BioAirNet

Establishing a National Surveillance Strategy for Antimicrobial Resistance in Bioaerosols: Challenges and Pathways

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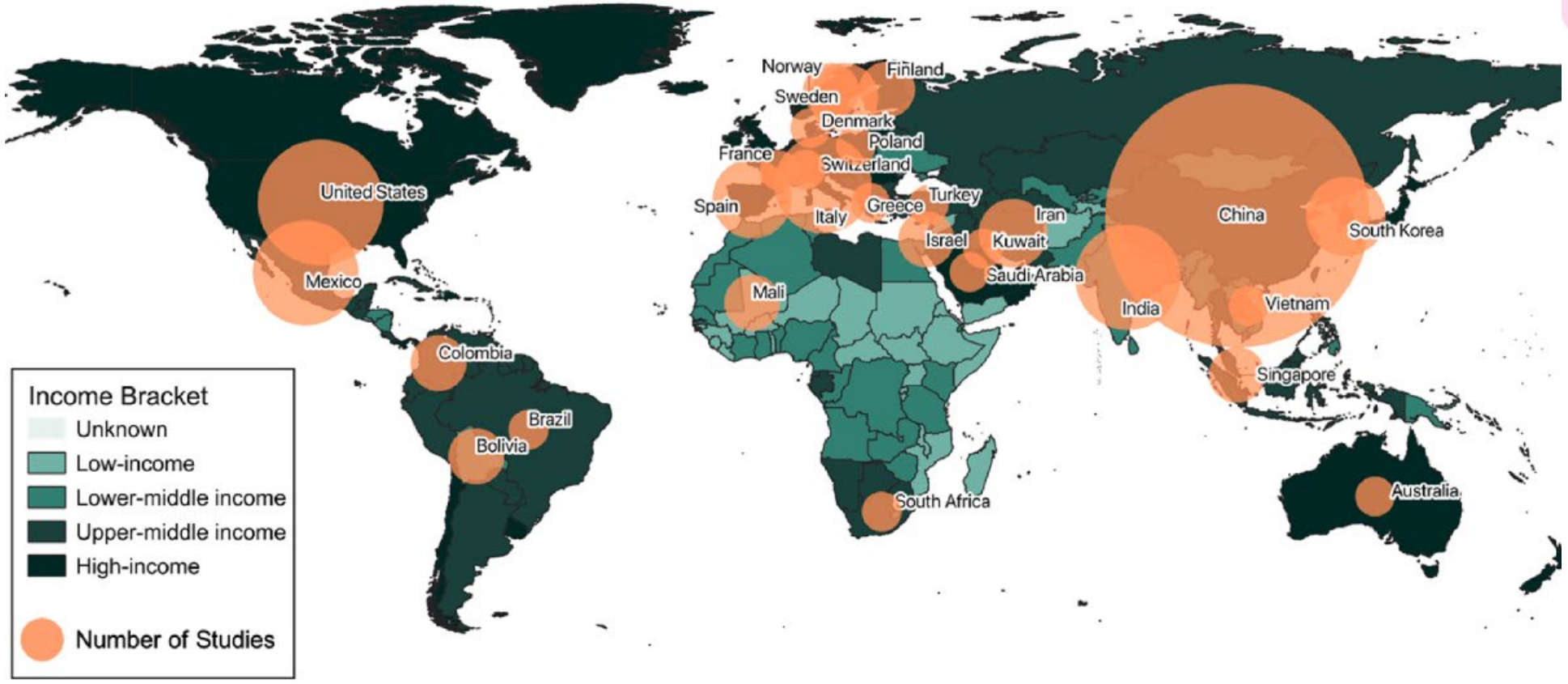
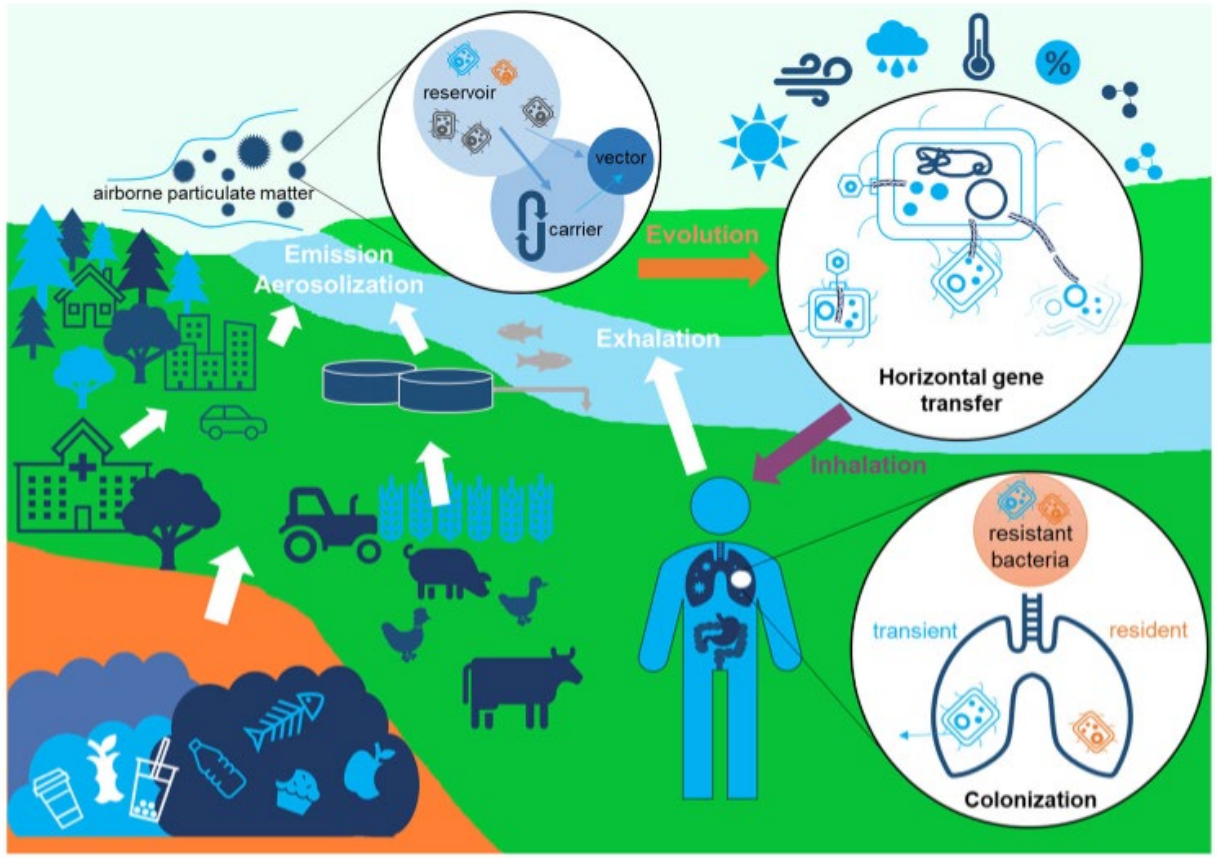
Global challenge of AMR, its impact on public health, and the specific relevance of bioaerosols in AMR transmission

Enteric pathogens and antibiotic resistance genes in outdoor urban aerosols



Ginn et al, 2022, Environ Research, 113097

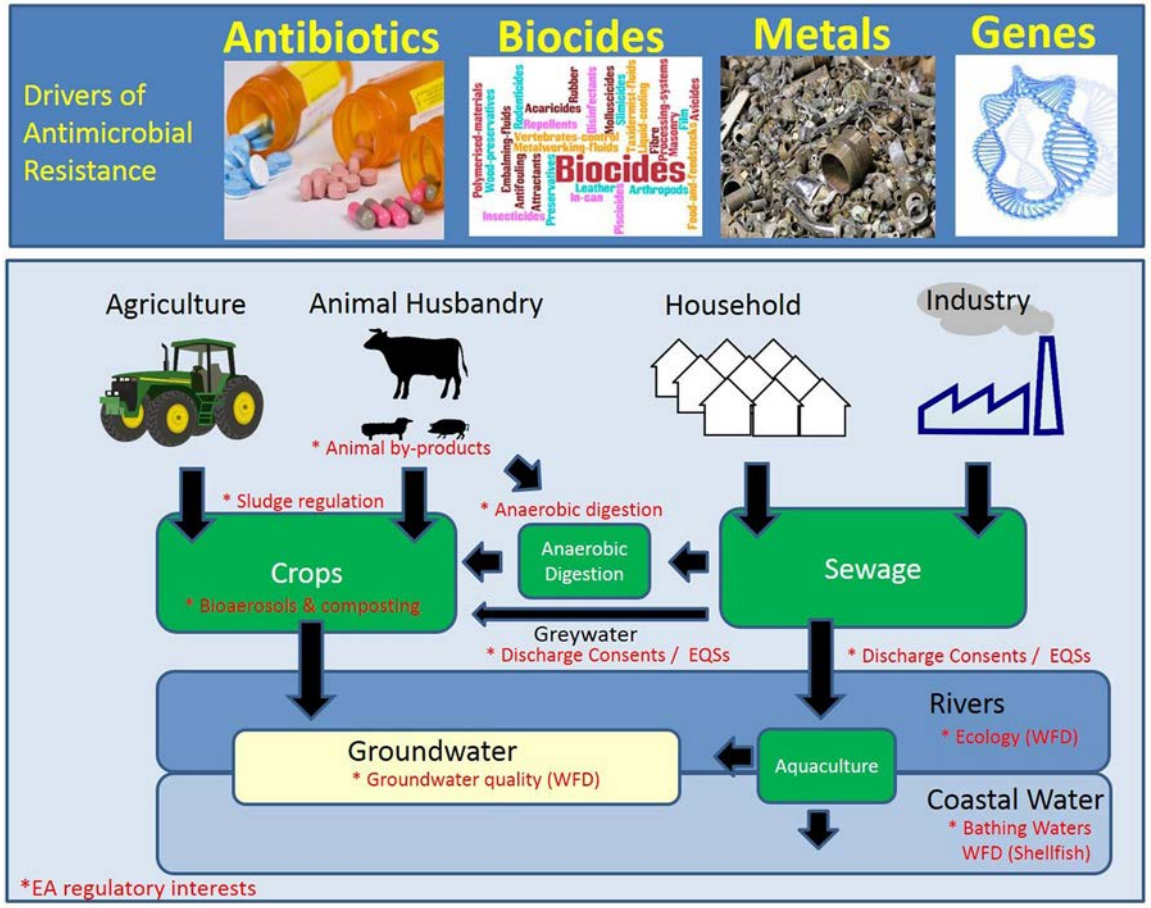
Key research questions on airborne AMR transmission



Jin et al, 2022, CREST, 23:4172-4193

- What are the **main sources** of airborne ARGs and resistant bacteria in urban areas?
- How do airborne ARGs and bacteria **spread and reach** human airways?
- What is the **extent of human exposure** to airborne ARGs and resistant bacteria?
- How are **environmental and human-associated ARGs genetically linked**?
- How do airborne resistant bacteria **colonise human airways**?
- How do **seasonal** and **environmental** factors affect airborne ARG levels?
- What **mechanisms drive ARG transfer** in airborne bacteria?
- How can airborne AMR transmission be **reduced through urban and public health strategies**?

Hot-spots and drivers of AMR

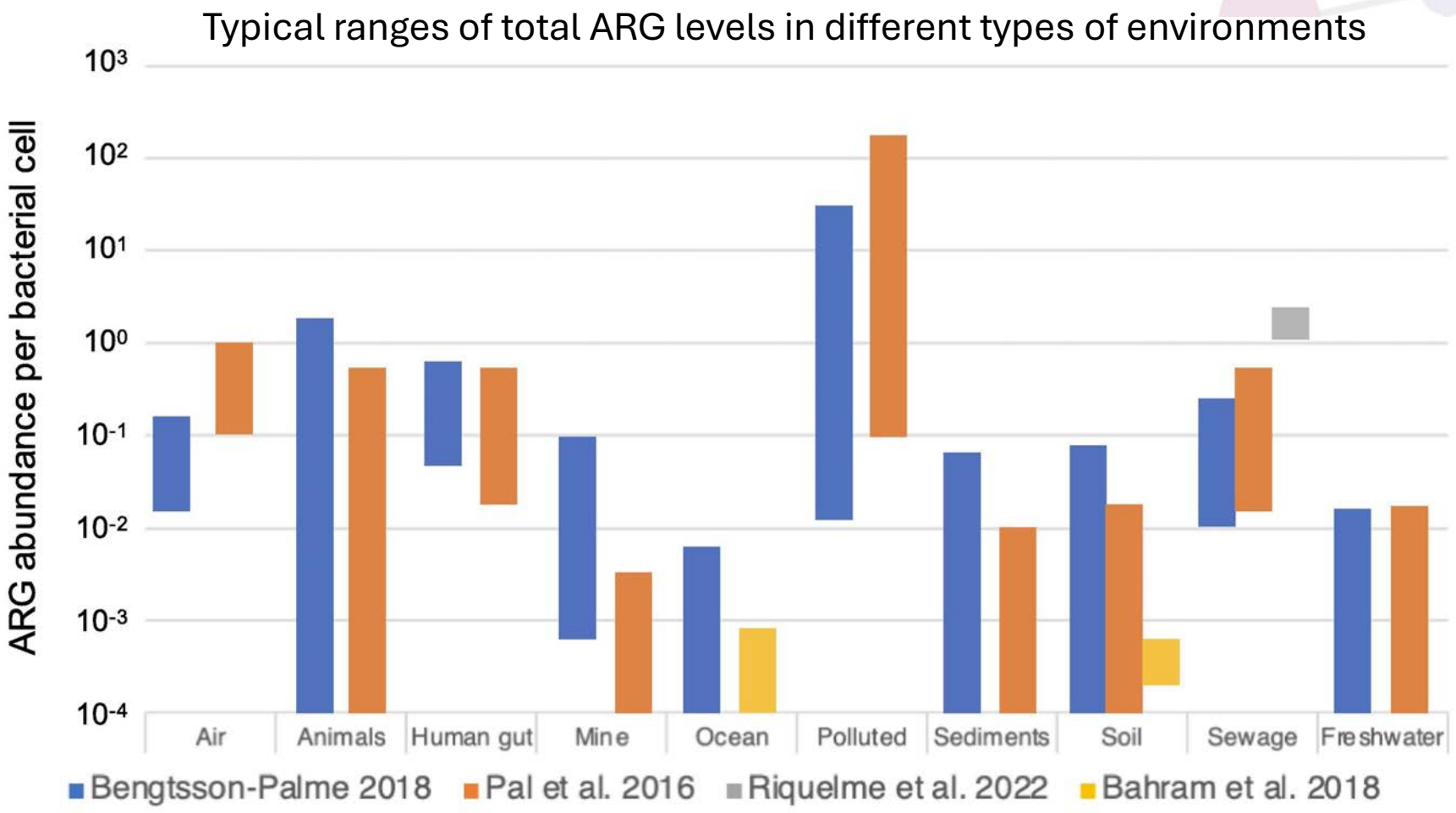


Singer et al, 2016, Front. Microbiol, 7

Current evidence gap that **hinders the ability of policymakers and environmental regulators** from delivering environmental protection from AMR.

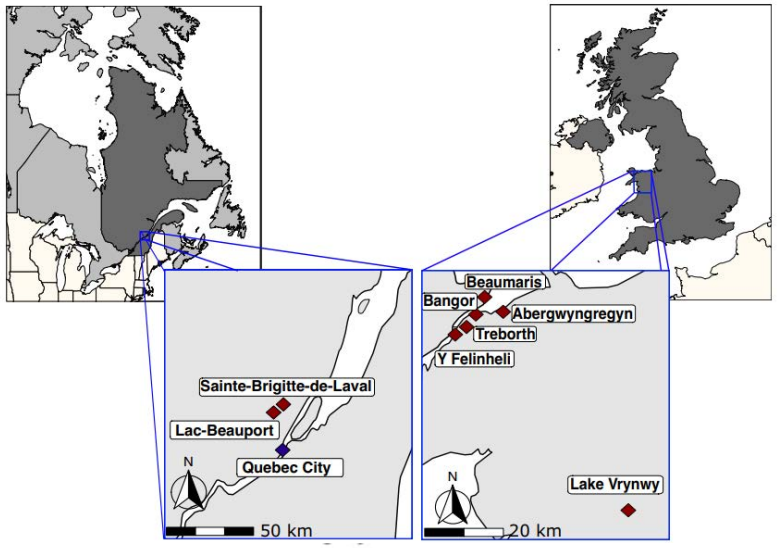
1. What are the benefits of controlling AMR in the environment beyond preventing human transmission?
2. How do antibiotics, metals, biocides, and ARGs contribute to AMR spread in the environment?
3. How effective are current technologies in limiting environmental AMR?
4. How should regulatory frameworks address AMR?
5. Does expanding pollutant regulation to include AMR reduce environmental AMR?

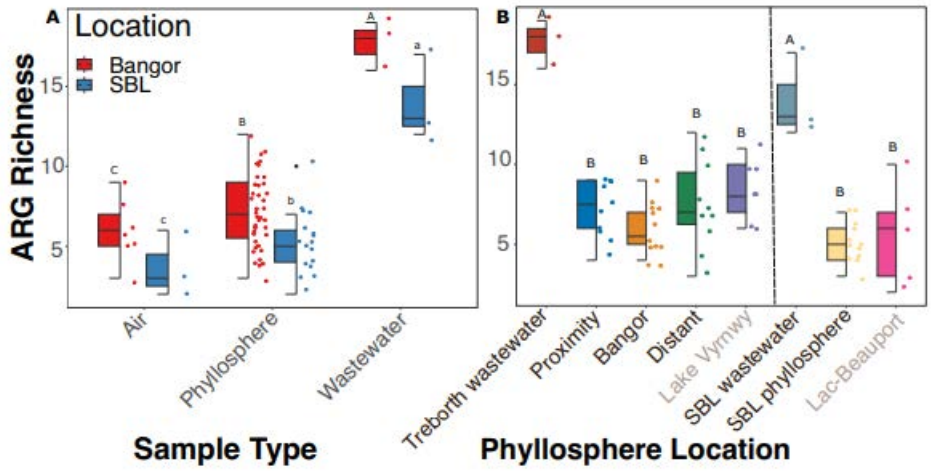
Environmental baseline for AMR

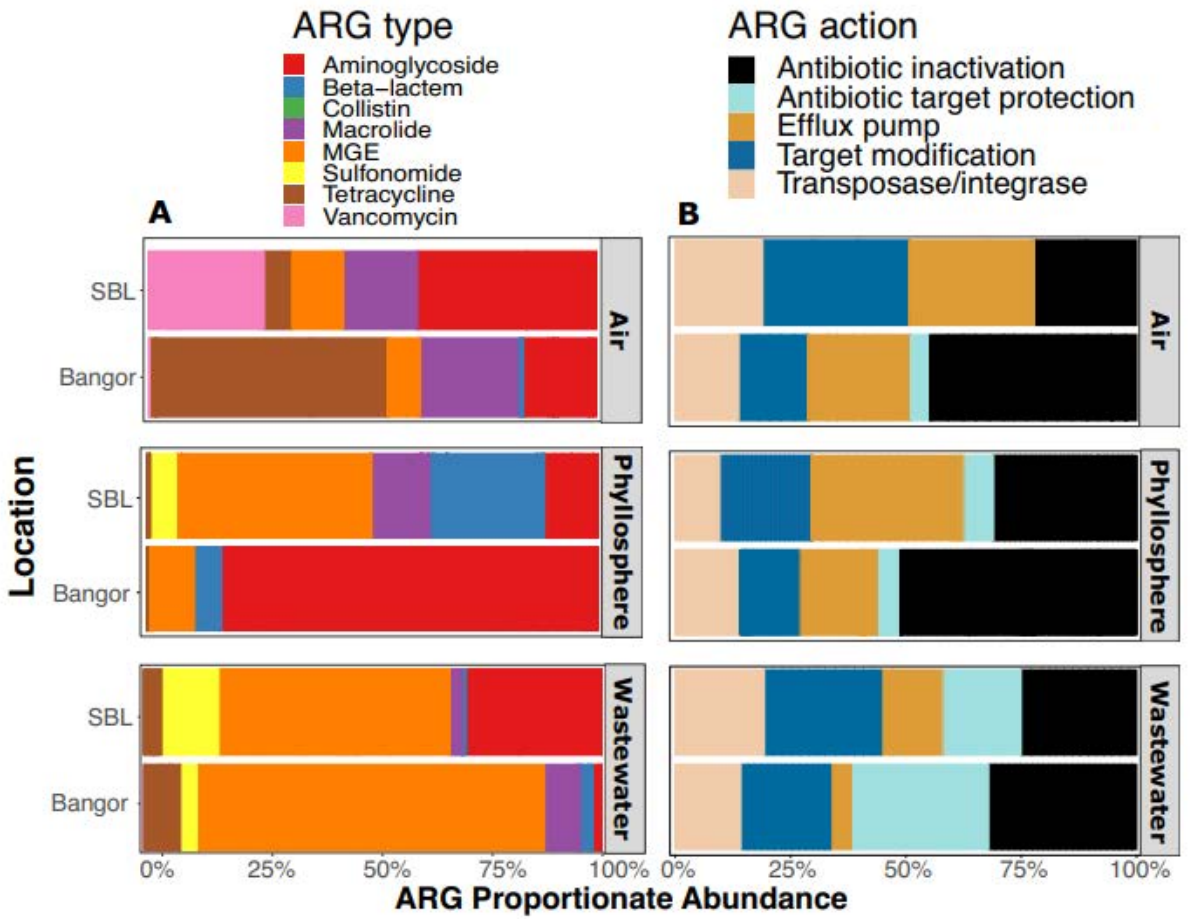


Bengtsson-Palme et al, 2023, Environ. Int. 178, 108089

Phyllosphere sampling a promising low-cost tool for monitoring airborne ARG emissions from WWTP



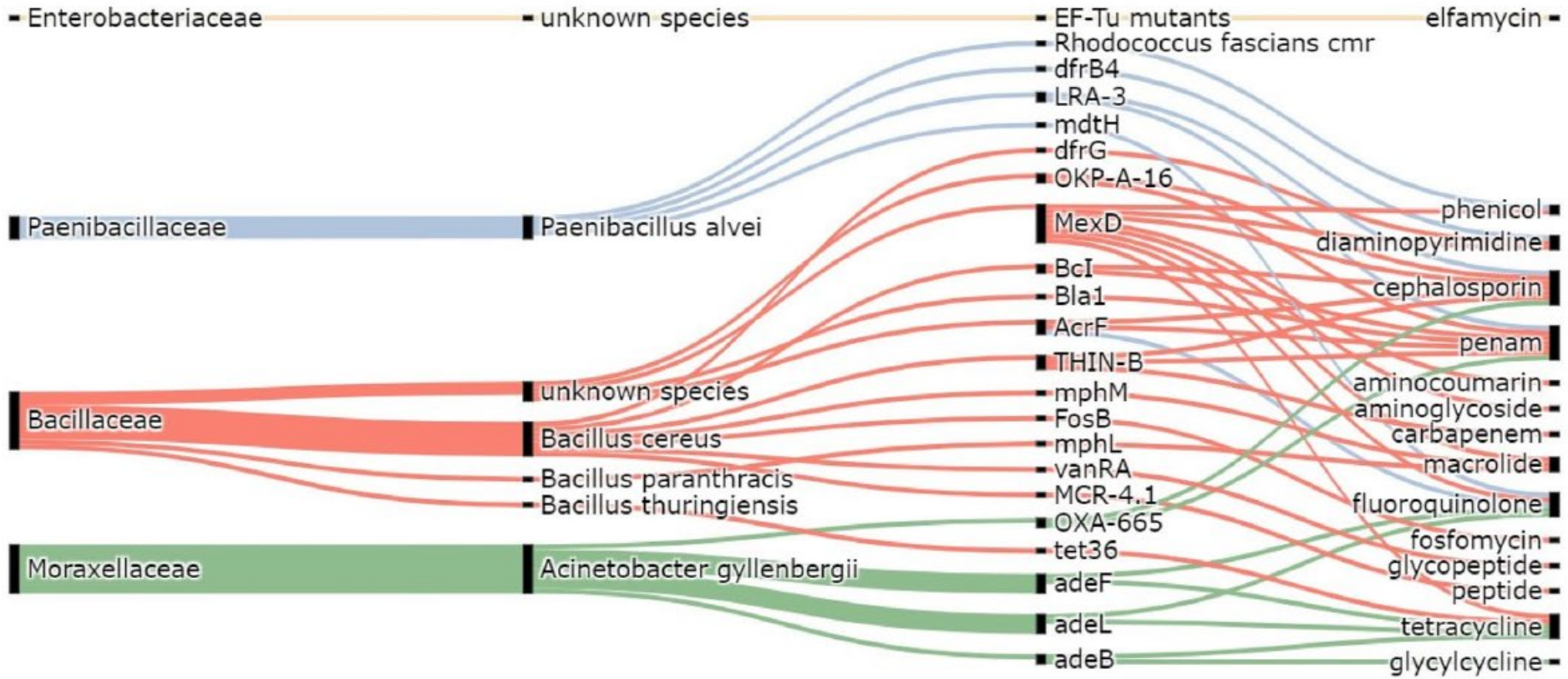




George et al, 2024, Canadian J Microb. 70: 348-357

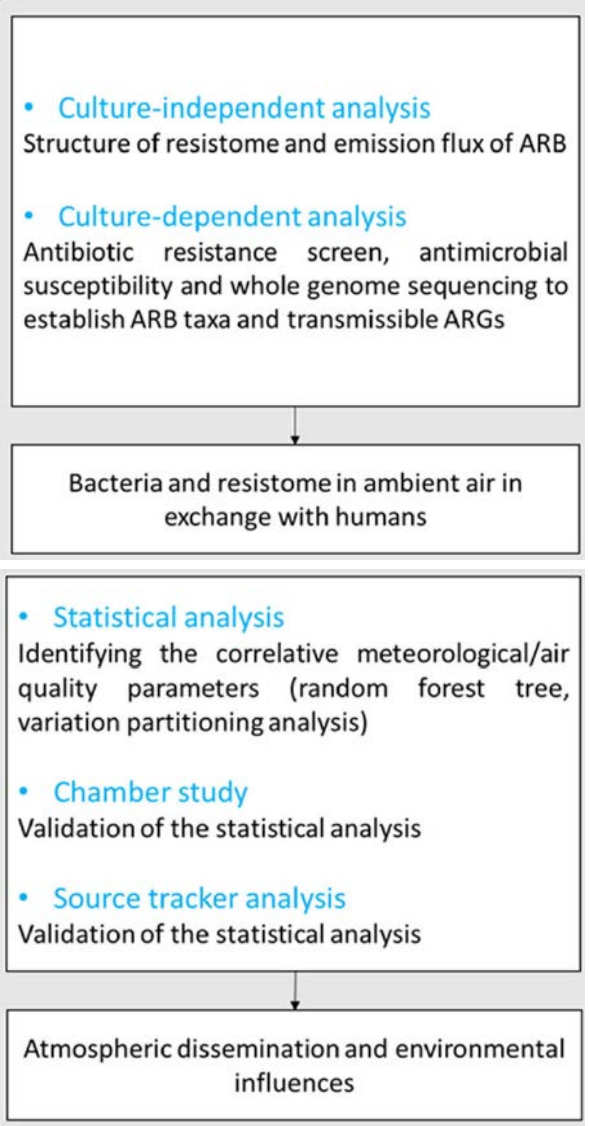
Selection of high-priority monitoring targets

Identified ARGs, their most probable taxon of origin, and the group of antibiotics they protect against.



One gene probably originated from a member of the family Enterobacteriaceae, 4 genes from *Paenibacillus alvei*, 3 from unknown members of the family *Bacillaceae*, 6 from *Bacillus cereus*, from *B. paraanthracis* and *B. thuringiensis*, one each and four from *Acinetobacter gyllenbergii*. Most of the detected genes protect beta- lactams (penams, carbapenems, cephalosporins). (Becsei et al, 2021, Microb Open, mbo3.1248)

Selection of high-priority monitoring targets



- AMR markers in bioaerosols have been quantified using culture-independent and culture-dependent methods, covering phenotypic resistance and genotypic measures such as antibiotic resistance genes (ARGs)
- Limited reports on antifungal resistance markers focus on azole-resistant *Aspergillus fumigatus* and cycloheximide-resistant fungi.
- Prevalence and density of AMR are reported in various ways, including phenotypic resistance %, resistance isolate density, and ARG abundance
- Overall, AMR marker selection is often biased toward pathogens or genes with public health implications, making general conclusions about prevalence and resistance density difficult.

The way forward

- Lessons from environmental AMR surveillance in terrestrial and aquatic matrices can inform air sampling for AMR.
- Clear objectives and endpoints are essential for air sampling programmes.
- Endpoints should focus on detecting specific AMR genes or organisms, using PCR or sequencing for resistance profiles.
- Appropriate sampling methods and protocols must be selected based on the type of AMR and the sampling locations. For example:
  - AMR associated with animal/agriculture, focus on livestock facilities or manure storage with high-flow rate sampling.
  - For hospital-associated AMR, target patient rooms and areas with high antibiotic use using discrete filtration methods.
- Environmental contamination, such as soil or water pollution, may affect air sample results and should be monitored and accounted for in sampling strategies.

Bioaerosol sampling methods for AMR detection in the atmosphere

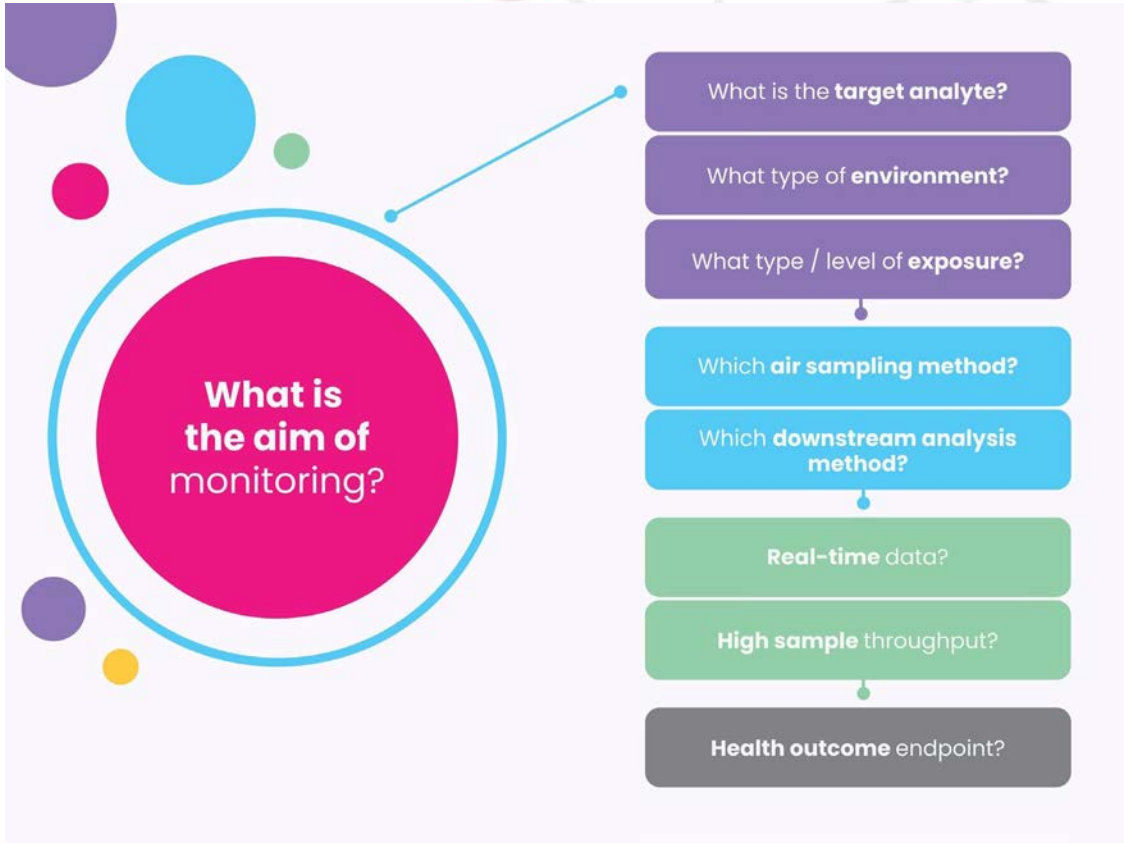
ELSEVIER

Advances in Ecological Research
Volume 67, 2022, Pages 101-229

Chapter Three - Compendium of analytical methods for sampling, characterization and quantification of bioaerosols

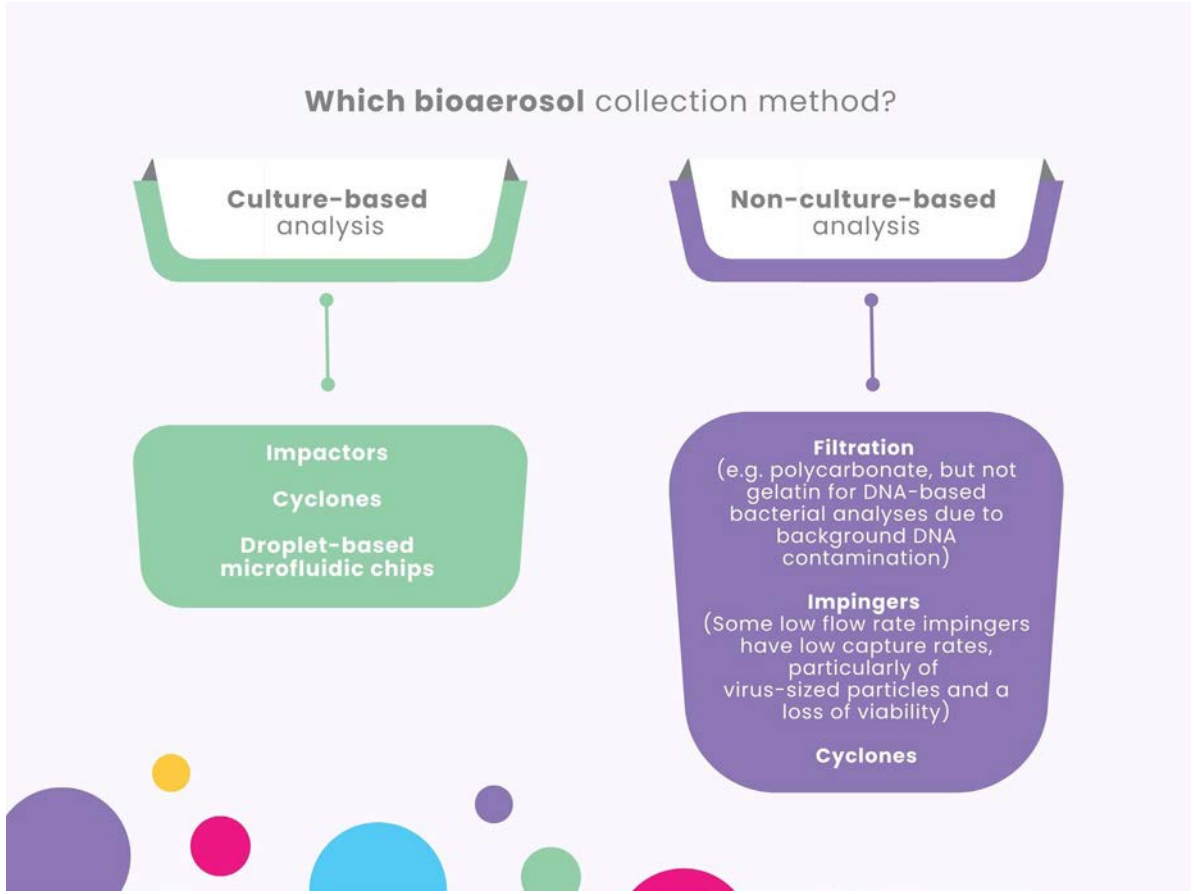
Corinne Whitby [a], Robert M.W. Ferguson [a], Ian Colbeck [a], Alex J. Dumbrell [a], Zaheer A. Nasir [b], Emma Marczylo [c d], Rob Kinnersley [e], Philippa Douglas [c d], Gill Drew [b], Kam Bhui [f], Mark Lemon [g], Simon Jackson [h], Sean Tyrrel [b], Frederic Coulon [b]

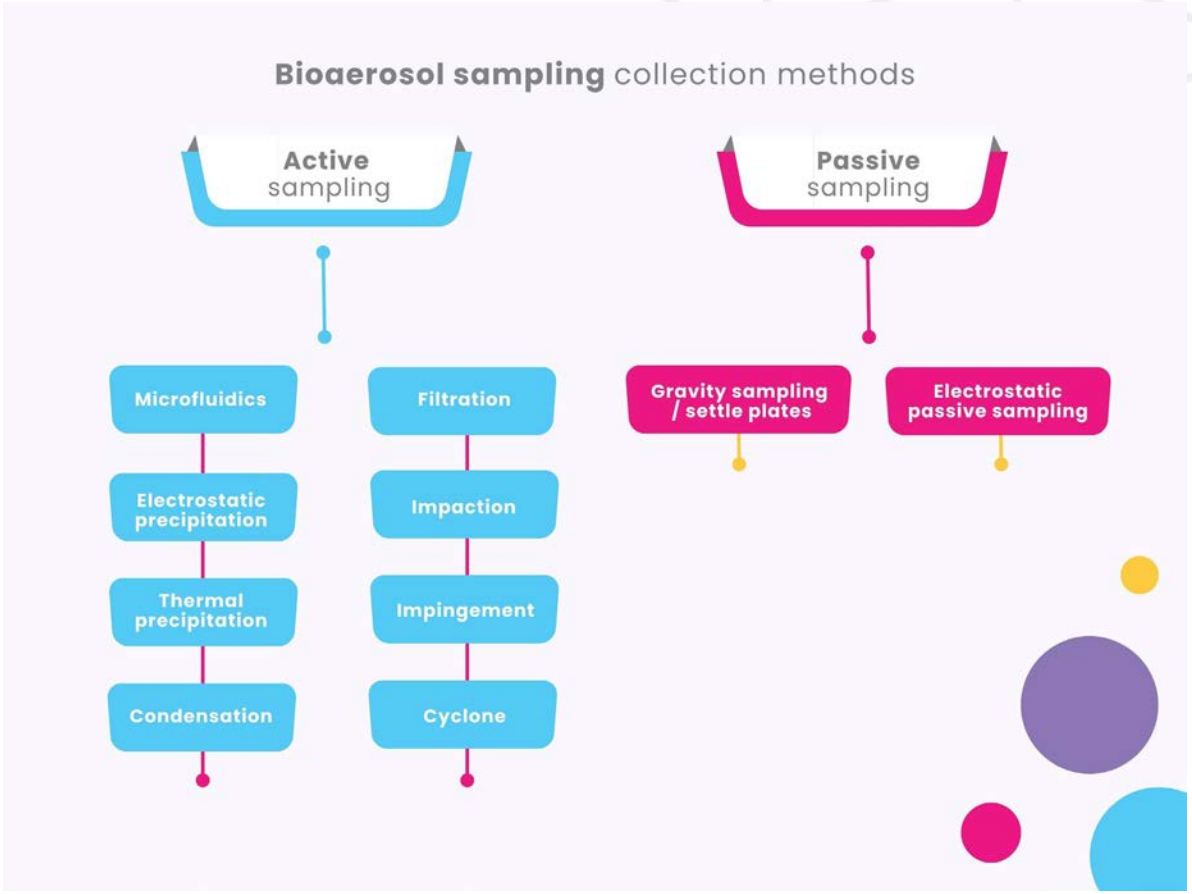
https://doi.org/10.1016/bs.aecr.2022.09.004



https://bioairnet.co.uk/publications/

Bioaerosol sampling methods for AMR detection in the atmosphere





Whitby et et al, 2022, Adv. Ecol. Res. 67, 101-229

Sample size, replication, and frequency considerations

Determine Sampling Purpose: Guide the number and strategy based on objectives (e.g., general air quality vs. worker/public health risks).

Use Statistical Methods: Apply methods like power analysis to determine optimal sample size, considering variability and confidence levels.

Consider Area Size/Layout & Sources: Larger or heterogeneous sites may require more samples. Locations should represent diverse areas within the site.

Review Existing Protocols: Utilise relevant regulatory or industry guidelines (e.g., M9, occupational guidelines) for consistency and compatibility with broader data.

Sampling Frequency: Set frequency based on exposure risk, periodic for low risk, continuous for high risk, ensuring statistical representativeness.

Field-based trials of bioaerosols sampling methods

Sampling plan based on a rapid evidence assessment, along with **M9 Technical Guidance Note** (Environmental monitoring of bioaerosols at regulated facilities), using a decision support tree as originally developed within the Environment Agency report "**Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms**" (EA, 2022)

Field-based trials of bioaerosols sampling methods

Sampling Methods and Results:

- Methods: Filtration (IOM, Leckel with Bio inlet), Impingement (SKC BioSampler), Cyclone (Coriolis Compact).
- Flow rates: 2l/min (IOM) to 50l/min (Leckel, Coriolis Compact).
- Duration: Short (1 hour) and long (2 hours) sampling at source and ambient locations.
- Total samples: 48 (excluding travel blanks).
- Samples preserved and shipped to UKHSA Laboratories for analysis.

Findings:

- Filtration (gelatine filters) and liquid impingement captured viable bacteria best.
- Impingement faced issues with evaporation and fragility.
- Viable bacteria counts declined with longer sampling durations.
- Based on results, **filtration and cyclones** were chosen for field trials.

Analysis options for AMR in bioaerosols

Culture-dependent approaches:

- Count total and resistant colonies from collected bioaerosols
- Calculate density and resistance proportions

Culture-independent approaches:

- Metagenomic sequencing: Results in FASTA files; store in secure repositories
- HT-qPCR: Gene identities and abundances
- Convert gene counts to relative abundances (genes/16S rRNA)

Metadata for interpretation:

- Sample volume, date/time, location, activity data
- Local meteorology, instrument performance, surrounding environment
- Additional data from nearby air quality monitoring stations

Statistical methods:

- Multiple linear regression for modelling airborne AMR
- Use models tailored to specific locations.

Data analysis and Reporting of AMR in air samples

Calibration & Consistency: Proper calibration of sampling equipment is essential for accurate results in all environments.

Culture-dependent methods:

- Quantify antibiotic-resistant colonies (CFUs/volume of air)Calculate proportion of resistant colonies
- Estimate airborne antibiotic-resistant bacteria diversity and abundance
- Compare resistance levels across sites/timepoints

Culture-independent methods:

- Quantitative PCR: Screen for targeted AMR genes (ARGs)
- Metagenomic analysis: More comprehensive, but requires bioinformatic expertise
- Identify and characterise ARGs, assess microbial community diversity, and source resistance gene dissemination

Combining Methods: Integrate both culture-dependent and independent methods for a complete understanding of airborne AMR levels, dynamics, and gene dissemination potential.

Design for an initial surveillance strategy for AMR within bioaerosols in the UK

Objectives of Airborne AMR Surveillance

1. **Characterise AMR at Source Sites** by measuring prevalence (% resistant determinants) and density (resistant determinants/volume of air) and identifying sources with significant aerosolized AMR and inform mitigation strategies.

2. **Monitor AMR at Receptor Sites** by estimating exposure levels of airborne AMR to humans to support health risk assessments and proportionate mitigation actions

Recommendation for an integrated Strategy :

- Develop sampling strategies for both source and receptor environments.
- Apply consistent bioaerosol sampling and microbiological approaches to connect sources and receptors effectively.
- Combine culture-dependent (pathogen-specific focus) and culture-independent (broader community analysis) methods

Design for an initial surveillance strategy for AMR within bioaerosols in the UK

Sentinel Pathogens for Targeted Surveillance:

- Azole-resistant Aspergillus fumigatus: Environmental reservoir; respiratory pathogen.
- Methicillin-resistant Staphylococcus aureus (MRSA): Airborne transmission concern.
- ESBL-producing Escherichia coli: WHO-recommended indicator species for AMR tracking.

Sampling Recommendations:

- Use gelatine filters or liquid impingement for bioaerosol collection.
- Maintain sample integrity and align analysis with public health priorities

Broad surveillance of sirborne AMR (Culture-Independent Methods)

Why Culture-Independent Methods?

- Over 99% of microorganisms are non-culturable, harbouring diverse resistance genes on mobile genetic elements.
- Essential for monitoring horizontal gene transfer risks and broad resistance in microbial communities.

Key Techniques

- **Metagenomic Sequencing**: Detects thousands of resistance genes; comprehensive but less sensitive to rare genes.
- **High-Throughput qPCR** (HTqPCR): Quantifies up to 384 genes per sample; highly accurate for rare and critical genes; Biomass markers (16S rRNA for bacteria, 18S rRNA for fungi) ensure microbial presence is validated.

Integrated Approach:

- Combine metagenomics for initial broad screening and HTqPCR for precise quantification of key genes.
- Supplement with amplicon sequencing to enhance microbial diversity insights.

Broad surveillance of airborne AMR (Culture-Independent Methods)

Applications

- Identify microbial diversity, resistance potential, and likely sources.
- Focus on total AMR gene density or diversity indices (e.g., Simpson's) to reveal patterns for targeted surveillance..

Costs

- Metagenomics: ~£200/sample; HTqPCR: ~£612/sample (£2,450 for analysis of 384 genes in four samples).
- Cost-effective with increased sample numbers; outsourcing recommended for scalability.

Outcome:

- Provides detailed, actionable data to inform AMR dynamics, source tracking, and surveillance refinement

AMR Source-focused strategy

Objective: Assess airborne AMR at high-emission source locations (e.g., WWTPs, farms, composting facilities) through a national surveillance network

Key Framework:

- **Define Objectives:** Identify AMR occurrence or changes at source categories.
- **Source Categories:** Prioritize high-risk sources via iterative sampling.
- **Representative Sampling:** Select candidate sites reflecting emission variability.
- **Sampling Frequency & Duration:** Tailor to source activity, risk, and bioaerosol densities.
- **Equipment & Protocols:** Use standardized equipment for consistent, representative data.
- **Analysis & Transport:** Ensure sterile handling, DNA extraction, and AMR quantification.

Considerations:

- Conduct regional pilot studies to determine variability and optimal sample size.
- Power calculations ensure statistically significant data across representative sites.

AMR Receptor-focused strategy

Objective: Quantify average airborne AMR levels in ambient air to assess human exposure risks

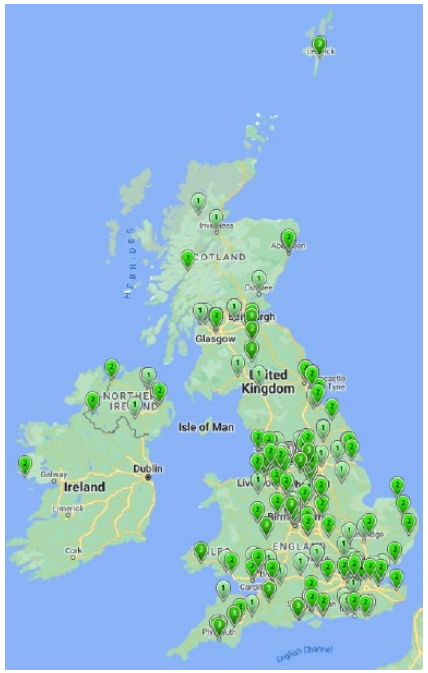
Key Framework:

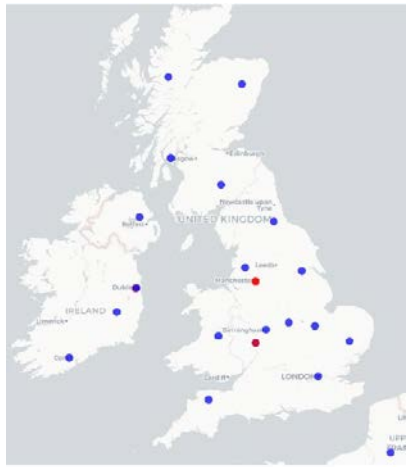
1. **Leverage Existing Networks:** Use sites like the AURN (170 stations) for sampling.
2. **Seasonal Sampling:** Deploy bioaerosol samplers alongside air pollution monitors.
3. **Correlations:** Link AMR data with air pollutants and meteorological factors.
4. **Sample Size & Power:** Pilot studies and power calculations determine sufficient data coverage.

Sentinel Pathogens: Target organisms (e.g., *A. fumigatus*, *S. aureus*, *E. coli*) monitored using active sampling to achieve >100 culture-positive samples per campaign.

Outcome: Combined source and receptor-focused strategies develop actionable insights into AMR transmission and exposure risks, guiding mitigation and public health protection.

Integration with existing networks in the UK ?





Air Quality Monitoring Sites (AURN): Provide established infrastructure, long-term data, and metadata for AMR analysis but may lack optimal exposure risk relevance.

Pollen Monitoring Network: Offers secure sites, complementary data, and daily visits, though location relevance and sampling duration might limit applicability.

Bioaerosol Monitoring Sites: Standardized sampling (M9 method) at waste sites adds value but relies on infrequent sampling and culture-based methods.

Key Considerations:

- Using existing networks for cost-efficiency, metadata integration, and secure site management.
- Address barriers such as location suitability, sampling consistency, and permission approvals to develop a robust AMR surveillance strategy.

Strategic alignment for AMR bioaerosol surveillance

Preferred approach: Aligning AMR bioaerosol sampling with the **Automatic Urban and Rural Network (AURN)** offers the most viable and resource-efficient option

Implementation needs:

- **Initial phase**: Establish a dedicated AMR bioaerosol team working in parallel with AURN resources
- **Medium term**: transition to a shared-duty model once AMR sampling methodologies are standardized.

Key Considerations:

- Evaluate AURN sites for relevance to AMR exposure and proximity to sources.
- Ensure sampling objectives of both networks are met without compromise

Challenges for Source-Specific Sampling: Collaboration with process operators poses risks, including inconsistent sampling practices and variable post-collection quality.

Take-Home Message: Early-Stage UK Airborne AMR Surveillance Strategy

Purpose: Address knowledge gaps on airborne AMR prevalence and risks to public health.

Approach: Combine bioaerosol sampling (culture-dependent and -independent methods) and existing air quality networks (e.g., AURN) for efficient and scalable surveillance

Key Recommendations:

1. Standardise sampling methods across source and receptor environments for consistency.
2. Use targeted culture-based methods for sentinel AMR pathogens and metagenomics for broader threat characterization.
3. Integrate with existing air quality regimes to leverage spatial/temporal coverage and metadata.

Future Needs: Advance detection methods, clarify AMR transmission pathways, and assess health impacts via a multidisciplinary approach.

Outcome: Develop actionable measures to mitigate airborne AMR and safeguard public health.

Disclaimer

Environment Agency

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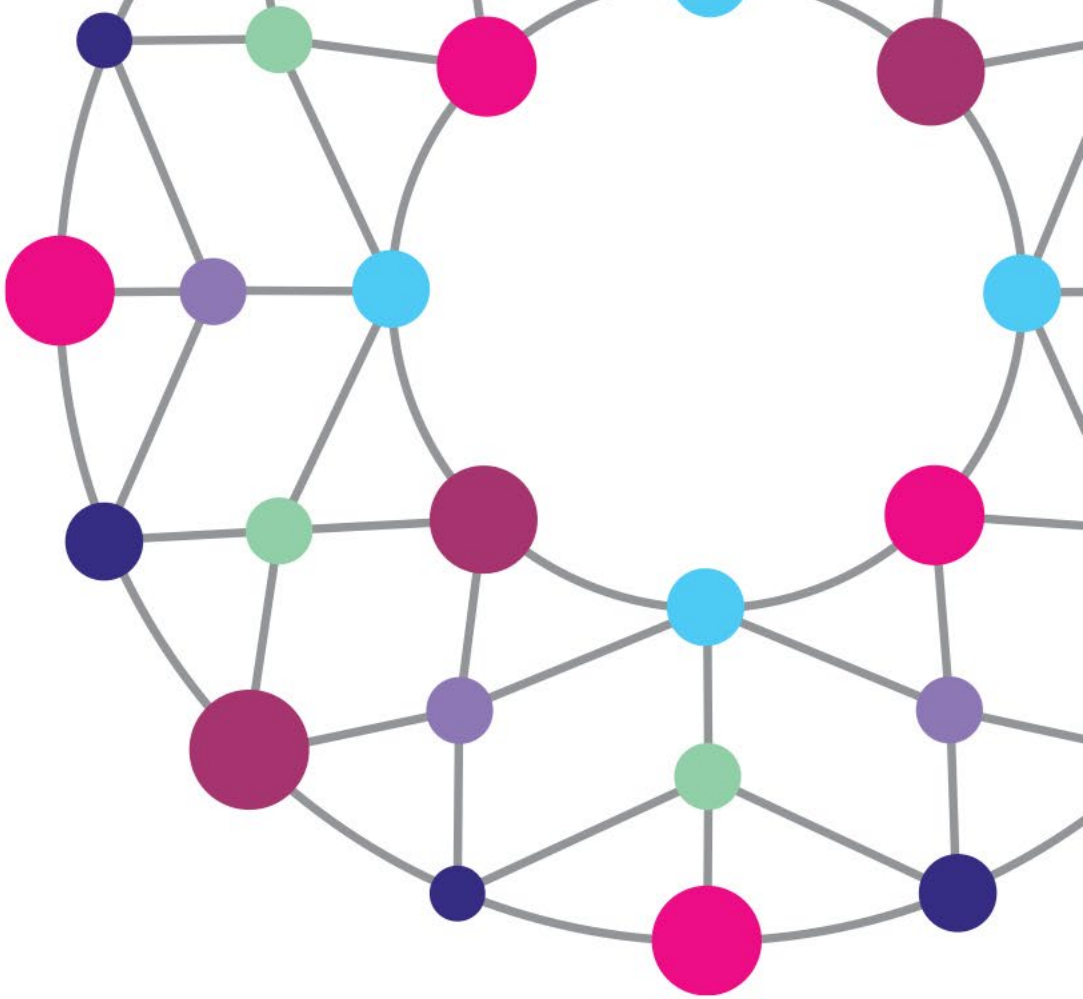

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