance (including cephalosporins). This was again in line with human influence.

Studies of households, offices and nurseries provided a natural experiment to validate these assumptions further. Because households are relatively similar, their air would be expected to be shaped by the wider environment in which they are found. Indeed, urban indoor environments demonstrated a higher resistance to erythromycin than tetracycline. In contrast, 2 studies of homes in areas influenced by livestock farming (Gandara et al. 2006, Madsen et al. 2018) indicated a qualitative similarity to nearby CAFO air (for example, in terms of antibiotic resistance profile and the presence of a pig-related strain of MRSA in farmhouse air).

In summary, there is ample evidence that airborne antimicrobial resistance profiles are related to the terrestrial habitat.

### **3.1.2 Industrial operations producing and/or processing faecal matter associated with the highest levels of multi-antibiotic resistance in the air**

**This conclusion has a limited level of support from current research.**

The environments with the highest MAR indices were the 3 types of CAFO and WWTPs, all of which processed large amounts of animal and/or human faeces. CAFOs (at least pig and poultry ones) appeared to have the highest MAR indices, adding weight to this conclusion given the amount of manure produced by the operations. These operations were also similar in that they involve almost constant air disturbance through moving parts and/or animals, providing ample opportunity for the aerosolisation of microorganisms.

Concurring with this, several studies found that areas and/or stages of the production process most associated with aerosolisation and enclosure (for example, screening rooms in WWTPs and during flocking indoors in poultry farming) had the highest levels of airborne resistance. As an 'outgroup' to this observation, one study (Soares et al. 2005) looking at the pathogenic fungus *Cryptococcus neoformans* identified the organism (including a fluconazole-resistant strain) in 11 out of 79 excrement samples but in none of the 37 air samples from pigeon feeding sides. This again suggests that faecal organisms require constant aerosolisation to become consistent members of airborne microbiota.

This is in line with the idea that, while Gram-positive staphylococci dominate airborne microbiota in poultry barns and can survive in air, they are adapted to desiccated environments (Chaibenjawong and Foster 2011). In addition, faecal bacteria survive for shorter periods and therefore require re-aerosolisation or a long-term reservoir (Schulz et al. 2016). Furthermore, several studies found that higher airborne antimicrobial resistance is promoted in CAFO operations that allow the build-up of faeces due to manure removal mechanisms and/or dirt, followed by re-aerosolisation (for example, through jet wash cleaning).

Thus there appear to be 2 major conditions driving the prevalence of airborne multi-antimicrobial resistance:

- large amounts of antibiotic resistant microbe-laden faeces
- continuous kinetic processes promoting aerosolisation and re-aerosolisation

## 3.2 Emerging major hypotheses for further testing

### 3.2.1 Intensive animal farming operations are the most problematic source of airborne antimicrobial resistance

**This conclusion is strongly supported by current research.**

Current research suggests that intensive animal farming operations are the major producer of antimicrobial-resistant bioaerosols. As already noted, this is logical given that CAFOs use large amounts of antibiotics, apply them onsite and produce large amounts of antibiotic-laden faeces. Conversely, WWTPs are likely to deal with faecal material in a less concentrated form, with less antibiotic selection pressure in the immediate environment.

The generalisability of this hypothesis, however, makes a number of assumptions about:

- how the risk of airborne antimicrobial resistance risk should be evaluated
- how translatable this hypothesis is to the UK's agricultural landscape

Almost all the studies of CAFOs identified were from the USA or China, where it is legal to use antibiotics as growth promoters in animal feed (especially tetracycline and erythromycin) and antibiotic use is at much higher levels than elsewhere. In the EU, the use of antibiotics as growth promoters in animal feed has been banned since 2006, meaning that the antibiotic resistance of bioaerosols from these operations in the UK is likely to look very different to those in the USA.

In addition, US and Chinese operations are much larger on average than EU/UK operations which could make the latter less of a risk. However, there was no evidence of a strong positive correlation between the number of animals in an operation and the number of bacteria/antibiotic resistance genes produced (indeed the limited assessment suggested a negative correlation), possibly because large operations have better hygiene practices.

Although WWTPs have lower MAR indices, they appear to be much more likely to harbour high resistance to antibiotics of importance to human health. WWTPs are also much more likely than CAFOs to be located near urban areas (especially in the UK), meaning the potential for exposure is probably also much higher. More thorough studies of WWTPs in the UK, as well as CAFOs, with air measured at varying distances from these facilities in a consistent way (see below) will help to clarify this.

### **3.2.2 Numbers of antimicrobial-resistant microbes reach ‘background’ levels by ~25m from point sources**

**This conclusion has a limited level of support from current research.**

Longitudinal studies of the distance–decay relationship of antimicrobial-resistant isolates from CAFOs and WWTPs suggest that downwind numbers decline to levels found upwind by about 25m from these facilities. However, this conclusion is very tentative given that data on distance numbers of microorganisms is spread across diffuse studies measuring different environments and organisms, and in different ways.

Much more data are needed to support this conclusion for CAFOs and WWTPs (especially in the UK) and to expand this ‘rule of thumb’ to other sources of antimicrobial-resistant aerosols.

### **3.2.3 Large particles carry the majority of antimicrobial-resistant microbes**

**This conclusion has a limited level of support from current research.**

Several studies used Andersen six-stage samplers – which separate air particles into chambers based on particle size bins – and reported the amount of 16S DNA isolated from these different particle size bins, or the number of colony forming units (CFUs) isolated. Although very few studies (3 out of 97) did this, these studies pointed to a positive correlation between aerosol particle size band and the number of bacteria isolated from that band between 0.65µm and 5µm (Heo et al. 2010, Gao et al. 2017, Kowalski et al. 2017). There were notable variations in this finding, associated with different production stages in WWTPs. For example, there appeared to be less strong relationships at the later stages of processing when most bacteria have been removed (Kowalski et al. 2017) or an extension of the positive correlation to 10µm for poultry confinement buildings (Heo et al. 2010).

What is perhaps more notable is that these similar types of patterns were identified from a poultry CAFO, a WWTP, a sanitary landfill, a rice field and a waste incinerator. This suggests there may be some underlying general relationship between particle size and the number of bacteria isolates. This makes physical and biological sense given that the air is a harsh environment in which microbes are heavily exposed to ultraviolet radiation, starvation and desiccation stress. Microbes are therefore more likely to survive when embedded within or attached to particles; notably fungi appeared to depart from a simple positive relationship in the study by Kowalski et al. (2017), perhaps due to their greater propensity to survive in air on their own.

It is also interesting because the abundance of antibiotic resistance genes in source environments that produce the majority of aerosols in their environment (for example, CAFOs and WWTPs) seems to be correlated with the total number of bacteria in the air. This suggests that antibiotic resistance phenotypes and genes are more likely to be found on larger particles. Direct evidence in support of this comes from the study by Gao et al. (2017), who showed that *tet(W)* and *tet(L)* are strongly positively correlated at poultry CAFOs with particle sizes between 1µm and 10µm.

Evidence for this hypothesis is still quite limited, but for the same reason as the distance–decay relationship (see Section 3.2.2), it has the potential to:

- be generalisable (with important adjustments for individual types of site)
- provide a relatively simple way to assess the risk of the spread of antimicrobial resistance via the airborne route

### 3.3 Knowledge gaps

The literature review allowed:

- clear conclusions to be made about the nature of airborne antimicrobial resistance
- the formulation of evidence-based hypotheses that could inform that assessment and management of risk

However, it also highlighted clear knowledge gaps in the current evidence base on airborne antimicrobial resistance. These gaps are discussed below.

#### 3.3.1 Is the air a primary route of antimicrobial resistance transmission to vulnerable hosts?

##### *Separating cause and effect when interpreting data*

One of the major problems with current research is the difficulty in separating cause and effect in interpreting airborne antimicrobial resistance data. It is clear that airborne antimicrobial resistance travels downwind from point sources and that airborne antimicrobial resistance profiles reflect the environments in which they are located. However, it remains difficult to conclusively say that the airborne route is a primary mode of antimicrobial resistance transmission. It is equally and perhaps more likely that urban air, for example, reflects WWTP air because of correlated anthropogenic influences (for example, the use of similar antibiotics in other contexts) and/or non-aerial transmission (for example, via the food chain, on footwear, through watercourses), followed by subsequent and transient re-aerosolisation. But to prove cause and effect, highly controlled and manipulative studies such as mark and recapture methods and experiments with sources and sinks under controlled conditions need to be carried out on a relatively large scale, making this difficult.

##### *Efficiency of transmission of airborne microbial resistance to vulnerable people*

There is also little information about how efficiently airborne antimicrobial resistance is transmitted to vulnerable individuals, for example how effectively is multi-drug resistance in airborne pathogens such as MRSA transmitted to humans? Especially with the advent of molecular methods, it is relatively easy to find MRSA or even *Bacillus anthracis* in air samples, but it is difficult to

understand the health implications and outbreak dynamics of this without more epidemiological studies.

Molecular-based studies, often with impressive scale, show that different environments are associated with different levels of different antimicrobial resistance genes. However, much of this research has proceeded without obviously considering the implications of these results. For example, are high abundances of antimicrobial resistance genes associated with 'clumps' of microorganisms in the air, a few organisms harbouring multiple gene copies or free genes?

On the latter point, a related question is how effectively genes alone, rather than just pathogens and other microbes, are taken up in human/animal airways versus skin. A study by Pal et al. (2016) suggested that airway microbial community compositions have a distinct community composition to airborne communities, but a very similar antimicrobial resistance profile. This finding indicated that airborne genes, rather than whole microorganisms, are being incorporated into otherwise relatively stable lung microbiome (for example, via the phage route). The use of personal air samplers as an air sampling method is one way to advance understanding in this area and human exposure to airborne antimicrobial resistance more generally.

### *Interpreting risk and impacts on non-health issues*

Research on airborne antimicrobial resistance has conventionally focused on pathogens – particularly aerosolisable, lung-infecting pathogens. Understanding of the transmission of antimicrobial resistance at the gene level is likely to:

- change interpretations of risk (for example, what particle sizes are important, who is likely to be effected)
- broaden concern about antimicrobial resistance spread to non-health issues (for example, the spread of resistance to antifungals has implications for agriculture as well as health)

### **3.3.2 Lack of UK studies**

The major omission gap in the current literature with most relevance to the Environment Agency is the lack of studies of airborne antimicrobial resistance in the UK. Neither the wider nor the refined literature searches identified a single study concerning airborne antimicrobial resistance in the UK. This is a very important problem when seeking to make inferences from the existing research to the UK situation. However, it is not thought that there is strong reason to believe that the main conclusions and some of the hypotheses identified should differ substantially in a UK context (with the exception of the CAFO versus WWTP relative risk).

UK studies should make a more concerted effort to relate airborne antimicrobial resistance to the types of antibiotic used or what is likely to appear in a UK context (for example, farming, hospital waste).

There are major legislative differences between the EU, USA and China on the use of antibiotics in agriculture. There are also less obvious differences, such

the greater influence of sheep farming in the UK compared with the USA, which may have an impact. For example, anti-helminthic resistance may be a larger problem in the UK, given that sheep worms cost the UK farming industry £84 million every year and are partly treated with azoles (Nieuwhof and Bishop 2005).

Other likely differences between the UK and other countries include:

- WWTP operation and the nature of waste being processed
- the geography of point sources and sinks (for example, location relative to built-up areas)
- interactions with other factors such as urban pollution (for example, London smog versus Beijing smog)

Although many other unidentified differences no doubt exist, current research provides a strong foundation and context on which to build research in the UK. No doubt there will be common features too.

### **3.3.3 Neglected environments**

#### *Escape of indoor CAFO air outside*

There is the potential for indoor CAFO air to escape outside, and while there will be dilution by the outdoor air, there remains a possibility that this could be a neglected point source that can spread widely due to lack of physical barriers. One study did measure airborne antimicrobial resistance in the air above a field where pig slurry was sprayed on the site of a pig farm, and compared with the air in the main pig farming area (Arfken et al. 2015). The farming area air and spray field air were found to have similarities in terms of composition, but the latter had fewer bacteria overall and more *Propionibacterium*, *Staphylococcus* and *Limnochabitans* than pig farm air. However, the authors looked only crudely at antimicrobial resistance, noting that fewer (25 versus >300 colonies) oxacillin-resistant isolates were captured on plates exposed to spray field air compared with pig farm air.

Another study of a similar agricultural practice (not included in the literature review because it did not explicitly sample air) looked at exposure to MRSA, methicillin-susceptible *Staphylococcus aureus* (MSSA), vancomycin-resistant enterococci (VRE) and vancomycin-sensitive *Enterococcus faecalis* (VSE) among spray irrigation workers but did not find evidence in nasal swabs that spray workers were colonised more than a control group of office workers (Rosenberg Goldstein et al. 2014).

#### *Arable farming*

Arable farming represents another major neglected environment that could be associated with airborne antimicrobial resistance. As with livestock farming grasslands, arable farms frequently fertilise land with slurry. Like animals, crops are treated with antimicrobials (for example, antifungals, antibiotics) and are subject to open air processes that promote aerosolisation (that is, harvesting).

Admittedly, the levels of antibiotic use in arable farming are probably lower than in livestock farming generally. As of 2002, for example, antibiotics applied to plants accounted for less than 0.5% of total antibiotic use in the USA (where oxytetracycline and streptomycin are applied to plants, especially via spraying, no data found for elsewhere) (McManus et al. 2002).

Figures are difficult to find for the UK, but nonetheless, arable farming is likely to represent a relatively higher threat in a UK given the ban on antibiotic use for growth promotion in livestock farming. Although antibiotics are sometimes used in plant agriculture, antifungals have more extensive usage in plant agriculture.

Increasing research on airborne antifungal resistance (see below) – especially in the context of arable farming – should be a priority area as UK research begins in this area. Although poorly reported, the limited research on airborne antifungal resistance in plant-based industries (for example, arable farming and sawmills) suggests this may be an emerging problem and the airborne adaptations of fungi could make them a more efficient vector of antibiotic resistance.

### *Other neglected environments*

Given the small number of environments currently being studied, the list of neglected ones is almost endless, but some are worth brief mention.

#### **Composting operations**

Compost heaps, for example, are another industrial environment that encourages the abundant growth of microorganisms (especially saprophytic fungi) alongside their aerosolisation. The Environment Agency has begun researching and regulating these operations with regard to bioaerosol contamination, and so it would seem a natural starting point for pilot studies on airborne antimicrobial resistance.

One study identified by the literature search did explicitly look at airborne antimicrobial resistance in a Chinese composting plant and identified high levels of antibiotic resistance (Gao et al. 2018a). This suggests that this is an area worth pursuing further in a UK context, especially as Environment Agency and Defra scientists and others are already pursuing bioaerosols work in this area (see Williams et al. 2013, Environment Agency 2018, Nasir et al. 2019).

#### **Hospitals**

Another neglected environment that is a potential high risk area suggested by the current literature is hospitals. Hospitals are interesting because they are both high risk sources and sinks. Current research suggests that hospital indoor air harbours multi-drug resistant pathogens and receives them from surrounding areas (for example, CAFOs). However, research in the indoor/outdoor spread and vice versa dynamics of hospitals is surprisingly scarce and so more research in this area is desperately needed, perhaps driven by public health bodies.

#### **Other environments**

Other environments that, if studied, could help to better piece together the story of antimicrobial resistance in the air include:

- rural residences and workplaces
- fish farms (which use heavy amounts of antibiotics)
- urban sewers (especially above drain grates in cities)
- the upper atmosphere

### **Diffuse sources of airborne antimicrobial pollution**

A focus on potential ‘hotspots of airborne antimicrobial resistance’ such as CAFOs, WWTPs and arable farming may present a misleading picture of exposure risk. Although the pigeon feeding/roosting site studies included in this review were inconclusive, it may be that smaller but more frequently encountered and diffuse sites like this have bigger consequences for the risk posed by airborne antimicrobial resistance – especially to immunocompromised populations. Considering such sources of airborne antimicrobial resistance – perhaps starting with a more concerted analysis of pigeon sites – should also be a priority for future research.

### **3.3.4 Non-bacterial/non-antibiotic resistance**

As highlighted above, one of the major omissions from the current literature is fungi and airborne antifungal resistance.

Antifungal resistance is currently considered to be less of an immediate threat than antibiotic resistance (Davies et al. 2013). Nonetheless, the ubiquitous use of azoles over all other groups of antifungals in agriculture, medicine, wood treatment and so on, combined with the propensity of fungi to travel long distances via the airborne route, makes antifungal resistance a particularly relevant problem regarding airborne antimicrobial resistance.

Nonetheless, very few studies looking at airborne antifungal resistance were found. Many studies included fungi alongside bacteria but only measured antibiotic resistance, while others looked at fungi but reported their results poorly or used non-standard methods.

The reasons for this largely reflect wider problems to do with a lack of standardised methods for antifungal susceptibility testing. Only a few antifungals have established EUCAST breakpoints and those that have are only for *Aspergillus* and *Candida* genera. While breakpoints are being established, a workaround for this issue is for authors to simply report MICs and compare them between studies (for the same species), though translating this into clinical relevance is more difficult.

What is encouraging is that many of the UK research groups currently working on airborne antimicrobial resistance and/or bioaerosols are focusing on fungi, indicating that UK research is plugging this knowledge gap from its foundation. The inclusion of studies of airborne antiviral and antihelminthic resistance would also be valuable.



### 3.3.5 Biophysics of airborne antimicrobial resistance

As also highlighted above, research on airborne antimicrobial resistance would greatly benefit from a better understanding of the biophysics of airborne antimicrobial resistance. A good starting point would be research into:

- the size distributions of particles from different point sources as they relate to the transport of bioaerosols from different sources with differing levels of aerosolisation
- the distribution and survivability of microbes carrying antimicrobial resistance

Such information would allow the better prediction of airborne antimicrobial resistance risk around point sources and better management of this. Modelling validated by empirical work could greatly help these efforts.

The survival of genetic elements outside of microbial hosts is also a potential mode of antimicrobial resistance transmission that needs to be investigated further.

## 3.4 Recommendations for future research

### 3.4.1 Antimicrobial susceptibility testing

The most amenable studies are those performing antimicrobial susceptibility testing using culture-based methods. Although these methods are limited by the fact that they can only measure the culturable (that is, not viable) fraction of a microbial community (approximately <1%), their long usage means that the methodology is usually more standard than newer molecular methods. Nonetheless, several studies identified in the literature were difficult to incorporate into this analysis and so minimum standards for future research in this area are suggested below.

1. Report fully the total number of colonies isolated, the total number of strains tested and the total number of strains (not just percentages) that are resistant, sensitive (optional) or susceptible for each antibiotic in a clear table format. The same strains should be tested for all antibiotics. As well as this summary format, the full database of strain-by-strain results should be provided in published supplementary materials or on a data-sharing platform, and signposted clearly in the main text of your published report.
2. Report the breakpoint (that is, threshold millimetre zone of inhibition for disk diffusion or concentration for dilution method, used to determine whether a strain is resistant, sensitive and susceptible) for each antibiotic susceptibility test. In the full database, also report millimetre zones of inhibition or MICs in the actual results table, and state clearly whether the isolate(s) was resistant, sensitive or susceptible alongside this. Report the standard from whence the breakpoint was obtained (for example, EUCAST or CLSI), but do not report this alone.

3. When isolating strains from multiple environmental compartments (for example, air, soil and water), report the number of resistant, susceptible and negative isolates in each compartment.
4. If there is some sort of taxonomic identification, report antibiotic susceptibilities of each taxonomic group at the finest resolution possible (for example, species).
5. For fungi, given breakpoints are not yet established for most species and antibiotics, report the MIC value in the results table so that this can be converted into a resistance, sensitivity or susceptibility table if and when breakpoints are established. If breakpoints are available for the study species, state clearly whether the isolate is resistant, sensitive or susceptible in brackets next to the MIC. Do not report this for species or antibiotics for which breakpoints are not yet available.
6. Upload results tables to data-sharing platforms such as Figshare ([www.figshare.com](http://www.figshare.com)) and state this clearly in the text of your published report. Such platforms make it easier to download tables of results for meta-analysis and open access to raw results; this is difficult from PDFs.

### 3.4.2 Air sampling

Despite dissimilarities in methods, there were broad commonalities in the results obtained using different methods of air sampling. However, quantitative comparison of some of these results (for example, concentrations of resistant bacteria in air) was hindered by insufficient and non-standard reporting of results. The following are therefore recommended for culture and non-culture based methods.

1. Where culture-based antimicrobial susceptibility testing has been performed alongside active sampling, the total number of colonies isolated should be reported as CFU per m<sup>3</sup> of air sampled. If those tested for susceptibility are a subset of these, this is sufficient. If not (for example, selective media were used to isolate a target organism), then CFU per m<sup>3</sup> should be reported for this part of the experimental work too. These should both preferably be in table format, uploaded to Figshare.
2. For passive sampling (for example, of dust), some kind of estimate of the air change rate in the location being sampled – or at least an estimate of the total air volume in the environment – should be included. Alternatively, if reporting say the numbers of microorganisms per grams of dust, some estimate of the concentration of dust in the air and a corresponding calculation of the concentration of microorganisms should be included.
3. For molecular methods (for example, qPCR, sequencing), the number of antibiotic resistance gene copies per m<sup>3</sup> should be reported as well as the number of 16S gene copies. For passive sampling, the same applies but as in item 2 above.
4. When using particle size-resolved sampling, the size range used should be clearly stated in the results table. Preferably, antibiotic resistance would be tested in the different particle size ranges separately to

understand which sizes are the most problematic, but costs or issues with DNA yield may sometimes make this impossible.

### **3.4.3 Other recommendations**

To better understand the risk of airborne antimicrobial resistance in outdoor environments, more information is needed about:

- particle size distributions from known point sources
- relatedly longitudinal studies to understand their spread outward from these sources into aerial and non-aerial sinks of clinical, economic and/or environmental relevance
- innovative epidemiological and manipulative science to understand the implications of exposure

More frequent use of six-stage Andersen sampling along sampling transects, with antimicrobial resistance reported in each size fraction and at each distance from the source, extending to distances covering residences and/or workplaces (other than the point source), would be a good foundation for this kind of research.

## **3.5 Closing remarks**

Airborne antimicrobial resistance is an emerging field of scientific enquiry in which much extensive and good quality science has already been conducted. This science indicates that airborne antimicrobial resistance profiles often reflect their environment, with sewage from animal and human activities appearing to be major influences. What is less known how precisely these profiles were assembled and what their implication is for the health of society, the environment and the economy.

Almost no work has been published on this subject in the UK, which is a challenge but also an opportunity, meaning there is a blank slate for UK academic and government research to establish a knowledge base while improving on many of the problems that have emerged in the largely health-science driven research already conducted. Further research will establish if there is a substantial risk from airborne antimicrobial resistance compared with other environmental pathways and provide more substantial recommendations for the management of a problem (if found).

## **3.6 ADDENDUM**

Since this report was completed one other relevant paper has been identified Bromley, M. J., et al. (2014) "Occurrence of azole-resistant species of *Aspergillus* in the UK environment". The authors investigated azole resistance in *Aspergillus fumigatus* from urban and agricultural settings with resistance detected in azole-treated field soil isolates. This suggests some potential for airborne transmission of azole resistant *Aspergillus* species.

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## List of abbreviations

CAFO	concentrated animal feeding operations
CLSI	Clinical Laboratory Standards Institute
CFU	colony forming unit
Defra	Department for Environment, Food and Rural Affairs
ESBL	extended spectrum beta lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MAR	multi-antibiotic resistance [index]
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
WWTP	wastewater treatment plant
qPCR	quantitative polymerase chain reaction

# Appendix A: Data and code availability

For access to the data and code used to produce this report, please refer to the project manager. This includes:

1. Bibliographic files (.bib and .nbib files) and code (airamr\_sysrevescript\_051018.R) used to process results of literature searches
2. Data frame 1: Full bibliography (biblio\_all\_final.csv) of 88 (92 with duplicates) articles used for the literature review, with some information about the types of organisms each focused on and how antimicrobial resistance was measured
3. Data frame 2: Data frame (AMSdataset\_051018.csv) of antimicrobial susceptibility testing data and metadata compiled from the available literature (24 studies), used for calculating antimicrobial resistance prevalence figures and MAR indices (and semi-quantitative analysis)
4. Code (report\_analysis051018.R) used to calculate antimicrobial resistance prevalence figures and MAR indices (and semi-quantitative analysis), as well as to produce the figures included in the text

## Appendix B: Additional analysis to validate meta-analysis

Meta-analytical comparison of MAR indices between studies indicated that the type or environmental source of the air was a key determinant of its multi-antibiotic resistance (see Figure B.1 for raw means and data). The MAR index summarises the number of tested strains that were resistant to multiple antibiotics, allowing comparison between studies that tested different antibiotics. That said, the index is strongly influenced by other factors:

- numbers of strains tested (incorporating in meta-analysis weighting)
- different types of strain tested
- antibiotics tested
- different air sampling methods used
- whether the sample was taken close to or inside the target environment or at a distance from it

With this in mind, these factors were incorporated into 3 additional analyses.

1. A generalised mixed binomial model indicated that the type of air – excluding the types of antibiotics tested given its incompatibility with NA (missing) values – was a significant determinant of the MAR index even after accounting for other types of variation as error ( $Z = 7.525$ ,  $P < 0.05$ ).
2. A random forest method ( $n_{tree} = 10,000$ ,  $m_{try} = 2$ , also excluding the types of antibiotics tested given its incompatibility with NA values) indicated that the type of air was by far the strongest determinant of the MAR index among the 60 other factors considered. This again indicated there was a genuine effect of the type of air despite differences between studies (increased mean square error (%IncMSE) = 204.13, total variance explained by model = 61.5%). The number of strains tested did appear to influence the index (%IncMSE = 77.55, total variance explained by model = 61.5%), but the air sampling method and the number of antibiotics tested were more influential (%IncMSE = 103.48, total variance explained by model = 61.5%).
3. A regression tree approach, which was robust to NA values unlike other approaches, was used to validate that the choice of antibiotics in each study was a bigger determinant of the MAR index. Again, this analysis suggested that the type of air was the main driver of antibiotic resistance (importance = 40%) rather than the number of strains tested (importance = 13%), the number of antibiotics tested (importance = 21%), or any of the antibiotics tested (all importances < 2%). Different average MAR indices were therefore clearly associated with different types of air environment, with the highest average MAR indices found in pig CAFO environments, intermediate levels found in cow CAFO environments, WWTPs (and possibly poultry CAFO environments, for which there was

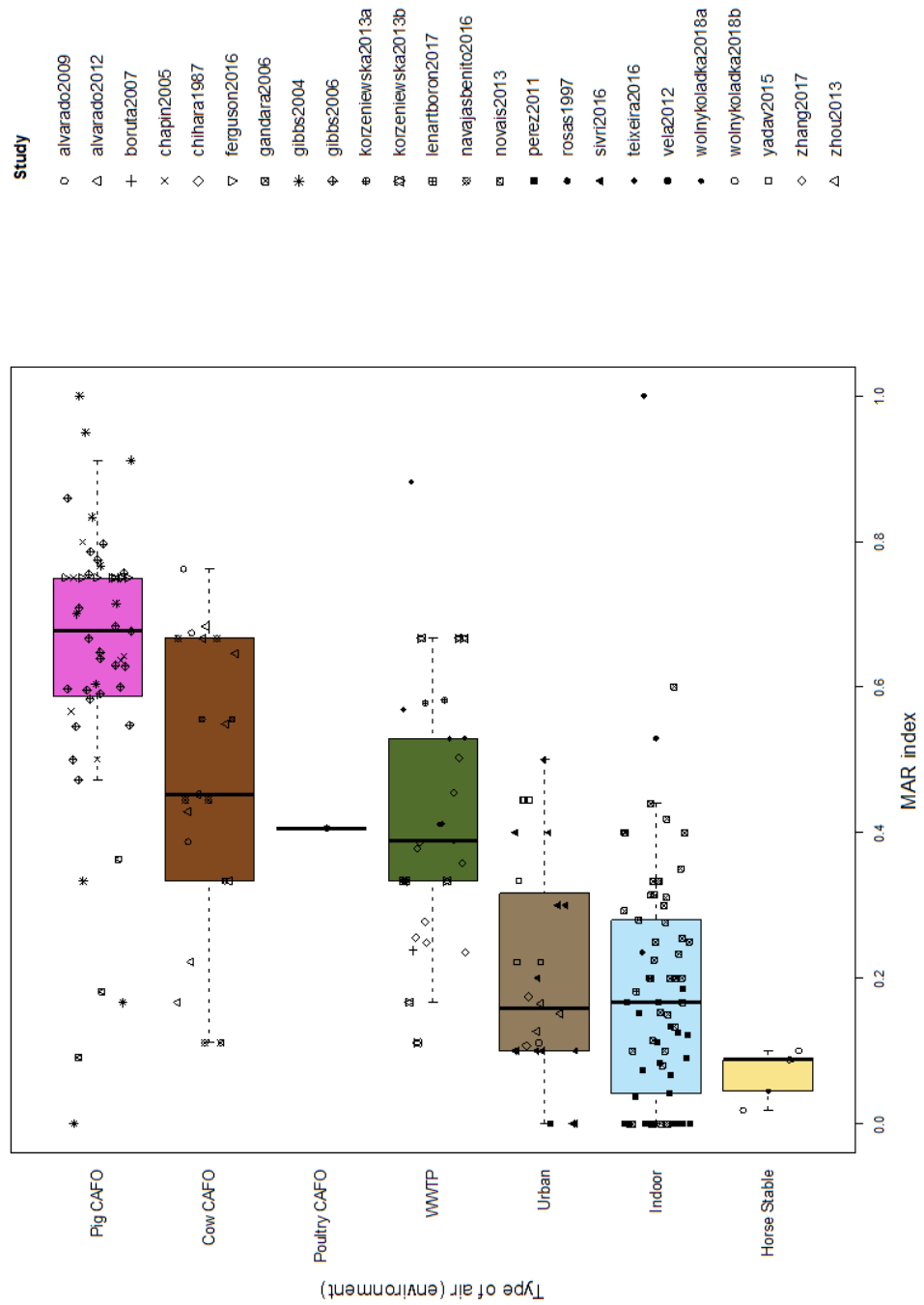


only one data point), and lower MAR indices found in urban and other environments.

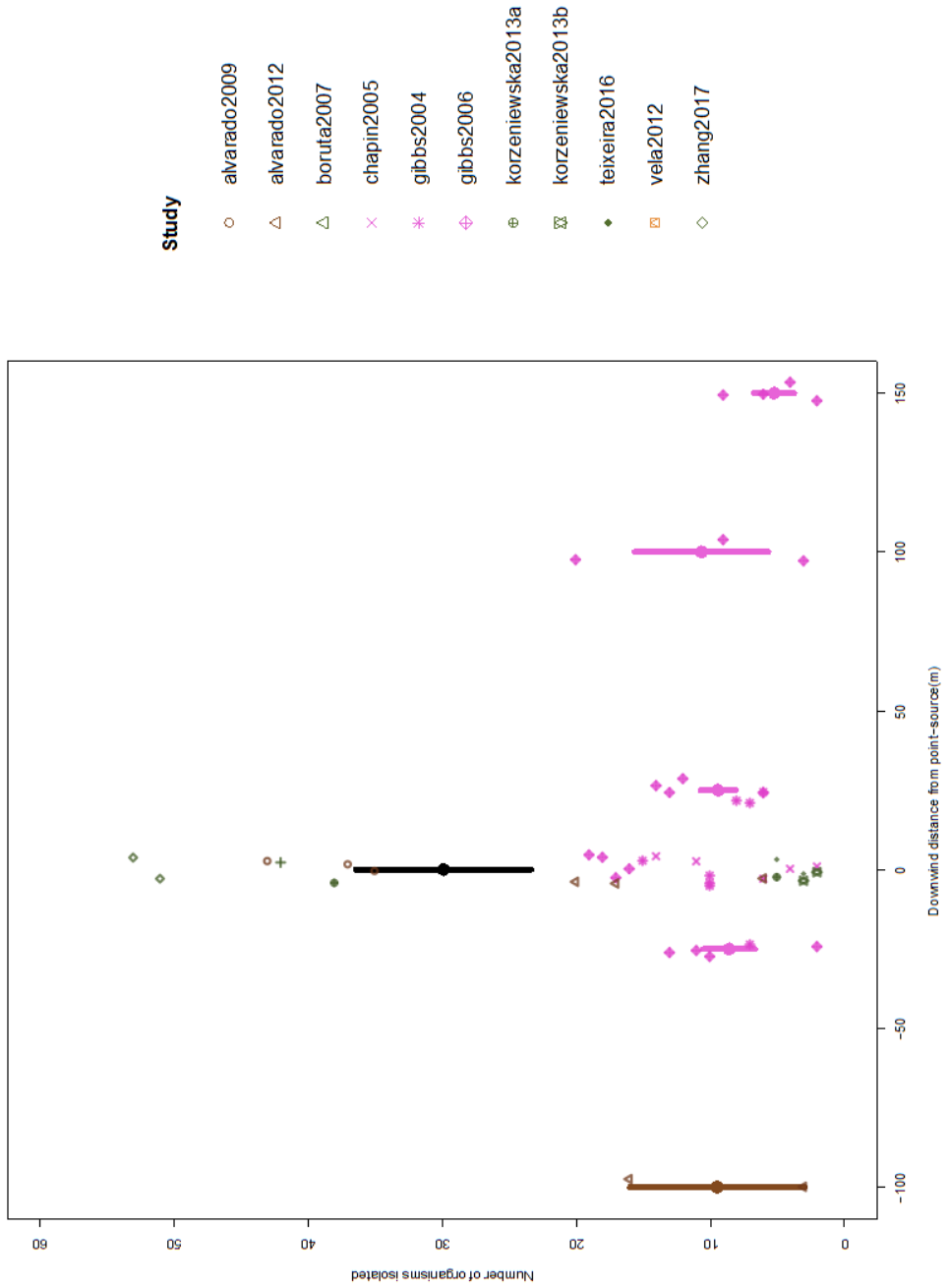
In terms of the levels of multi-antibiotic resistance in air around airborne antimicrobial resistance environments, distance was the least influential factor affecting the MAR index. Although several studies suggested a distance–decay relationship with declining numbers of microorganisms and antibiotic resistance further away from point sources, this information was not collected widely enough and/or reported in sufficient detail to permit cross-study comparison. Only a few of the CAFO and WWTP studies provided transect-based studies for comparison.

Qualitatively, the available research suggests that numbers of airborne microorganisms (strains isolated) follow a distance–decay type pattern, reaching upwind levels by 25m downwind across all CAFOs (Figure B.2). However, this very approximate inference was based only on pig CAFO studies, as these are the only type of studies that measured transects extensively enough to make calculate average numbers at each distance (and the pattern was much less strong within these studies). Thus there is currently insufficient information available to say to what extent antimicrobial resistance spreads out from environmental hotspots into the wider environment.

Furthermore, the MAR index is only one way to assess the risk of airborne antimicrobial resistance. It might, for example, be more relevant to account for the hazard associated with certain resistance to certain groups (for example, resistance to ‘last resort’ antibiotics). Again, data were too sparse to understand whether different environments were associated with resistance to different groups of antibiotics. For example, while WWTP research qualitatively suggested high levels of resistance to cephalosporins, there was a high degree of variation and this group of antibiotics was rarely studied outside of WWTP research, making comparison between environments difficult. This highlights the need for a more standard set of antibiotics to be tested across different environments, regardless of whether that environment is hypothesised to be associated with that type of resistance, in order to separate bias from results.



**Figure B.1 Comparison of MAR indices between different types (environments) of air**



**Figure B.2 Distance–decay relationship of number of bacteria isolated across different studies**

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