Session 5
Exploring Novel Biosurveillance Methods

PATH-SAFE

WS3a

Rapid diagnostic technologies for foodborne pathogens

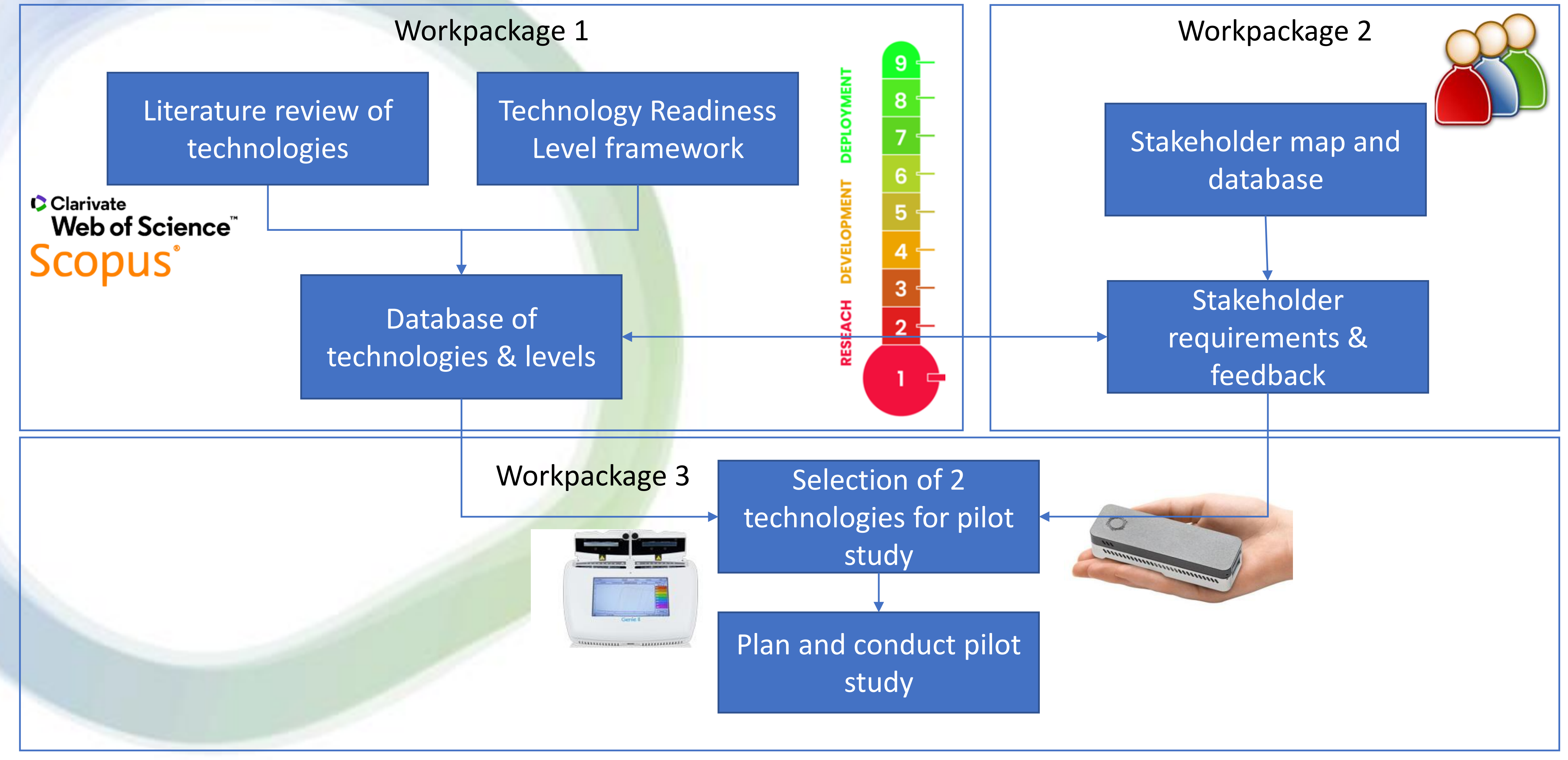
PATH-SAFE Biosurveillance conference

28-29th February 2024

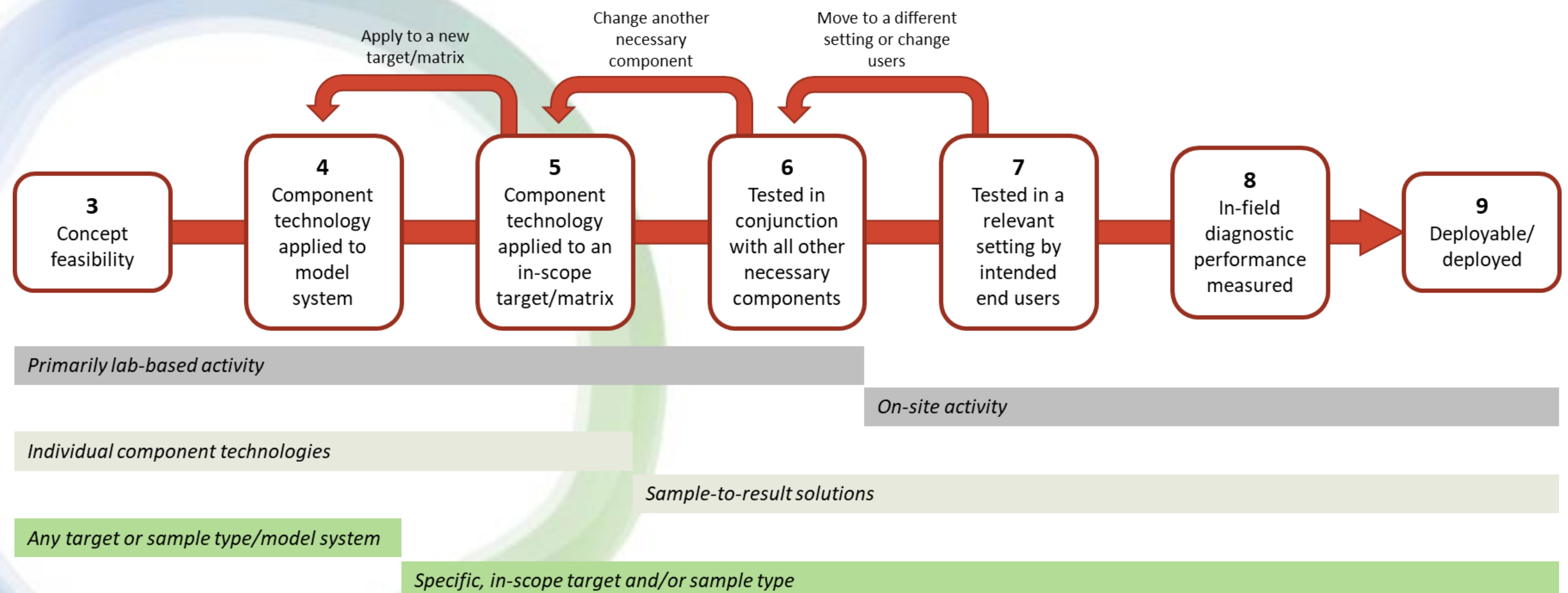
Ashleigh Elliott

Manisha Gupta

Pathsafe - Workstream 3a Technology Readiness Level (TRL) study



WP1 – TRL framework



TRL assessment tool was used to answer questions about each technology using information from the literature search to guide the TRL assignment.

WP2 Stakeholder engagement

Strategic steer

2 focus groups & 3 interviews (FSA, DEFRA, APHA, CEFAS, UKHSA)

↓

Operational feasibility & need

7 end-user interviews

Needs and opportunities

Statutory testing vs Non-statutory / additional testing

Statutory testing

- Pen-side testing
- Product testing (production & points of entry i.e. ports)

Non-statutory / additional testing

- Customer assurance (e.g. norovirus in shellfish)
- Hygiene testing (e.g. counter-tops)
- Production decisions (e.g. irrigation water)

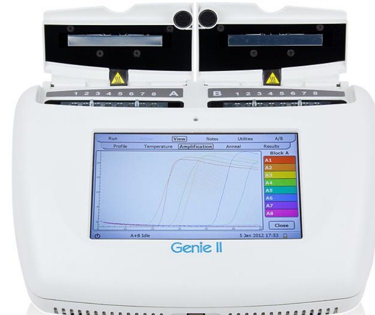
Test requirements (scenario specific)

- Pathogen viability
- Presence / absence vs quantification
- High sensitivity – but no enrichment (live cultures)
- Cost, speed, ease of use: training possible

WP3: Pilot in-field diagnostic testing

Monitoring of *E. coli* in irrigation water for fresh produce using portable real-time PCR.

Detection of *Salmonella* in high-risk foods of non-animal origin at ports using LAMP.



Monitoring of *E. coli* in irrigation water for fresh produce using portable real-time PCR.

Validation in Lab- Selection of real-time PCR assay

Assay 1 Commercial kit

Assay 2 Silkie et al. 2008

Assay 3 Walker et al. 2017

Analytical specificity

Target bacteria – inclusivity
E. coli (49)
Non-target bacteria – exclusivity
Salmonella Enteritidis
Salmonella indiana
Salmonella Agana
Salmonella Bredeney
Salmonella enterica
Acinetobacter lwoffii (2)
Proteus mirabilis (3)
Citrobacter braakii (2)
Citrobacter werkmanii
Enterococcus faecalis
Klebsiella pneumoniae
Klebsiella oxytoca
Listeria monocytogenes
Pantoea agglomerans
Pseudomonas aeruginosa
Vibrio parahaemolyticus
Bacillus cereus
Clostridium perfringens
Lactobacillus delbrueckii

Analytical sensitivity: Dilution series *E. coli* DNA from 10 ng – 1 fg.

Water filtration method

Ct 29.4 ± 0.1 (n=5)

Vs

Ct 30.7 ± 0.4 (n=5)

DNA extraction methods

Ct 30.5 ± 0.2 (n=5)

Vs

Ct 29.4 ± 0.1 (n=5)

Final method for validation experiments

1.

Sample water source

2.

Disposable filter funnel

Vacuum hand pump

Water poured into the filter unit and filtered using a hand pump to collect bacteria on the filter paper.

3.

Filter paper folded using forceps and added to a tube with ball bearing.

4.

DNA extraction cartridge

5.

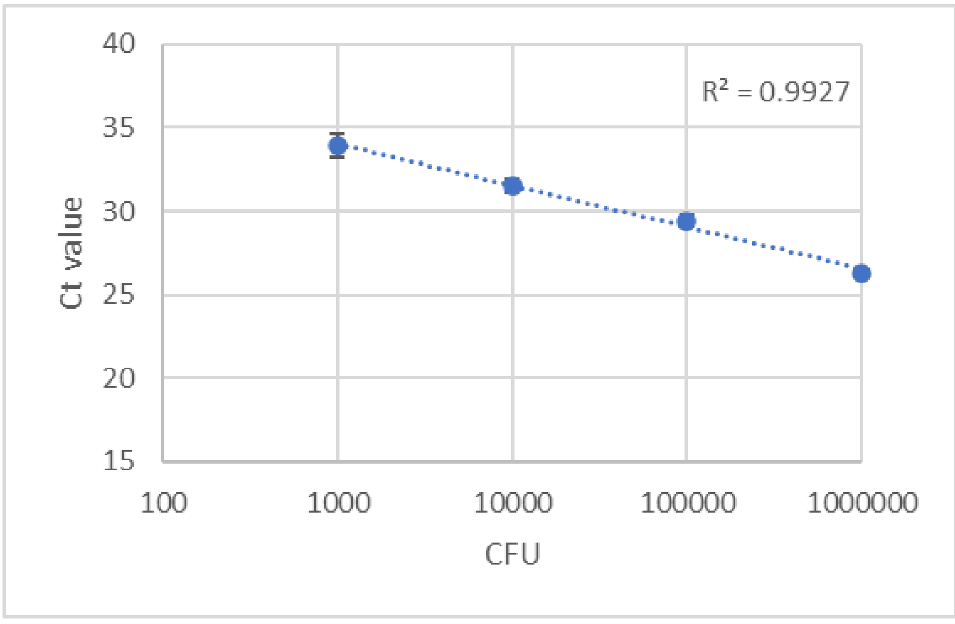
PCR set-up

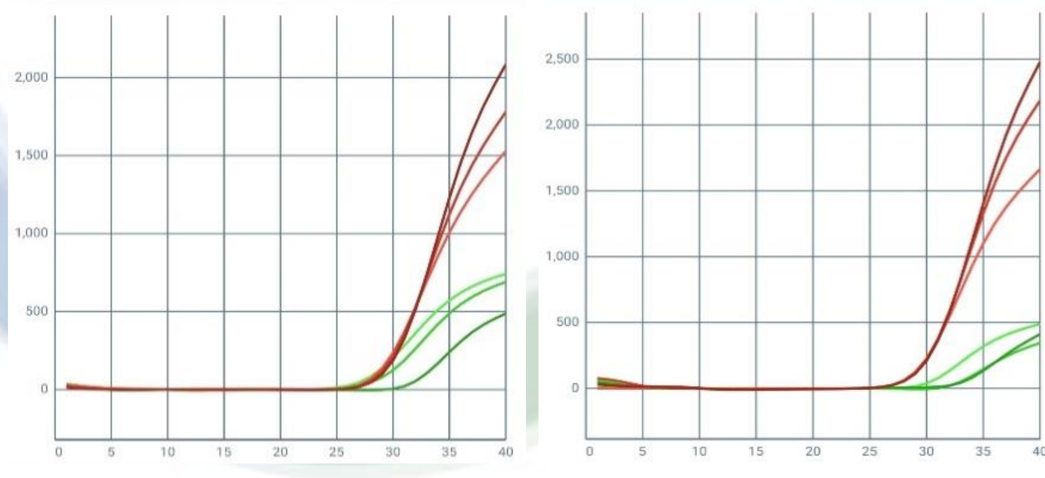
Portable PCR machine

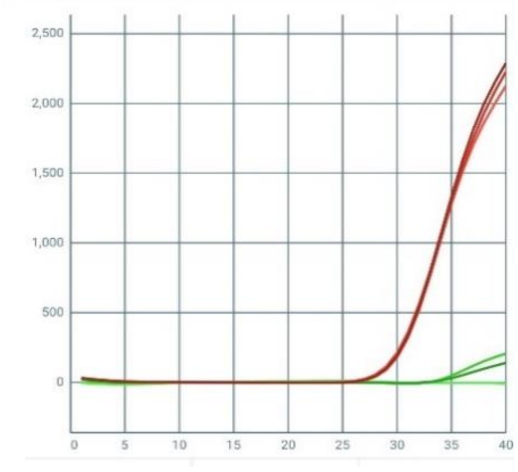
Sensitivity of the test

Limit of detection determined to be ~1000 CFU/ 100 ml

E.coli CFU/100 ml	Average Ct	SE	% detection	Replicates
10^6	26.3	0.1	100	3
10^5	29.43	0.4	100	6
10^4	31.5	0.4	100	6
10^3	33.9	0.7	90	10
10^2	34.2	n/a	17	6
10^1	-	n/a	0	6







Portable real-time PCR - Results graphs

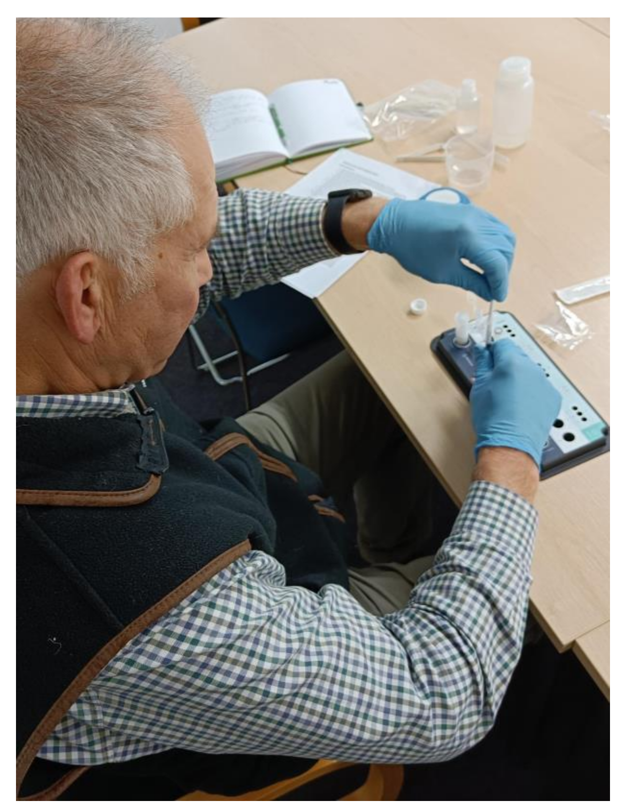
E.coli, IPC

Real samples – side-by-side testing

Sample	Source	Total confirmed *E. coli* CFU/100ml	*E. coli* Ct	IPC Ct
1	Reservoir	87	-	25.27
2	Reservoir	17	-	25.27
3	Reservoir	56	-	25.2
4	Reservoir	84	-	24.22
5	Drain	5	-	25.11
6	Borehole	1	-	25.89
7	Beck	200	-	26.59
8	Beck	1160	28.65	25.21
9	Pond	62	-	24.98
10	Pond	22	-	24.18
11	Beck	202	28.2	25.57
12	Pond	8	-	25.41
13	Dyke	460	-	25.89
14	Dyke	250	-	25.28
15	Well	2	-	25.31
16	River	150	-	26.27
17	River	94	-	26.47
18	River	330	30.24	25.99
19	River	1540	29.14	26.16
20	River	370	29.58	26.24
21	River	94	-	25.33
22	River	196	-	26.34
23	River	11	-	25.16
24	River	59	-	25.21
25	River	146	-	25.12

- Samples over >1000 CFU were detected in line with the limit of detection.
- Samples > 100 CFU were sporadically detected.
- Ct values did not show a relationship to CFU counts.

Training with End Users



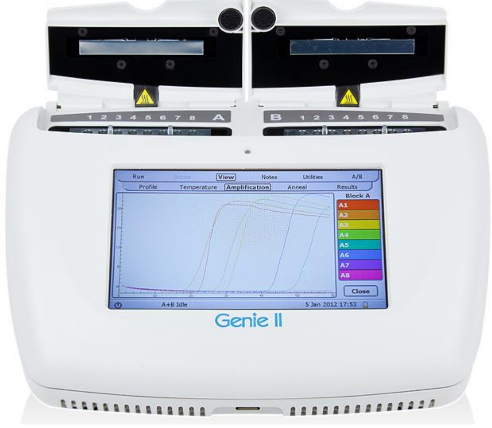
- Training took place with two end-users during two training sessions. First at Fera, where negative water samples were processed.

- Second at an on-site location where real irrigation water samples were collected and tested. DNA extracts and parallel samples were re-tested at the lab.

Summary

- The test showed good specificity during validation.
- Simple and equipment free workflow.
- Feedback from end-users about performing the method was generally positive.
- Sensitivity is likely not high enough for some of the strictest testing criteria.
- The real-time PCR Ct values did not show a high correlation with the gold standard method CFU counts.

Detection of *Salmonella* in high-risk foods of non-animal origin using LAMP.



Verification in Lab- Selection of the LAMP assay

Assay 1 SAL4 (Ge *et al.*, 2019 and WS3b)

Assay 2 invA (Hara Kudo *et al.*, 2005)

Assay 3 OptiGene Ltd. (BK-S.enterica-050)

Target bacteria
Salmonella Agona (3)
S. Bredeney
S. Enteritidis (3)
S. Hadar
S. Indiana
S. Infantis
S. Livingstone
S. Mbadanka
S. Ohio
S. Senftenberg
S. Stanley
S. Thompson
S. Virchow
Salmonella sp.
S. enterica
Non-target bacteria
Acinetobacter iwoffi (2)
Citrobacter brakii (3)
Citrobacter sp. (2)
Citro werkmanii
Enterobacter cloaceae
E.coli (2)
Proteus mirabilis (4)

Verification in Lab- Crude DNA extraction method

Segregating seeds and solution

Cell strainer

Filter paper and Funnel

Filtration Methods to extract cells

Syringe filtration

Filter funnel with a hand vacuum pump

DNA extraction method

Heat treatment of cells with filtration and without filtration

Dipstick DNA Extraction Kit

Final Crude DNA extraction method

1.

Nalgene bottle with 25gm seeds

Add 100ml sterile distilled water (till red line) and shake for 10-15 seconds

2.

Cell strainer with 100µm pore size

3.

Vacuum hand pump

Water extract poured into the filter unit and pumped used hand pump to collect cells on filter paper.

4.

Filter paper folded using forceps and added to a 2ml tube with ball bearing.

5. Add 500µl sterile distilled water and shake the tube for 1 min and pipette out in liquid in a tube.

6. Heat the extract at 95°C for 5 min.

7. Spin the extract and take upper clear liquid for LAMP

Sensitivity of the test

- Limit of detection determined to be ~1000 CFU/ 25gm of seeds.
- Inhibition was observed in case of black seeds, therefore, dilution and BSA was incorporated in the reaction.
- Annealing temperature for *Salmonella*: 87.5±1°C.

Reagent	Total volume per reaction (µl)
ISO-004	14.5
Primer Mix	5
BSA	0.5

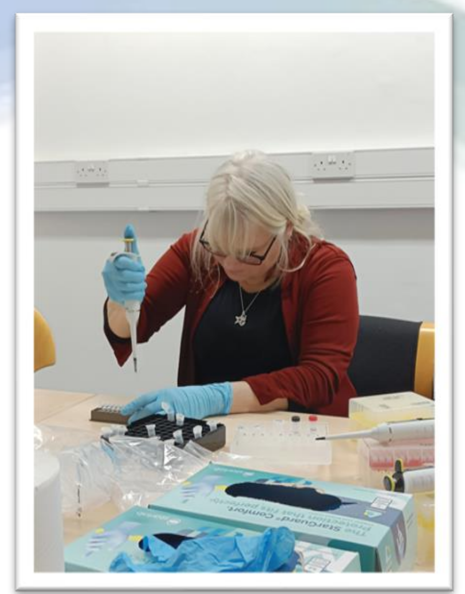
Amplification	Anneal	Ramp rate
65°C for 20 minutes	95°C to 75°C	0.05°C /s

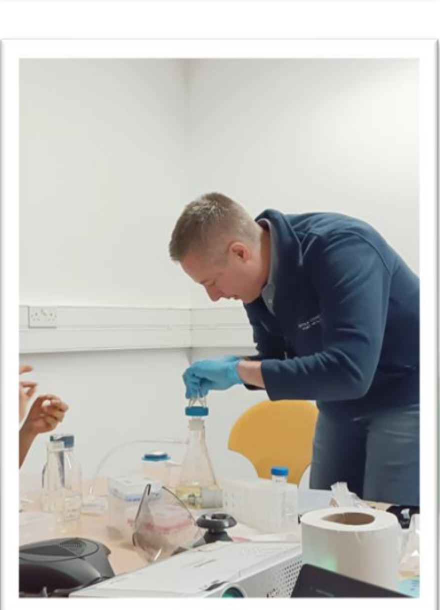
Salmonella CFU/25gm	Mixed seeds		White seeds		Brown seeds		Black seeds (1:5 dilution)	
	Tp	Ta	Tp	Ta	Tp	Ta	Tp	Ta
10*5	00:07:00	87.69	00:06:30	87.98	00:08:00	87.98	00:07:45	87.54
10*4	00:10:15	87.93	00:09:45	87.93	00:12:45	87.89	00:11:45	87.83
10*3	00:15:15	87.78	00:08:15	88.18	00:14:30	87.49	00:15:00	87.44
10*2	-	-	00:17:45	87.93	00:11:45	88.13	-	-
10*1	-	-	-	-	-	-	-	-

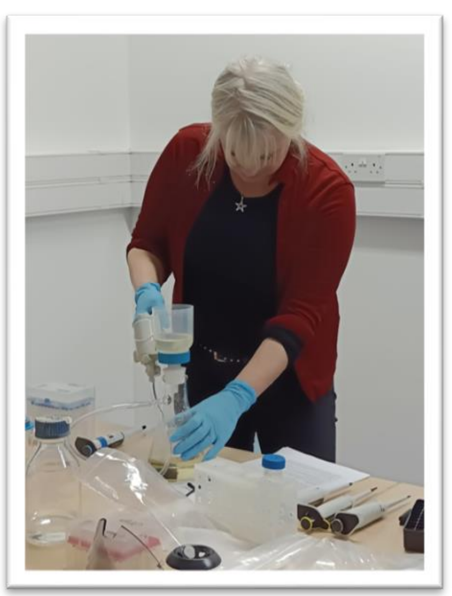
Visiting end-users during sampling session and testing real samples

Sample no.	Description	Country of origin	UKHSA result	FERA result
1428590/2	Organic Sesame seeds (Black)	Bolivia	Negative	Negative
1445109/2	Black Sesame seeds (Black)	India	Negative	Negative
1444794/2	Roasted mixed Sesame seeds	Taiwan, Province of China	Negative	Negative
1445012/3	Sesame seeds (White)	India	Negative	Negative
1445012/3	Sesame seeds (White)	India	Negative	Negative
1448549/2	Black Sesame seeds (Black)	China	Negative	Negative
1449537/3	Sesame seeds (White)	India	Negative	Negative
1451860/3	Sesame seeds (Brown)	India	Negative	Negative
1455735/3	Sesame seeds (White)	China	Negative	Negative
1460495/3	Sesame seeds-Hulled (White)	India	Negative	Negative

Training to end-users at Fera Science Ltd.



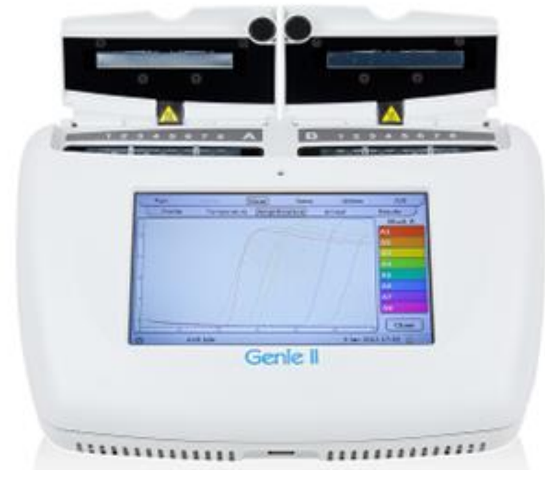




On-site training and parallel testing

Summary

- Specificity of the assay was good; no cross reaction was observed.
- Feedback from end-users was positive, able to perform the test independently after training.
- Sensitivity: 1000 CFU per 25gm of seed sample.
- Developing crude DNA extraction method for seeds is challenging.
- Approximate cost per test: ~20-25GBP per sample plus one time investment of instrument cost, time to result: 1 hr.

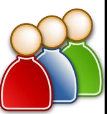


Outcomes from WS3a

Workpackage 1

- 16485 title and abstract screening, 3168 full text review.
- Technology Readiness Level framework was developed.
- Database of technologies and their readiness level were delivered.

Workpackage 2



- Engagement with stakeholders to understand the testing requirements.
- Opportunities around statutory and non-statutory testing were discussed.

Workpackage 3

- Based on the information from WP1 and WP2, two technologies and scenarios were selected for pilot in-field diagnostic testing.
- During validation, both assays showed good specificity; sensitivity was ~1000 CFU/sample. Real samples were tested using finalised method.
- End-users were trained at Fera, followed by on-site testing and feedback was gathered.

Future work/ next steps

Technical issues

- Challenges in developing crude extraction method for food matrices:
  - Quantity per sample as per regulations is usually high.
  - Presence of inhibitors.
- Achieving sensitivity of the assay compared to gold-standard methods.
- Interpretation and troubleshooting of results.

Logistical issues

- Requirement of infrastructure: staff and appropriate facilities to perform the testing on-site.
- Regular trainings, maintaining proficiency, quality control.
- Changes in legislations for decision making on-site and implementation.

Thank You

Acknowledgments

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Catherine Harrison
Jayne Hall
Deb Jones

University of Lincoln

Bukola Onarinde

End-users:

Port health authorities, Felixstowe
SDF Agriculture
Sails farm

CEFAS

How to design environmental surveillance for AMR
Chapter 1: A new hope or the phantom

Alwyn Hart, Martin Spurr, Jono Warren, Wiebke Schmidt

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Chief Scientist's Group

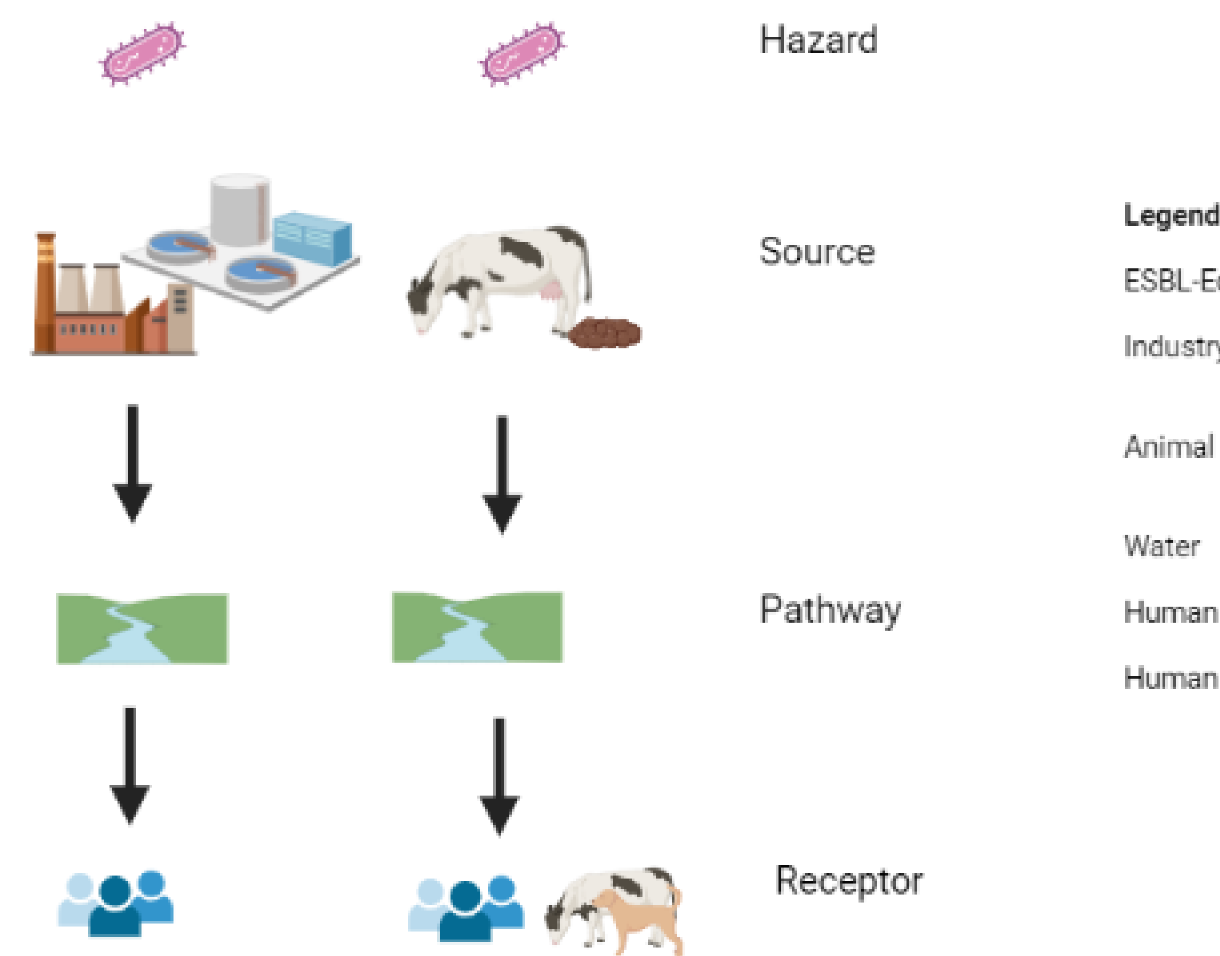
Recap: UK commitments

UK 20-year vision for AMR (by 2040) and UK 5-year action plan for AMR 2019 to 2024, was developed across the government, its agencies and administrations in Scotland, Wales and Northern Ireland, with support from a range of stakeholders

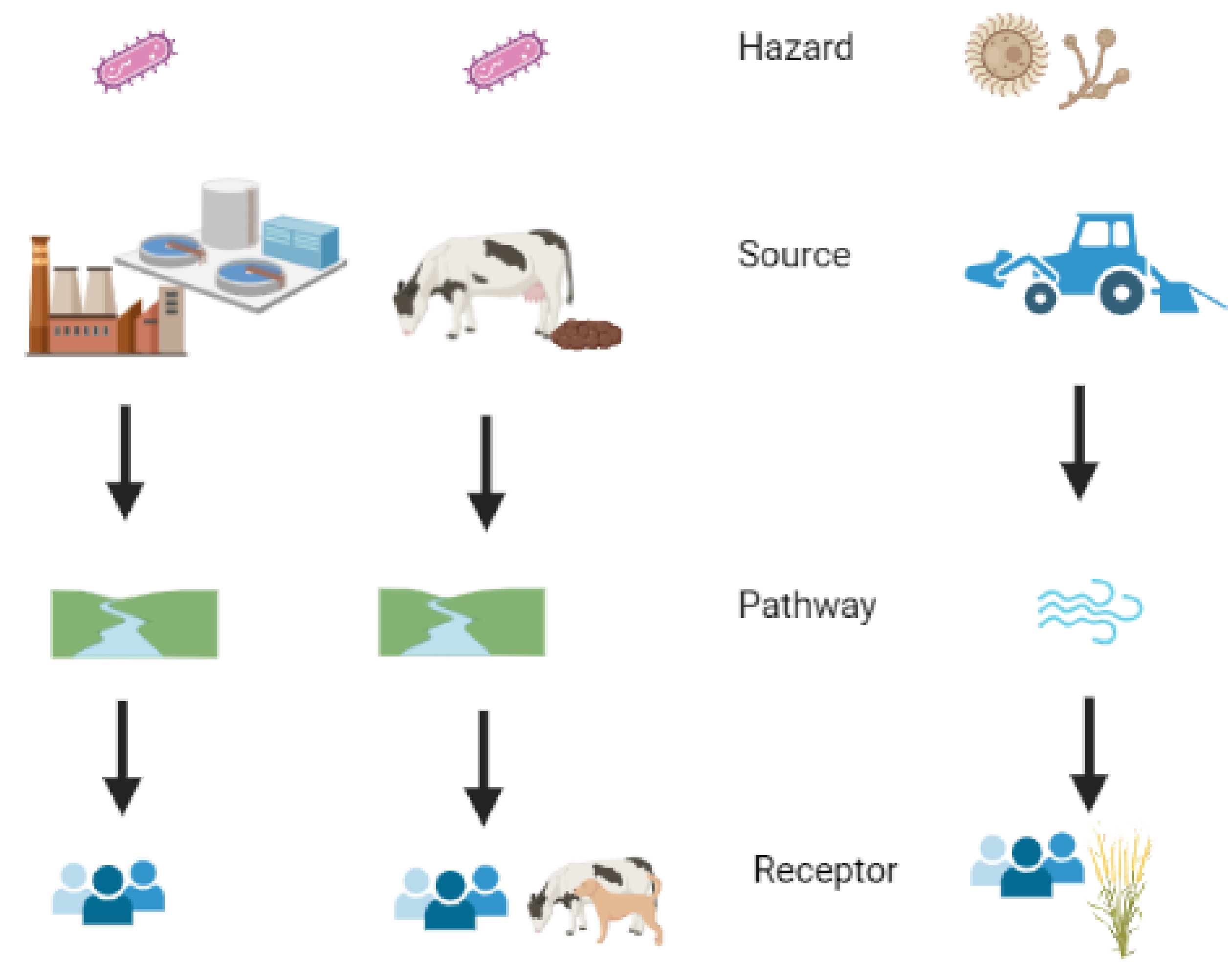
To deepen understanding about AMR in the environment, the UK will:

- Support research to reduce evidence gaps and improve understanding of the hazards and risks from AMR in the environment.
- Explore the establishment of a river catchment based research programme with clear standards for sample collection, analysis and review, with the aim of delivering AMR monitoring data that can be used to evaluate existing management interventions and inform any new policy initiatives.
- Increase public awareness of the hazard and risk of AMR in the environment.

So how do we this?



But what about…?



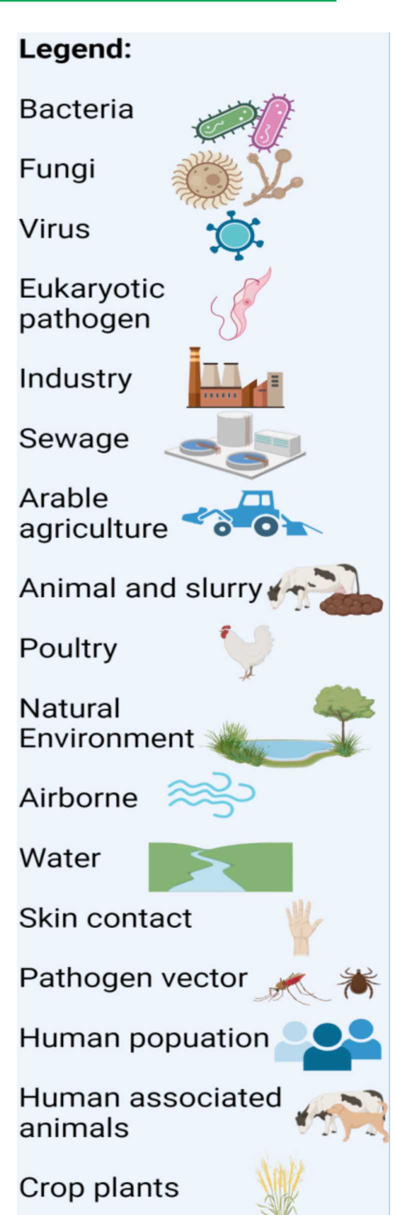
In other words,…

Hazard

Source

Pathway

Receptor



Aim of WS4: Environmental surveillance

1. Identify appropriate methods suitable for monitoring a range of resistant organisms, genes, and antimicrobial substances in air, water, and solids.
2. Test these methods at pilot scale in river catchments that exhibit a range of land uses and inputs.
3. Increase our understanding of selection pressures of antimicrobials, including antifungals.
4. D
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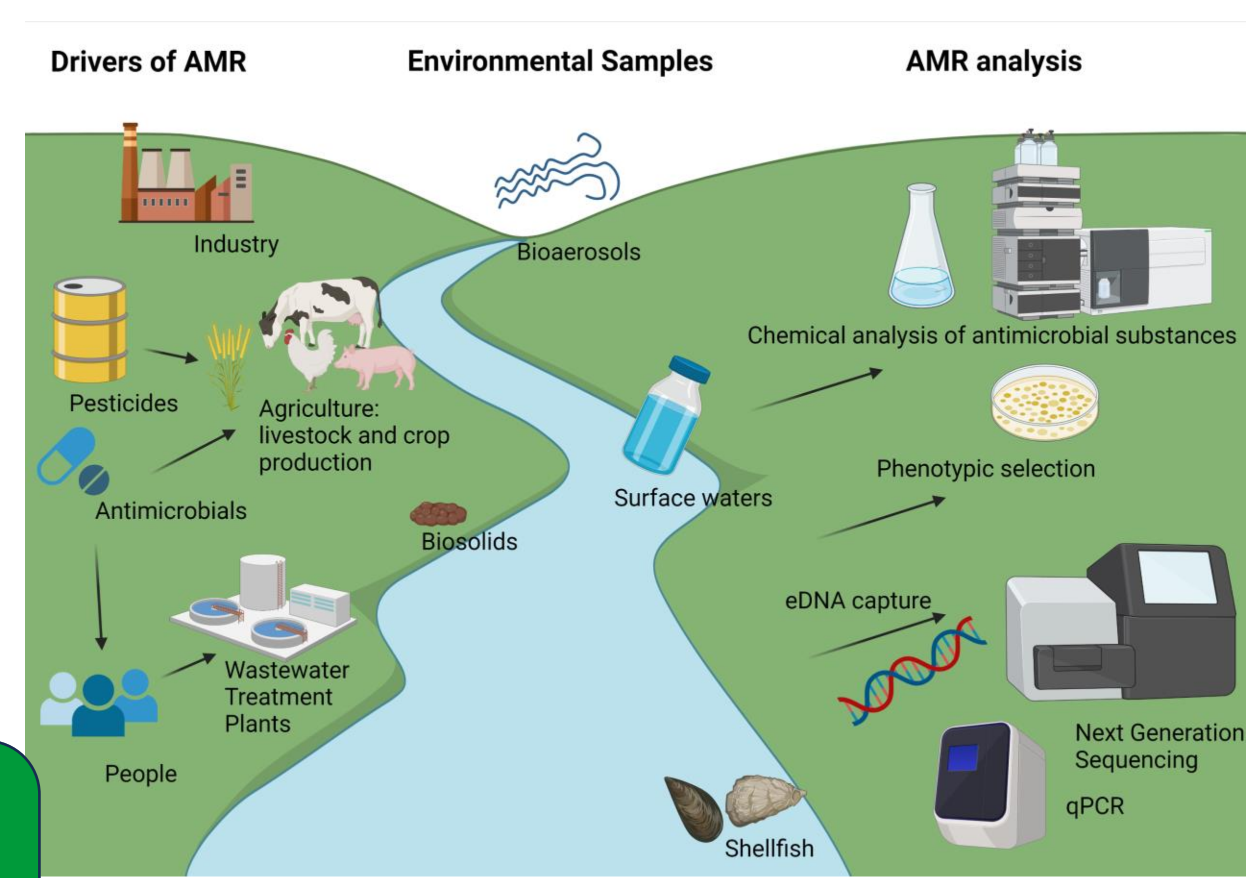
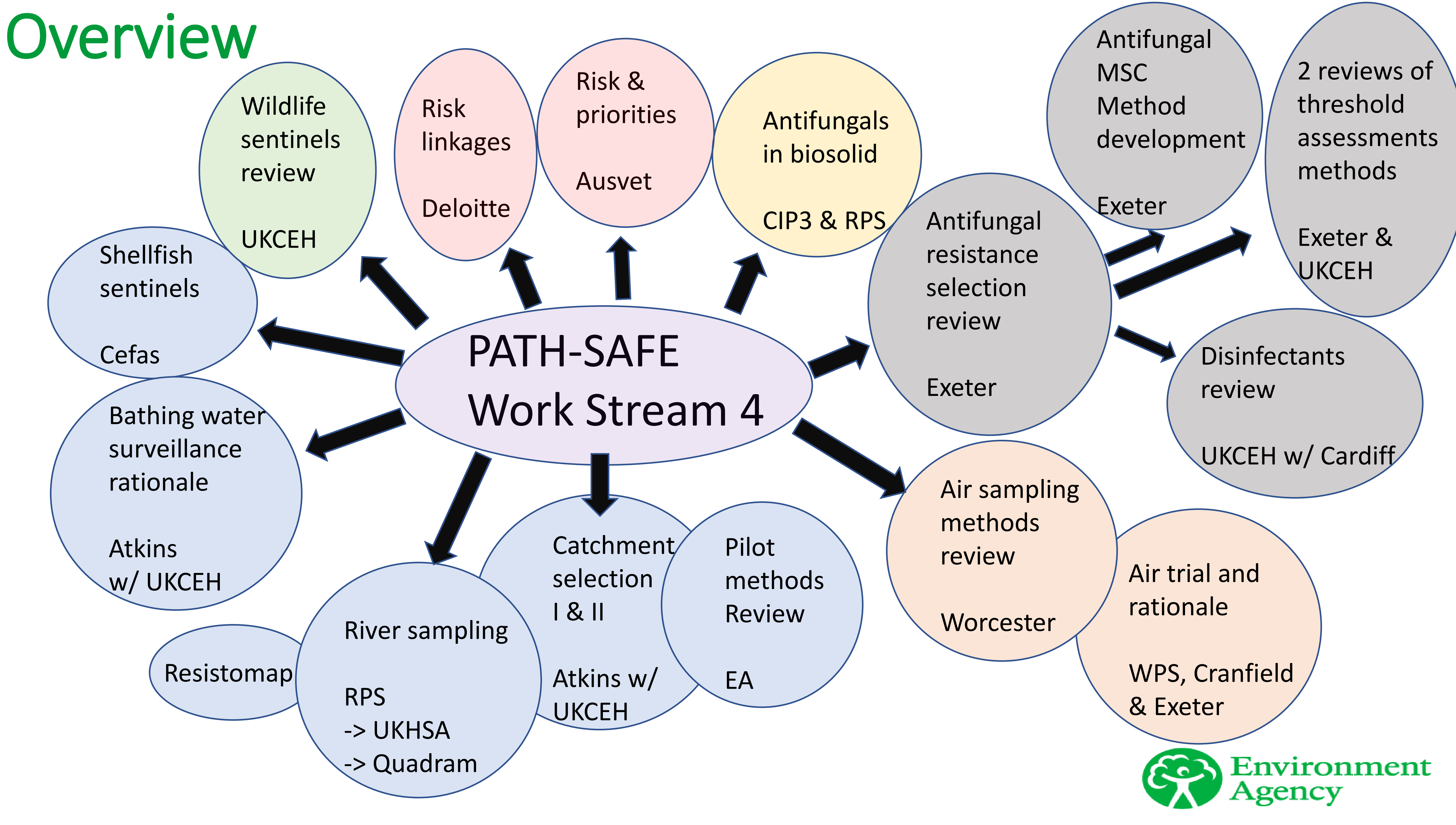
Olisa's talk introduced this part of the workstream yesterday

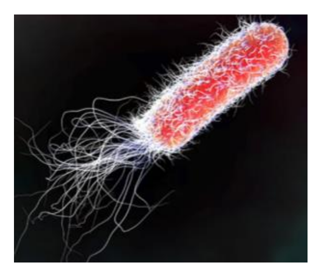



Overview
PATH-SAFE
Work Stream 4
Wildlife sentinels review
UKCEH
Risk linkages
Deloitte
Risk & priorities
Ausvet
Antifungals in biosolid
CIP3 & RPS
Antifungal resistance selection review
Exeter
Antifungal MSC Method development
Exeter
2 reviews of threshold assessments methods
Exeter & UKCEH
Disinfectants review
UKCEH w/ Cardiff
Air sampling methods review
Worcester
Air trial and rationale
WPS, Cranfield & Exeter
Pilot methods Review
EA
Catchment selection I & II
Atkins w/ UKCEH
River sampling
RPS
-> UKHSA
-> Quadram
Resistomap
Bathing water surveillance rationale
Atkins
w/ UKCEH
Shellfish sentinels
Cefas
Environment Agency

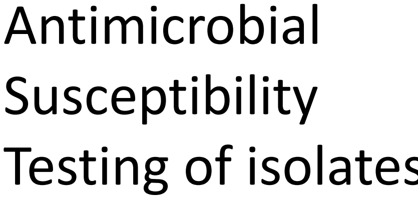
River Pilot

See Katie's poster for more info

1. We selected three pilot river catchments that exhibit a range of land uses and inputs.
2. Sampled river surface waters from May 2022 to Feb 2023 at different times, frequencies and locations within a catchment
3. We applied a range of testing methodologies:

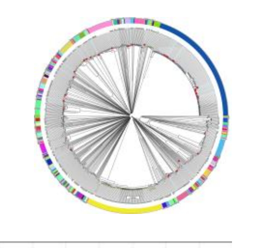


Phenotypic Indicator Organisms

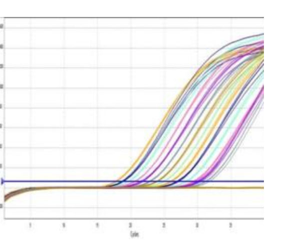


Antimicrobial Susceptibility Testing of isolates

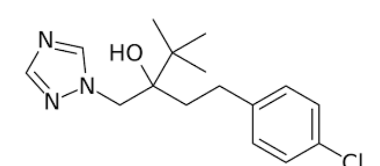
Whole-genome sequencing of isolates



Metagenomic sequencing of water samples to identify and detect microbial composition within the samples

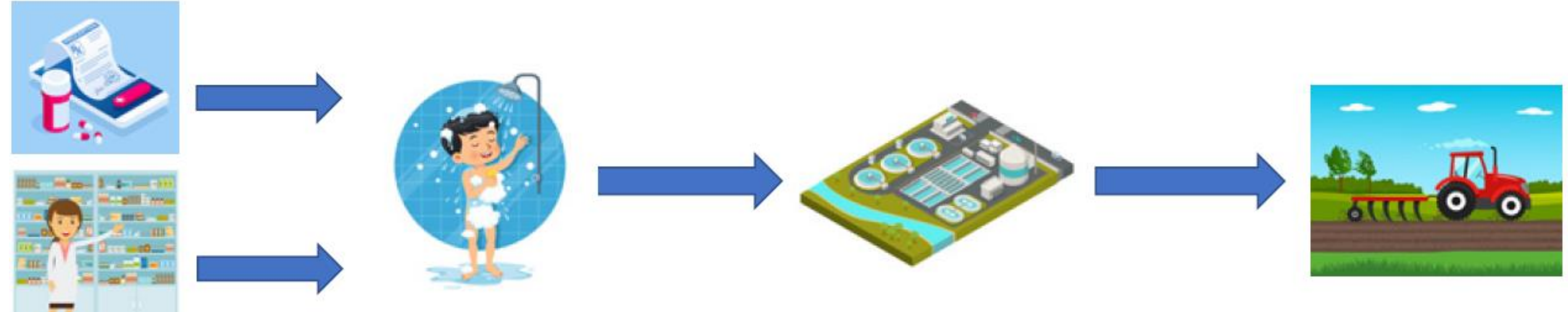


qPCR for High Throughput Detection and Quantification of Antibiotic Resistance Genes



Chemical analysis of a range of antimicrobial substances, covering antibiotic classes, antifungals, disinfectants and heavy metals

Clinical antifungals in biosolids



Samples from 11 Sludge Treatment Centres in England and Wales were collected over a 12-month were tested for 14 selected antifungals

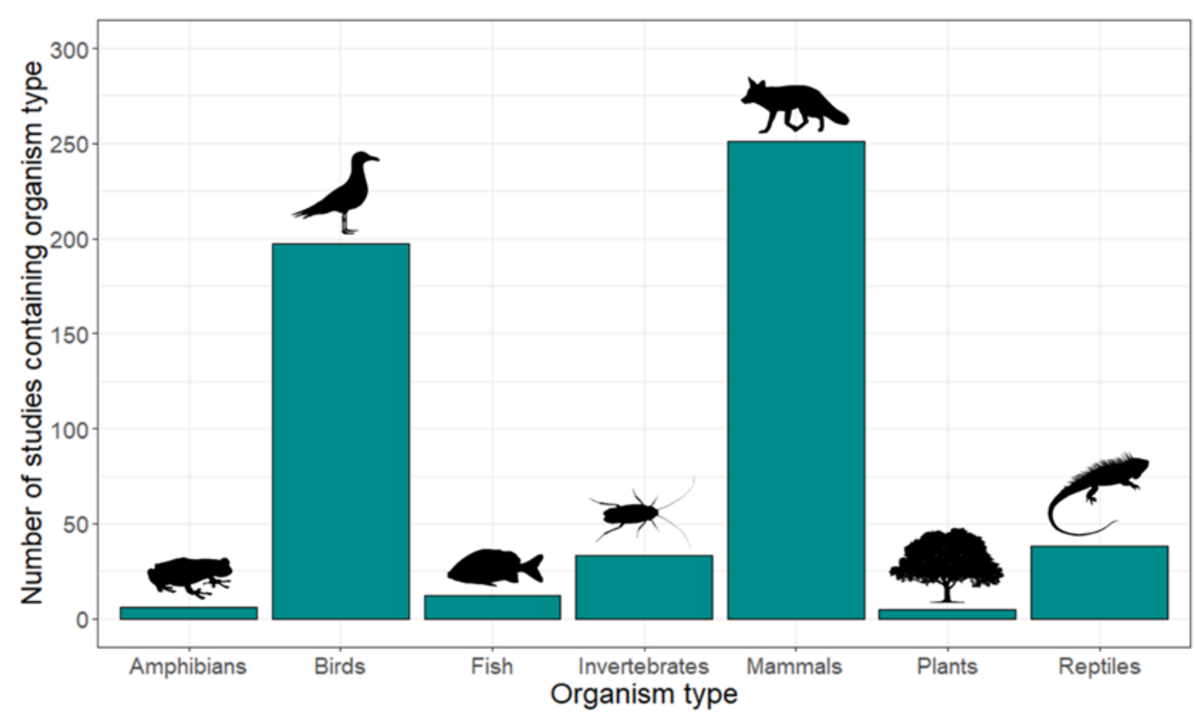
Antifungals	Use systemic (S), topical (T)	Antifungals	Use systemic (S), topical (T)
Amorolfine	Clinical: T	Miconazole	Clinical: S, T
Clotrimazole	Clinical: T	Posaconazole	Clinical: S
Enilconazole	Veterinary: T	Terbinafine	Clinical: S, T
Fluconazole	Clinical: S	Voriconazole	Clinical: S
Griseofulvin	Clinical: S, T	Climbazole	Preservative
Itraconazole	Clinical: S	Flucytosine	Clinical: S
Ketoconazole	Clinical: S, T	Nystatin	Clinical: S, T

Antimicrobial substance & selective pressures

- There are not many values available that inform us at which concentration of an antimicrobial substance, resistant microbes have an advantage compared to non-resistant microbes.
- Most values that exist are based on estimations and mainly for antibiotics.
- Exposing *Candida glabrata* strains to a range of concentrations of antifungals and explored their growth over time.

Tested substance	Clinical antifungals	Agricultural antifungals
Voriconazole	☑	
Fluconazole	☑	
Posaconazole	☑	
Itraconazole	☑	
Difenoconazole		☑
Triticonazole		☑
Tebuconazole		☑
Epoxiconazole		☑

Wild flora and fauna & shellfish



Evaluation of wildlife surveillance schemes in England
*Diseases of Wildlife scheme**
Passive bat surveillance scheme
*Garden Wildlife Health**
Rothamsted Insect Survey
Fish tissue archive
Predatory Bird Monitoring Scheme
National Honey Monitoring Scheme
Fish disease surveillance
*Otter surveillance**
National Bat Monitoring Programme
Forest Research, Tree Alert
Tick Surveillance Scheme
Nationwide mosquito surveillance project

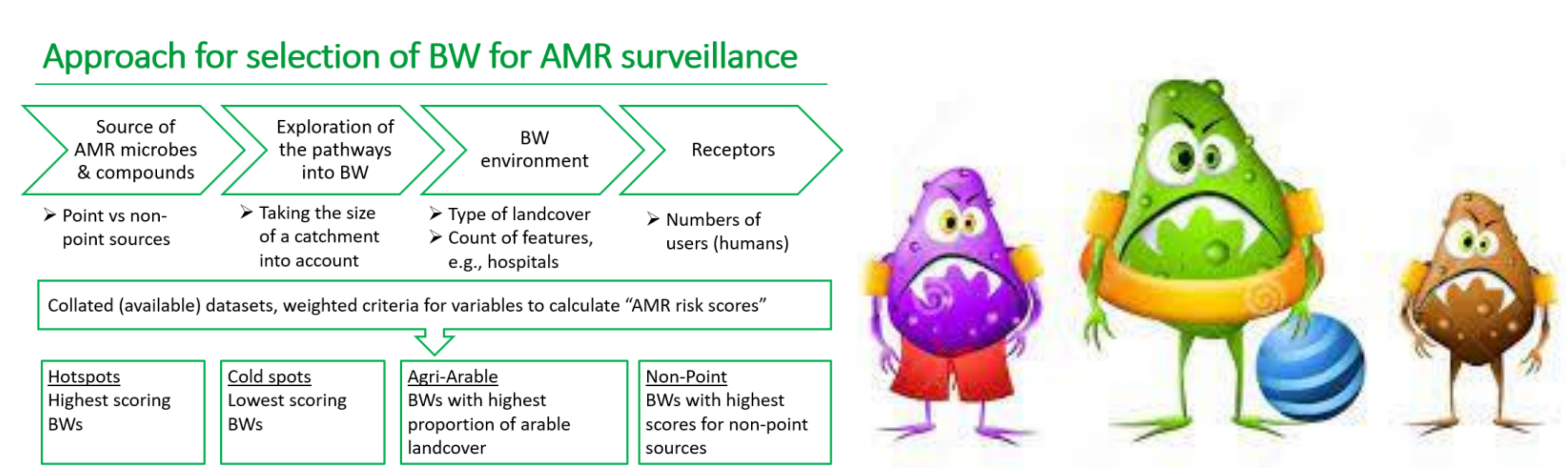
The atmospheric microbiome

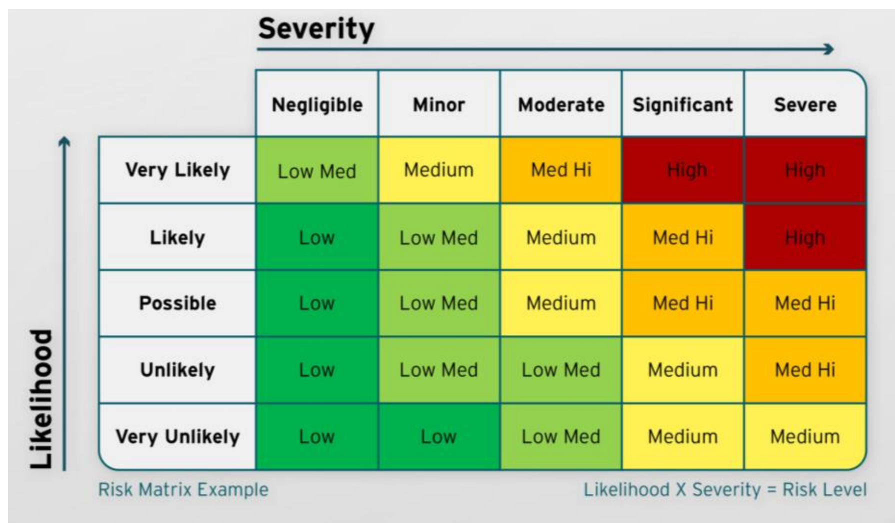
- To date much of the work about the sources, distribution and pathways of AMR in the environment has been gathered from aquatic and/ or terrestrial systems, often neglecting the atmospheric microbiome.
- Yet there are increasing reports of human infections caused by airborne resistant organisms derived from environmental sources.
- We reviewed the available sampling options for antimicrobial resistant microorganisms, including their antimicrobial resistance genes, from the atmosphere.
- And examined further the prevalence of AMR near potential sources or points of human exposure.

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

Towards risks

- We have developed an approach to assess a range of different environmental scenarios for human exposure to microorganisms resistant to antimicrobials and hence compare their significance for human health.
- Although there is currently waters, provide also first steps towards how waters and bno statutory obligation to monitor AMR in bathing waters, our review on approaches to monitoring and surveillance of AMR in bathing eaches used for recreation could be selected.

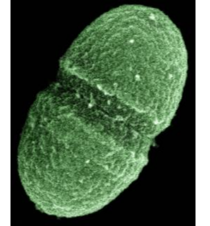


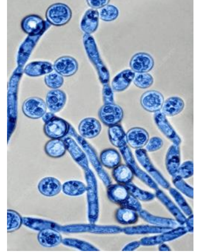


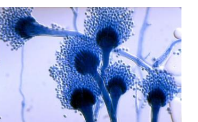
	Negligible	Minor	Moderate	Significant	Severe
Very Likely	Low Med	Medium	Med Hi	High	High
Likely	Low	Low Med	Medium	Med Hi	High
Possible	Low	Low Med	Medium	Med Hi	Med Hi
Unlikely	Low	Low Med	Low Med	Medium	Med Hi
Very Unlikely	Low	Low	Low Med	Medium	Medium

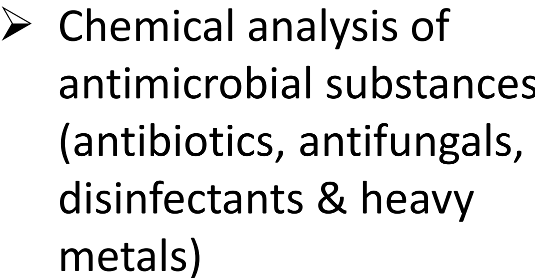
In summary

Identify & test hazards/ drivers









- Chemical analysis of antimicrobial substances (antibiotics, antifungals, disinfectants & heavy metals)
- Review of disinfectants
- Minimum selective concentrations

- Culture-based methods (bacteria & fungi)
- Molecular based methods (qPCR, metagenomic)

Exploration of the pathways

- River surface water
- Biosolids
- Bioaerosols
- Shellfish
- Bathing Waters
- Review of wild fauna and flora

Options & Appraisals

- Surveillance options
- Risk approaches

- Presence & Prevalence AMR data

- Information for future environmental quality standards (e.g., MSCs)

More details on gov.uk & to come...

1. Environmental surveillance of antimicrobial resistance (AMR), perspectives from an environmental regulator
2. Antimicrobial resistance surveillance pilot site selection and database extension
3. Sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms
4. Antifungal medicines in the terrestrial environment: Levels in biosolids from England and Wales
5. Scoping review into environmental selection for antifungal resistance and testing methodology
6. Environmental antimicrobial resistance: review of biological methods
7. Antimicrobial resistance in bioaerosols: towards a national surveillance strategy
8. Shellfish as bioindicators for coastal antimicrobial resistance
9. Review: approaches to monitoring and surveillance of antimicrobial resistance in bathing waters
10. Antimicrobial resistance surveillance strategies within wild flora and fauna of England
11. Pilot Surveillance of Antimicrobial Resistance in River Catchments in England
12. Development of experimental approaches for determining concentrations of antifungals that select for resistance
13. Applying a published approach for deriving resistance selection concentrations for antibiotics to antifungals
14. Determining concentrations of substances which influence development of AMR
15. Disinfectant use in the UK and consideration of their impact on AMR development
16. Antimicrobial resistance in the environment – risk screening and prioritisation tool

Next steps…

- **Where do we want to go?**
- Choices and decision on ‘who’ or ‘what’ we want to protect and to what extent will need to be made.
- Having learnt how to do surveillance, an option could be to integrate with existing monitoring initiatives.

- **Continuation of EA’s work**

1. Currently undertaking work to better understand the fate of resistant microbes as they are transported in an urban river (Trent) setting.
2. Investigating the development and genetic basis for resistance to azoles in yeast strains.

Thank you

for listening,
to the AMR team at the Chief Scientist's Group,
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