

# PATH-SAFE: Novel Foodborne Pathogen, and associated antimicrobial resistance, knowledge and surveillance approaches

Underpinning any surveillance system, are sample collection and analysis methodologies, baseline data to draw comparison to and data analysis tools.

## Overview

The work within this theme has focused on three key areas:

- development of novel surveillance methodologies, approaches and tools
- generation of data on the genomic diversity of a range of foodborne pathogens, and associated AMR, across the four nations of the UK to establish baselines and address knowledge gaps
- exploration of whole genome sequence data to investigate foodborne pathogen and antimicrobial resistance transmission routes

Drawing on the strengths and expertise of a range of delivery partners, and the power of working collaboratively, it has been possible to deliver a large portfolio of projects that have incorporated perspectives and input from across the agri-food system.

**Food Standards Scotland (FSS)** have sequenced thousands of Escherichia coli (E.coli) isolates, from a diverse range of sources across Scotland, to help elucidate transmission dynamics through the agri-food chain and to develop machine learning source attribution models for predicting the source or host of origin for E.coli isolates. The team are now sequencing further agri-food related isolates to verify the application of the E. coli source attribution models, looking to demonstrate proof of concept for use of the model in the investigation of foodborne illness and risk management, and extend the approach to develop a similar model for Salmonella Typhimurium (STm).

**The Animal and Plant Health Agency (APHA)** and **Veterinary Medicines Directorate (VMD)**, have generated new data and knowledge on AMR presence, diversity, and transmission by applying genomics to existing surveillance sampling and initiating novel sampling in understudied compartments of the agri-food chain, particularly ruminants (via bulk milk, and cattle and sheep at slaughter) and animal feed. Whole genome sequence data produced by these projects is now undergoing enhanced genomic to enhance understanding of AMR dynamics, transmission pathways and mobile genetic elements dissemination. Further novel sampling is being conducted to determine the prevalence of AMR bacteria in imported Raw Pet Food and to explore the application of novel abattoir environment sampling approaches, as a potential alternative surveillance method that could act as a proxy for existing, labour intensive, livestock sampling methods.

**The Environment Agency (EA)** have explored surveillance options for AMR in the environment including river water and bathing waters, wild flora and fauna, shellfish and bioaerosols, and developed a pilot environmental surveillance system This work is continuing as part of the Environment Agency research portfolio.

**The Centre for Environment, Fisheries and Aquaculture Science (Cefas)** undertook a river catchment pilot to compare wastewater, river water and shellfish for monitoring of a range of foodborne pathogens and have worked with UKHSA and the Public Analyst laboratories in Scotland to demonstrate the utility of wastewater for monitoring norovirus and salmonella at a national scale. The team is now continuing the application and advancements of methodologies developed in PATH-SAFE to demonstrate the utility of bivalve molluscan shellfish (BMS), a species routinely sampled through pre-existing surveillance frameworks, as a sentinel in a 'sample once test many' approach.

**Bangor University** are examining the flow, dynamics, and risk posed by AMR and FBP in wastewater discharges, particularly those originating from healthcare settings. Further work is ongoing to investigate seasonal patterns and potential wastewater treatment opportunities and barriers.

**Food Standard Agency (FSA), UK Health Security Agency (UKHSA), NHS Lothian and Cefas** are undertaking a project to generate FBD prevalence and WGS data from business-as-usual UKHSA polio wastewater monitoring, utilising methodologies developed by Cefas within their PATH-SAFE project. This data will subsequently be compared to data from clinical samples collected through [the third study of Infectious Intestinal Disease in the UK](#), presenting an exciting opportunity to align two large surveillance efforts to generate insights into the effectiveness of wastewater monitoring for FBP.

**Queen's University Belfast (QUB)** have been investigating the application of air and near-source wastewater monitoring for AMR and FBP in a care home setting in Northern Ireland. This work is continuing to explore seasonal trends, increasing understanding of AMR and foodborne pathogens prevalence and transmission and further evidencing the utility of the novel surveillance methods.

**University of Oxford** have sequenced thousands of Campylobacter isolates, from a diverse range of collaborators, to help elucidate source attribution and transmission dynamics through the agri-food chain, and the rise in AMR. Sequencing and analysis of further human and agri-food Campylobacter isolates is ongoing, to determine levels of AMR in Campylobacter isolates currently circulating in the UK and enhance understanding of AMR gene transmission.

**Agri-food and Biosciences Institute (AFBI)** in Northern Ireland selected and sequenced archived isolates of Salmonella and Listeria monocytogenes from food and animal sources, to provide important historical context against which future outbreak sequences can be compared.

## Delivery Partners

In addition to the lead delivery partners highlighted in the overview section above, a number of other collaborators have supported the delivery of these projects, including:

- Scottish Environment Protection Agency
- Public Health Scotland
- Scotland's Rural College
- Moredun Research Institute
- The University of Edinburgh
- Northern Ireland Environment Agency
- Department of Health (NI)
- Agriculture, Environment and Rural Affairs (NI)
- Department of Infrastructure (NI)
- HSC Public Health Agency
- Northern Ireland Water
- Welsh Government

- Public Health Wales
- Welsh Water
- Cardiff University
- South West Water
- Ribble Rivers Trust
- Biosphere North Devon
- Virosafety
- Resistomap
- Quadram Institute
- University of Exeter
- Cranfield University
- UK Pet Food
- Agricultural Industries Confederation
- National Milk Laboratories
- Arla
- UK Centre for Ecology and Hydrology
- Aecom
- Arup
- Capita
- Deloitte
- MicrobesNG

## Status

All projects are progressing well through their sample collections and analysis stage.

Key examples of achievements in this quarter include:

- the abattoir environment project has had some success in bringing abattoirs on board for the project after a limited response during the early stages of the project
- a number of ports and a raw pet food manufacturer have been recruited to the imported raw pet food project; samples have been received and are being processed
- phylogenetic analysis to identify related isolates and comparison of AMR genotypes across Sheep, Animal feed and raw bulk milk have been completed in the genomic characterisation project.

Data for the Campylobacter project can be viewed on [PubMLST](#), PATH-SAFE year 2 #160. The food sampling programme element of the E.coli and Salmonella infections in Scotland project is now complete. Dissemination of outputs from Phase 1 is ongoing (please see outputs section above).

## Key successes

Across the theme:

- more than 7,000 samples have been collected, across a range of sample sources and four nations
- additional analysis has been undertaken on over 2,300 samples collected as part of existing surveillance activities
- exploration of more than 3,500 archived samples and 4700 archived pathogen isolates
- in excess of 18,000 pathogen isolates have been sequenced
- increased understanding of methods suitable for environmental monitoring across a range of different media and development of a pilot environmental surveillance system

Novel surveillance methods, approaches and tools developed and tested using real world samples and data for the first time, including:

- protocols for isolation and testing of foodborne pathogens from river water, wastewater and shellfish
- wastewater-based AMR and FBD surveillance sample collection schedules, sample analysis and data analysis pipelines
- machine learning models to determine source/host attribution of pathogens

Contamination and transmission models:

- demonstrable benefit of wastewater-based surveillance inspiring nursing homes in Northern Ireland to consider wastewater systems design in future builds to support wastewater-based surveillance
- large sample volume, broad scope and high throughput nature of PATH-SAFE projects have accelerated development and refinement of existing and established methods, including long read sequencing methodologies used by APHA for routine AMR surveillance
- utility of routine whole genome sequencing demonstrated through patterns, trends and insight gained across the programme, including the identification of resistant bacterial isolates of concern within the isolates sequenced for PATH-SAFE
- strengthened cross government and academia collaborative approach to identifying and pursuing surveillance opportunities, methods development and results analysis
- industry actively involved in the design and conduct of government surveillance activities, and promoting benefits of such collaborations, including milk producers and an animal feed mill, in collaboration with National Milk Record and The Agricultural Industries Confederation respectively

## Outputs

All projects were presented at the PATH-SAFE Biosurveillance Conference on 28 and 29 February 2024 in London. [Recordings, slides and posters are available on the conference webpage.](#)

## Summary articles

[Monitoring antimicrobial resistance in the environment.](#) Published 24 October 2024 on gov.uk, a piece within the Environment Agency's [Creating a better place blog](#).

[How the PATH-SAFE programme has driven forward our understanding of AMR in UK animals:](#) Published 18 November 2024 in The Microbiologist, Applied Microbiology International's digital magazine. This piece highlights how evidence generated within PATH-SAFE has progressed the understanding of AMR prevalence and transmission and illustrates the significant benefits that well-funded, coordinated, cross-sectoral initiatives can deliver.

## Environment Agency Publications

[Scoping review into environmental selection for antifungal resistance and testing methodology.](#) Published on 22 July 2022 on gov.uk, this report reviews the current understanding of the mechanism for selection for antifungal resistance in fungal species following exposure to antifungals.

[Antimicrobial resistance surveillance pilot site selection and database extension.](#) Published on 22 July 2022 on gov.uk, this project developed selection criteria to identify suitable river catchments for piloting a surveillance programme for environmental antimicrobial resistance.

[Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms.](#) Published on 22 July 2022 on gov.uk, this report reviews the available sampling options for antimicrobial resistant microorganisms, including their antimicrobial resistance genes, from the atmosphere.

[Antifungal medicines in the terrestrial environment: Levels in biosolids from England and Wales.](#) Published on 02 February 2023 in Science of The Total Environment, this article reports on biosolids from ten sites in England and one in Wales that were sampled and analysed for clinical antifungals.

[Environmental surveillance of AMR, perspective from a national environmental regulator in 2023.](#) Published 30 March 2023 in Eurosurveillance, this is a perspective paper discussing some of the issues and choices that arise in selecting the most appropriate methods for surveillance for AMR in the environment from the perspective of a government agency that conducts routine environmental monitoring for other health related purposes.

[Environmental antimicrobial resistance: A review of biological methods.](#) Published 26 October 2023 on gov.uk, this report reviews biological methods for the detection of environmental antimicrobial resistance.

[Antimicrobial resistance surveillance strategies within wild flora and fauna of England.](#) Published 26 October 2023 on gov.uk, this report reviews potential antimicrobial resistance surveillance strategies for wild flora and fauna.

[Shellfish as bioindicator for coastal antimicrobial resistance.](#) Published 26 October 2023 on gov.uk, this report covers development of testing approaches to assess the presence and identity of antimicrobials and resistant microorganisms in marine shellfish.

[A review of approaches to monitoring and surveillance of antimicrobial resistance in bathing waters.](#) Published 26 October 2023 on gov.uk, this report improves understanding of antimicrobial resistance in relation to human exposures via bathing waters.

[Antimicrobial resistance in bioaerosols: towards a national surveillance strategy.](#) Published 26 October 2023 on gov.uk, this report considers the decisions that must be made in designing a national surveillance strategy for antimicrobial resistance in bioaerosols.

[Pilot surveillance of antimicrobial resistance in river catchments in England.](#) Published 24 October 2024 on gov.uk, a pilot approach to detection, identification and quantification of antimicrobial resistance in three selected river catchments in England.

[Development of experimental approaches for determining concentrations of antifungals that select for resistance.](#) Published 24 October 2024 on gov.uk, methods for the determination of the lowest concentration of antifungals that can lead to a selective advantage for resistant organisms.

[Determining selective concentrations for antibiotics and antifungals in natural environments.](#) Published 24 October 2024 on gov.uk, this project determined the concentrations of specified antimicrobials at which selection for resistance may occur.

[Determining concentrations of substances that influence development of antimicrobial resistance.](#) Published 24th October 2024 on gov.uk, this report reviews the available data on concentrations at which selection for antimicrobial resistance has been reported for different antimicrobials and the approaches used to determine these concentrations. This work was also published as a peer-reviewed paper: [A critical meta-analysis of predicted no effect concentrations for antimicrobial resistance selection in the environment.](#) Published 20 August 2024 in Water Research.

[Potential impact of disinfectants on antimicrobial resistance development.](#) Published 24 October 2024 on gov.uk, this report identifies the range of disinfectants currently used in the UK and reviewed available information on their potential role in the development of antimicrobial resistance.

[Risk screening and prioritisation tool for antimicrobial resistance in the environment.](#) Published 24 October 2024 on gov.uk, a risk screening and prioritisation tool to assess antimicrobial resistance in the environment.

## Cefas Publications

[Development and validation of a duplex RT-qPCR assay for norovirus quantification in wastewater samples.](#) Published November 2023 this article covers development of a duplex RT-qPCR assay that [enables the sensitive detection of both NoVGI and NoVGII](#) in wastewater-derived RNA eluents, in a time and cost-effective way and may be used for surveillance to monitor public and [environmental health](#).

[Long Amplicon Nanopore Sequencing for Dual-Typing RdRp and VP1 Genes of Norovirus Genogroups I and II in Wastewater.](#) Published on 27 March 2024 on bioRxiv, this article covers the development and optimisation of a long amplicon nanopore-based method for dual-typing the [RNA-dependent RNA polymerase \(RdRp\)](#) and major structural protein (VP1) regions of norovirus, with wastewater as the sample matrix.

[Realising a global One Health disease surveillance approach: insights from wastewater and beyond.](#) Published 22 June 2024 in Nature Communications, this article presents case studies centred around the recent global approach to tackle antimicrobial resistance and the current interest in wastewater testing, with the concept of “one sample many analyses” to be further explored as the most appropriate means of initiating this endeavour.

[Piloting wastewater-based surveillance of norovirus in England.](#) Published 1 October 2024 in Water Research, this study took approximately 3,200 samples of wastewater from across England, previously collected for quantification of SARS-CoV-2, and re-analysed them for the quantification of norovirus genogroup I (GI) and II (GII). [Comparisons of national average norovirus concentrations in wastewater against concomitant norovirus reported case numbers showed a significant linear relationship.](#)

[Food-borne disease risk: biosurveillance in water networks.](#) Published 12 September 2024 in Eurosurveillance, this meeting report details to presentations, group discussions, conclusion and recommendations of a workshops hosted by Centre for Environment, Food and Aquaculture Science (Cefas) and Bangor University at the Royal Institution, London, on 31 January 2024.

## Methodology publications

Cefas: [Long Amplicon Nanopore Sequencing for Dual-Typing RdRp and VP1 Genes of Norovirus Genogroups I and II in Wastewater V.2](#)

## Data

FSS: [Scottish E.coli](#) (locate sequencing by searching the ‘Comment’ field for ‘PATH-SAFE’ and ‘PATH-SAFE project’)

Cefas: [Norovirus, ammonia and orthophosphate in wastewater samples collected from across England 2021 - 2022](#)

Cefas: [Whole Genome Sequencing Salmonella in Scottish Wastewater](#)

University of Oxford: [Campylobacter isolate collection on PubMLST](#)

AFBI: [Listeria Sequences](#)

AFBI: [Salmonella sequences](#) (locate sequencing by searching the 'Comment' field for 'PATH-SAFE' and 'PATH-SAFE\_23\_24')