

Metagenomics for AMR detection in food and feed: *Lessons learned from One Health applications and challenges ahead*

**Bram Bloemen - Sigrid De Keersmaecker
Sciensano**

Transversal activities in Applied Genomics (TAG)

AS2AMR conference
Brussels, May 26th, 2025

If you wanted a quick overview of all the fish living in your backyard pond, would you ...

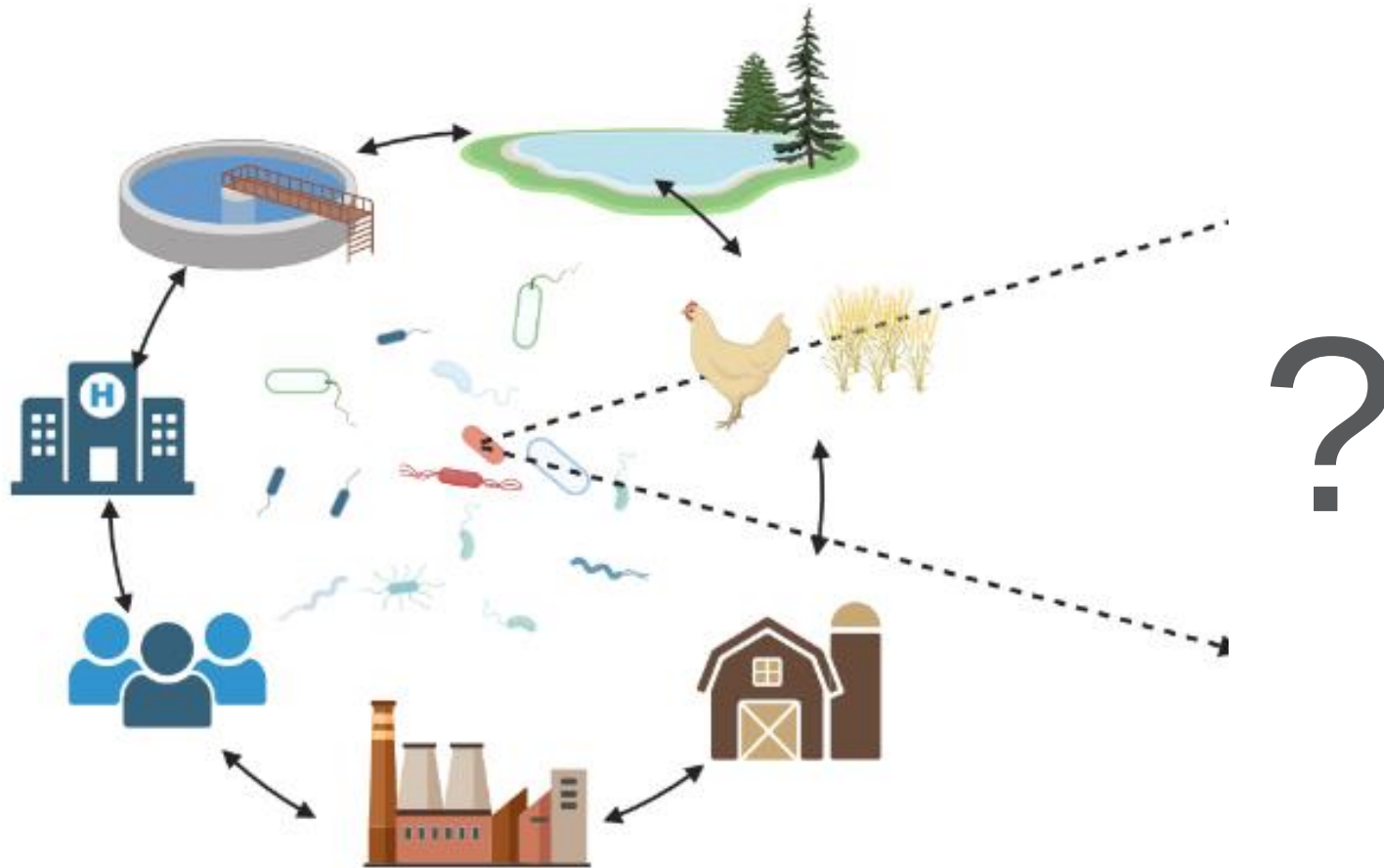


fish for one at a time

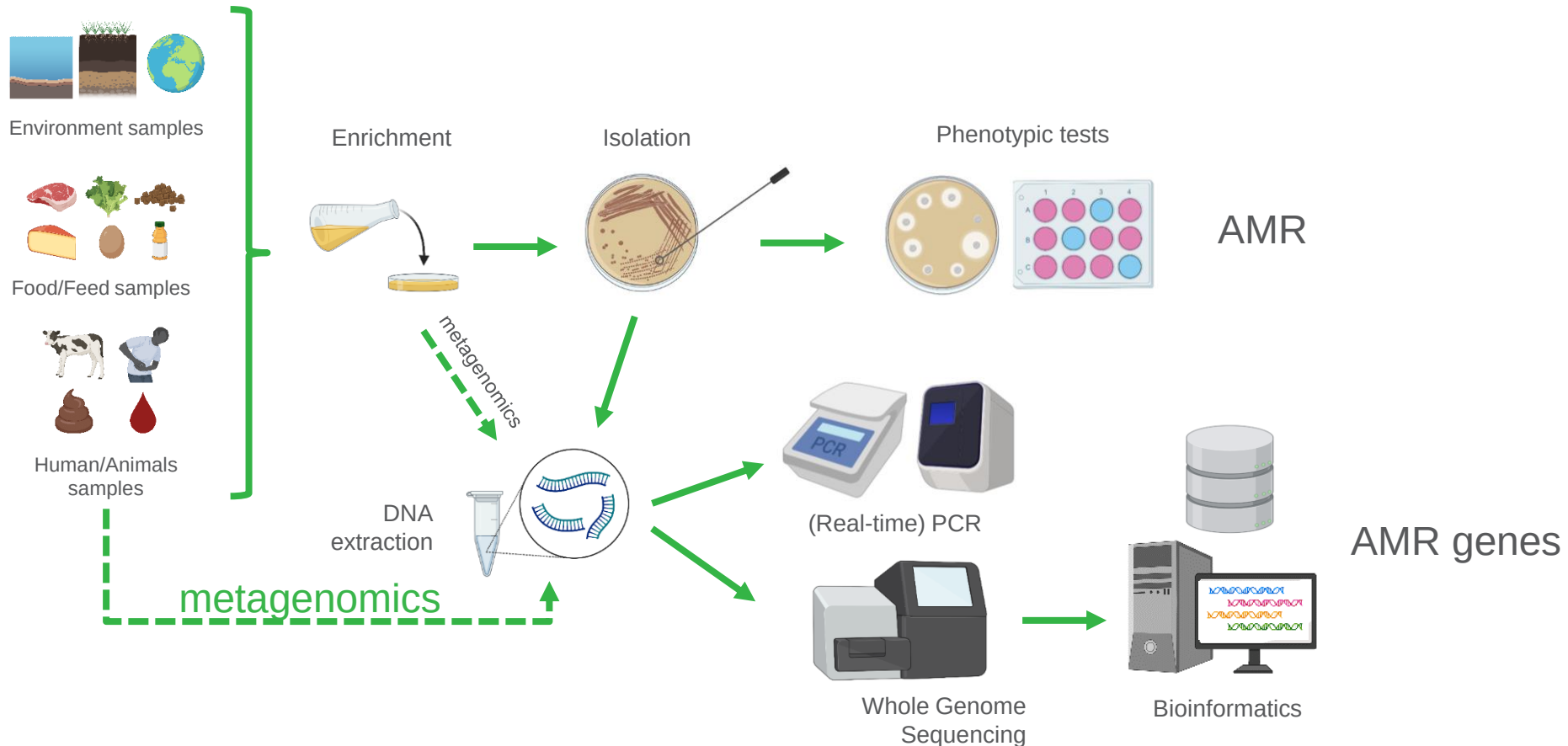


... or catch them all?

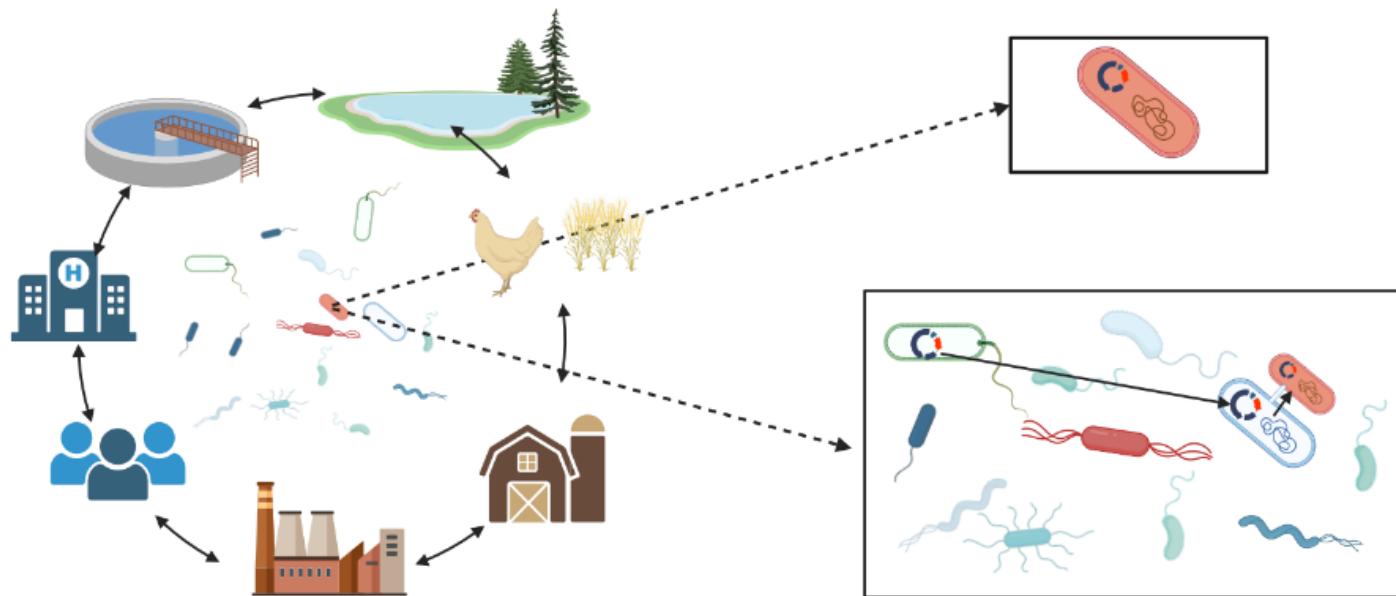
AMR in a OH context, with the food chain as key AMR transmission



Pathogen and AMR detection



Metagenomics for AMR detection?



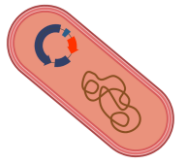
Conventional testing

- A priori knowledge
- Limited picture
- Culture dependent
- Time-consuming

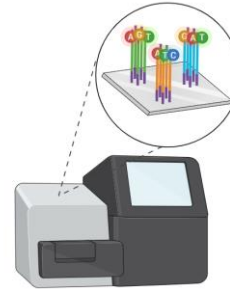
Metagenomics

- Open approach
- Comprehensive
- Culture independent
- Rapid

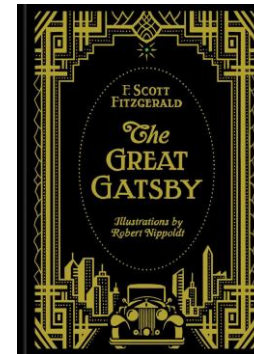
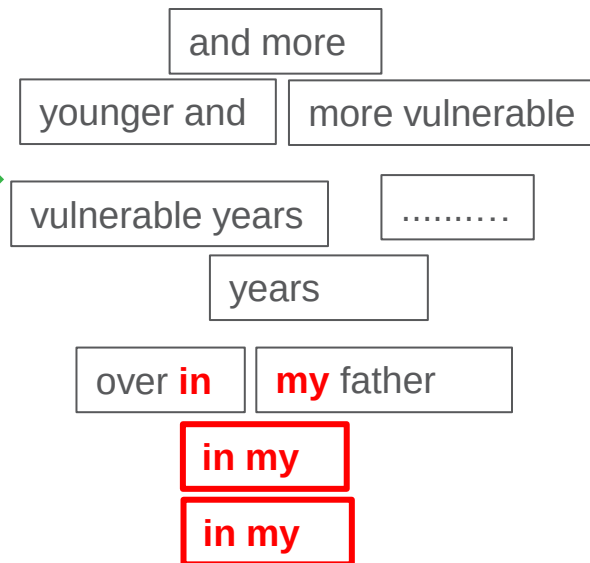
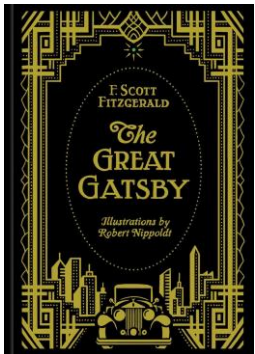
Metagenomics: the challenge



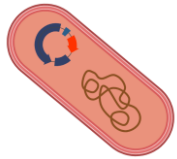
Short “reads”



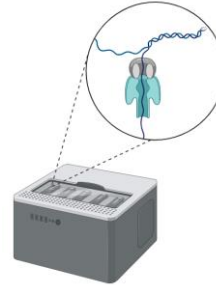
- e.g. Illumina:
- <300 bases
- High accuracy



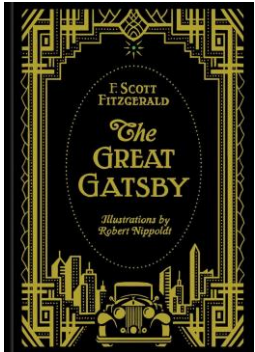
Metagenomics: the challenge



Long “reads”



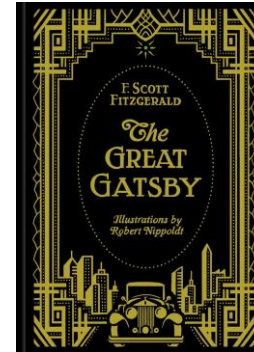
- e.g. Oxford Nanopore:
- **Read length ~ input DNA fragments**
- Real-time
- Higher error-rate



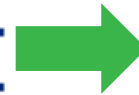
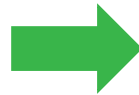
In my younger and more vulnerable years my father gave me some advice



me some advice that I've been turning over in my mind ever since.

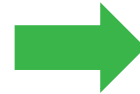
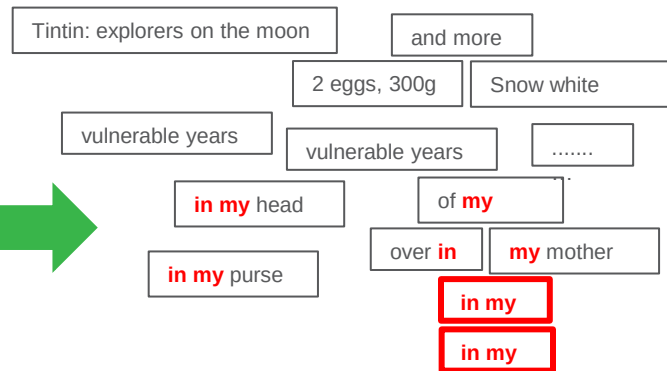


Metagenomics and AMR: the challenge



AMR gene

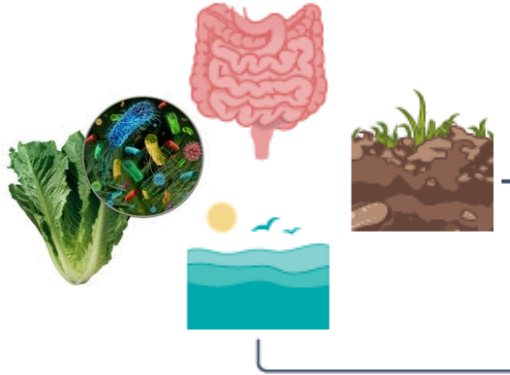
```
ATGAAAGGACCAATAATAATG
ACTAGAGAAGAAAGAATGAA
GATTGTTTCATGAAATTAAGGA
ACGAATATTGGATAAATATGG
GGATGATGTTAAGGCAATTG
GTGTTTATGGCTCTCTTGGTC
GTCA
```



In my younger and more vulnerable years my father gave me some advice that I've been turning over in my mind ever since.

What is metagenomics?

a Sample collection and storage



Bioinformatics: re-creating the story from the snippets

Goal: to investigate all AMR present = **Resistome**

- untargeted
- need for databases

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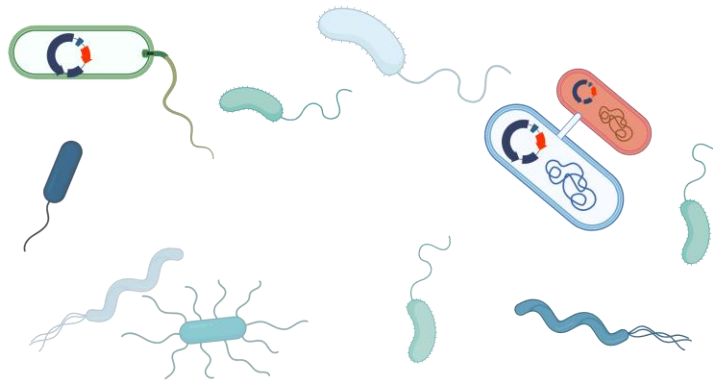


- **Genetic context** of AMR gene
 - chromosome? plasmid? mobile?
 - Similar strains: very difficult scenario

Bioinformatics: re-creating the story from the snippets

Goal: to investigate all AMR present = **Resistome**

- untargeted
- need for databases
- **Genetic context** of AMR gene
 - chromosome? plasmid? mobile?
 - Similar strains: very difficult scenario
- **Host:** to which species does the AMR gene belong?
 - To which species does the plasmid belong?



Use Cases – Food Safety Applications: *Pathogen detection in food, AMR part of it*

MICROBIAL GENOMICS

RESEARCH ARTICLE

Buytaers et al., *Microbial Genomics* 2021;7:000547
DOI 10.1099/mgen.0.000547



Application of a strain-level shotgun metagenomics approach on food samples: resolution of the source of a *Salmonella* food-borne outbreak

Florence E. Buytaers^{1,2}, Assia Saltykova^{1,2}, Wesley Mattheus³, Bavo Verhaegen⁴, Nancy H. C. Roosens¹, Kevin Vanneste¹, Valeska Laisnez⁵, Naïma Hammami⁵, Brigitte Pochet⁶, Vera Cantaert⁶, Kathleen Marchal^{2,7,8}, Sarah Denayer⁴⁺ and Sigrid

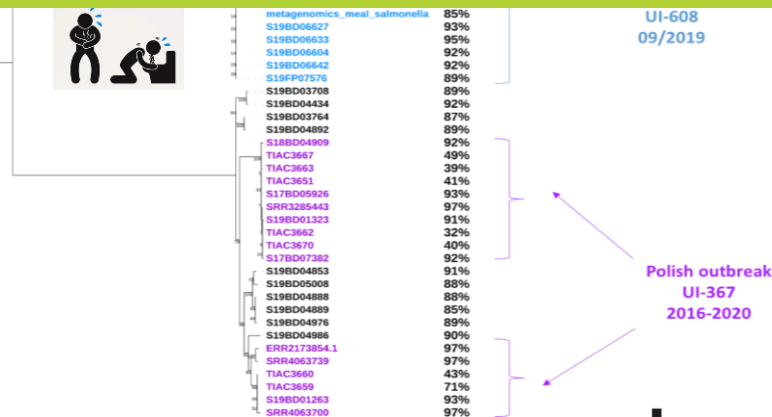
Typing: *Salmonella enterica* subsp. *enterica* serovar Enteritidis

+ AMR detection

Context: no food isolate, no resolution of outbreak

Challenge: low abundance

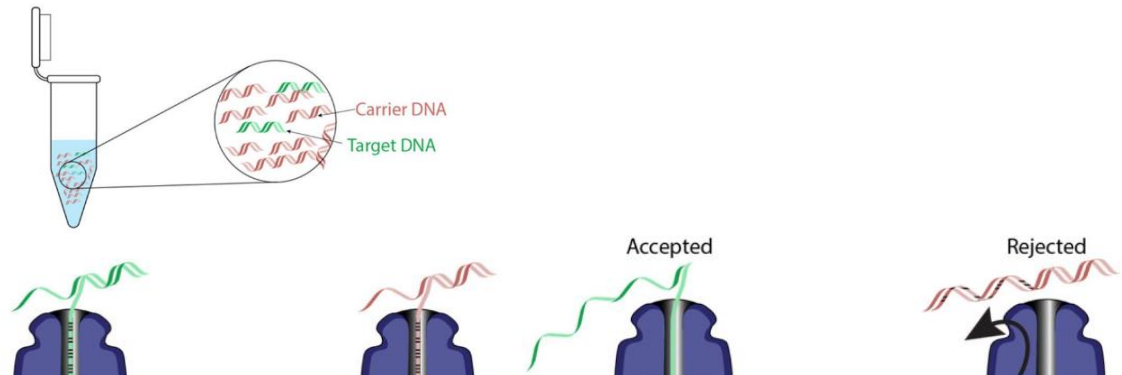
Solution: cultural enrichment (cfr routine analysis)



Use Cases – Food Safety Applications: *Pathogen detection in food, AMR part of it*

 **frontiers** | Frontiers in **Microbiology**

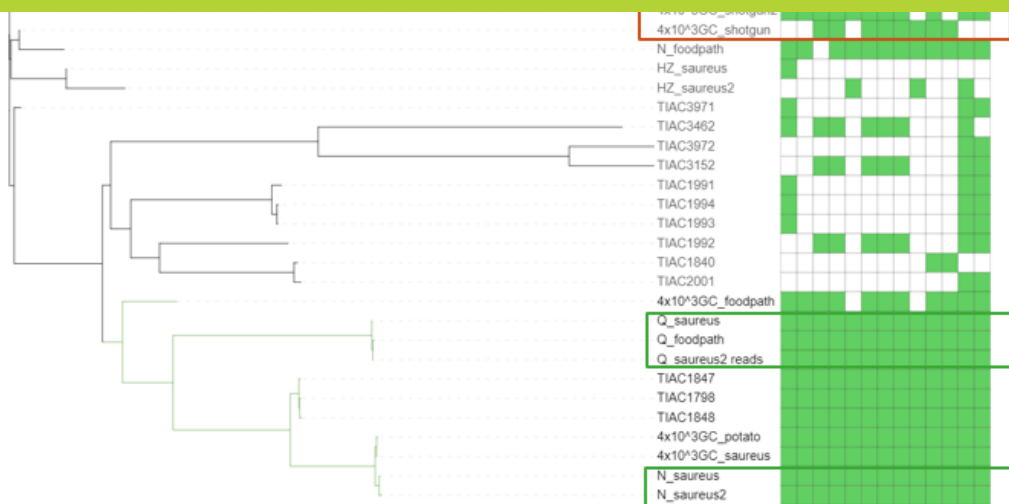
Strain-level characterization of foodborne pathogens without culture enrichment for outbreak investigation using shotgun metagenomics facilitated with nanopore adaptive sampling



Context: no food isolate, no resolution of outbreak

Challenge: low abundance

Solution: optimized DNA extraction; Nanopore adaptive sampling (selective sequencing)



- 2/3 DNA ext. kits: Correct placement in outbreak cluster using AS *S. aureus*
- All genes detected in the pathogenic strain

Use Case – Detection of GM Microorganisms Carrying AMR genes as selection marker



Microbial fermentation products



Often produced by **GMM**, carrying **AMR** genes as **selection marker** – transfer?

Context: control GMM – AMR; open approach

Challenge: complex matrix, sensitivity, no reference strain/sequence available

Solution: optimized DNA extraction; high sequencing depth; advanced bioinformatics methods (incl. tailored database AMR)

→ Open approach needed

Food Chemistry: Molecular Sciences 2 (2021) 100023

Contents lists available at ScienceDirect

Food Chemistry: Molecular Sciences

journal homepage: www.elsevier.com/locate/fochms



Article

Metagenomic Characterization of Multiple Genetically Modified *Bacillus* Contaminations in Commercial Microbial Fermentation Products

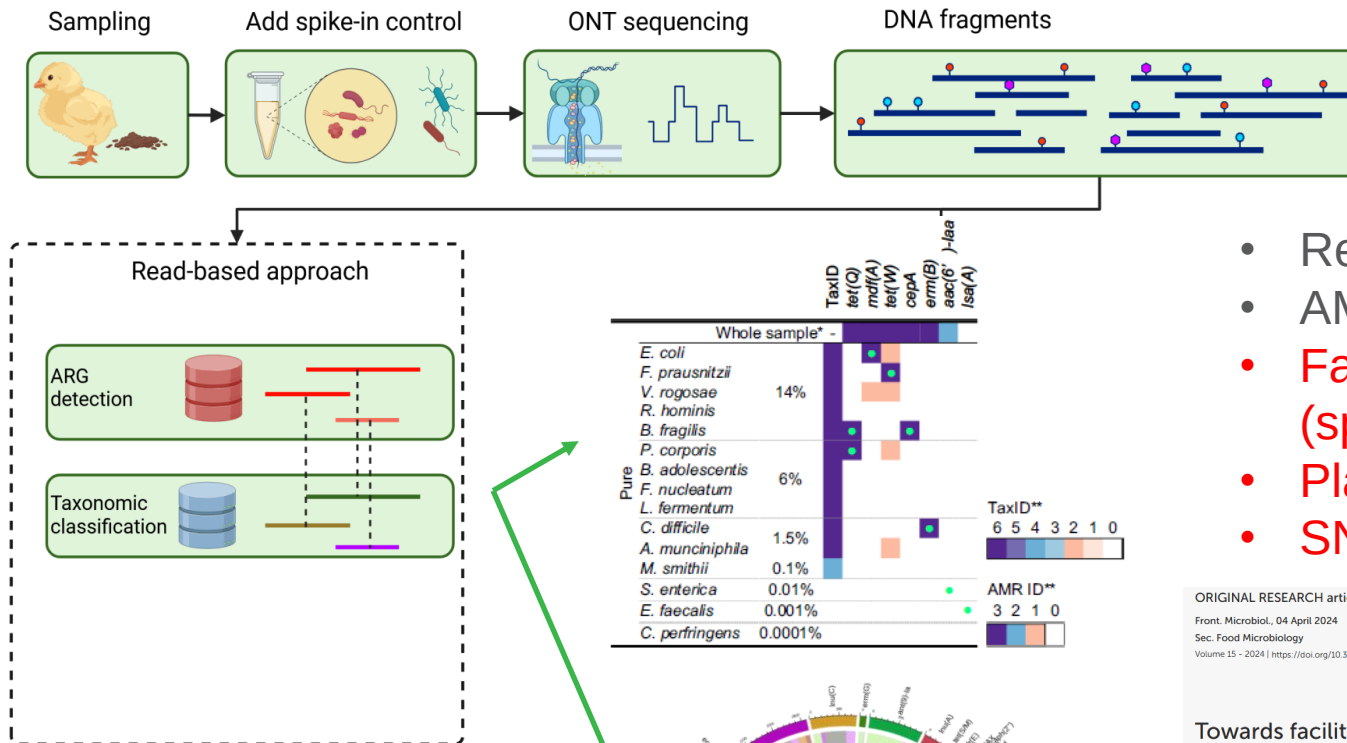
Jolien D'aes[†], Marie-Alice Fraiture[†], Bert Bogaerts, Sigrid C. J. De Keersmaecker, Nancy H. C. J. Roosens^{*,†} and Kevin Vanneste[†]

A shotgun metagenomics approach to detect and characterize unauthorized genetically modified microorganisms in microbial fermentation products



Florence E. Buytaers^{a,b}, Marie-Alice Fraiture^a, Bas Berbers^{a,b}, Els Vandermassen^a, Stefan Hoffman^a, Nina Papazova^a, Kevin Vanneste^a, Kathleen Marchal^{b,c}, Nancy H.C. Roosens^{a,1}, Sigrid C.J. De Keersmaecker^{a,1,*}

Use Cases – Animal Feces Proxy for (on-farm) AMR monitoring



- Resistome determined
- AMR gene – host linkage
- False-positive linkage (spike-in control strains)
- Plasmids?
- SNP-related AMR?

ORIGINAL RESEARCH article

Front. Microbiol., 04 April 2024
Sec. Food Microbiology
Volume 15 - 2024 | <https://doi.org/10.3389/fmicb.2024.1336532>

This article is part of the Research Topic
Zoonotic Antimicrobial Resistance and Virulence: One Health
Integrated Approaches to Monitor and Reduce Food Chain Hazards
[View all 8 articles >](#)

Towards facilitated interpretation of shotgun metagenomics long-read sequencing data analyzed with KMA for the detection of bacterial pathogens and their antimicrobial resistance genes

Article | [Open access](#) | Published: 11 November 2023

Development of a portable on-site applicable metagenomic data generation workflow for enhanced pathogen and antimicrobial resistance surveillance

[Bram Bloemen](#), [Mathieu Gand](#), [Kevin Vanneste](#), [Kathleen Marchal](#), [Nancy H. C. Roosens](#) & [Sigrid C. J. De Keersmaecker](#) 

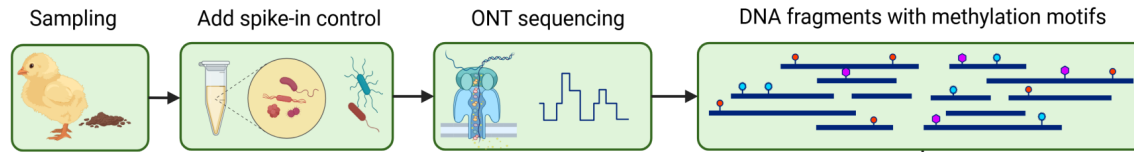
[Scientific Reports](#) 13, Article number: 19656 (2023) | [Cite this article](#)

5451 Accesses | 2 Altmetric | [Metrics](#)



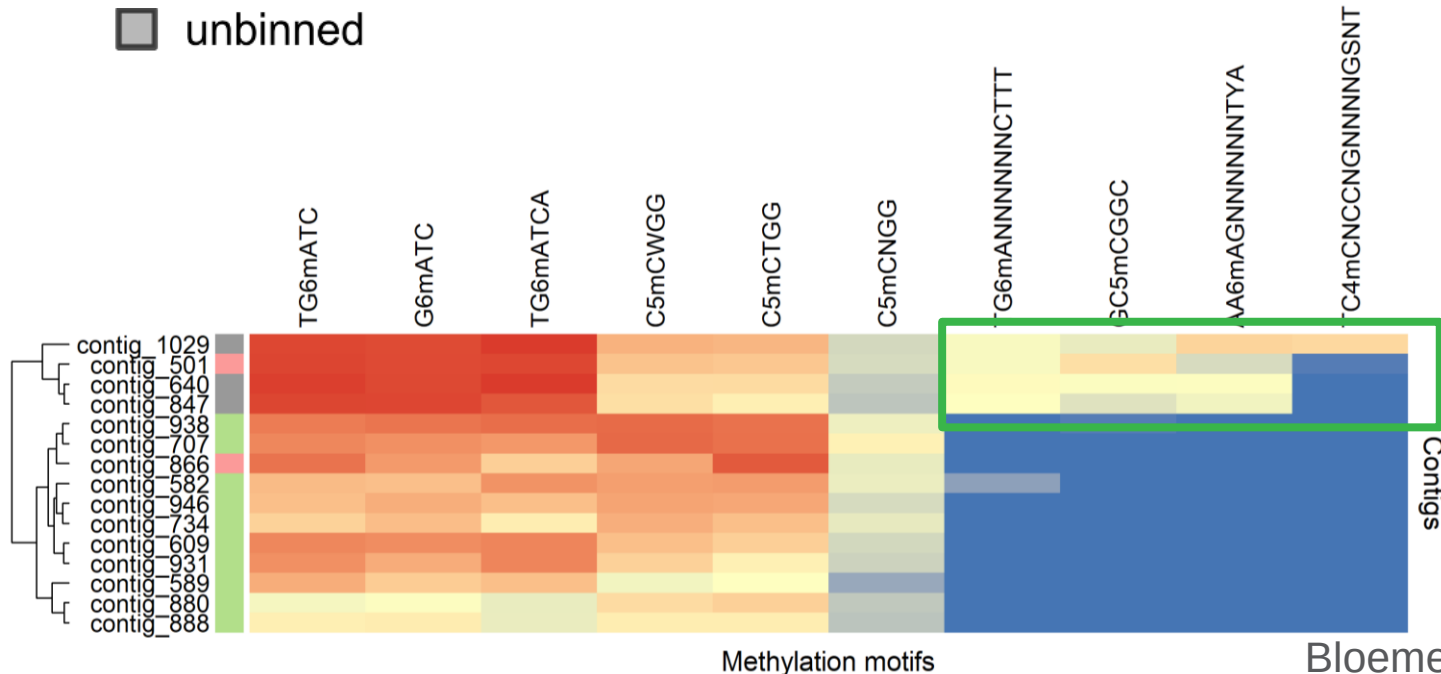
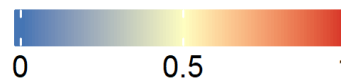
Use Cases – Animal Feces

Proxy for (on-farm) AMR monitoring



- Escherichia coli
- Klebsiella pneumoniae
- unbinned

Percentage methylated



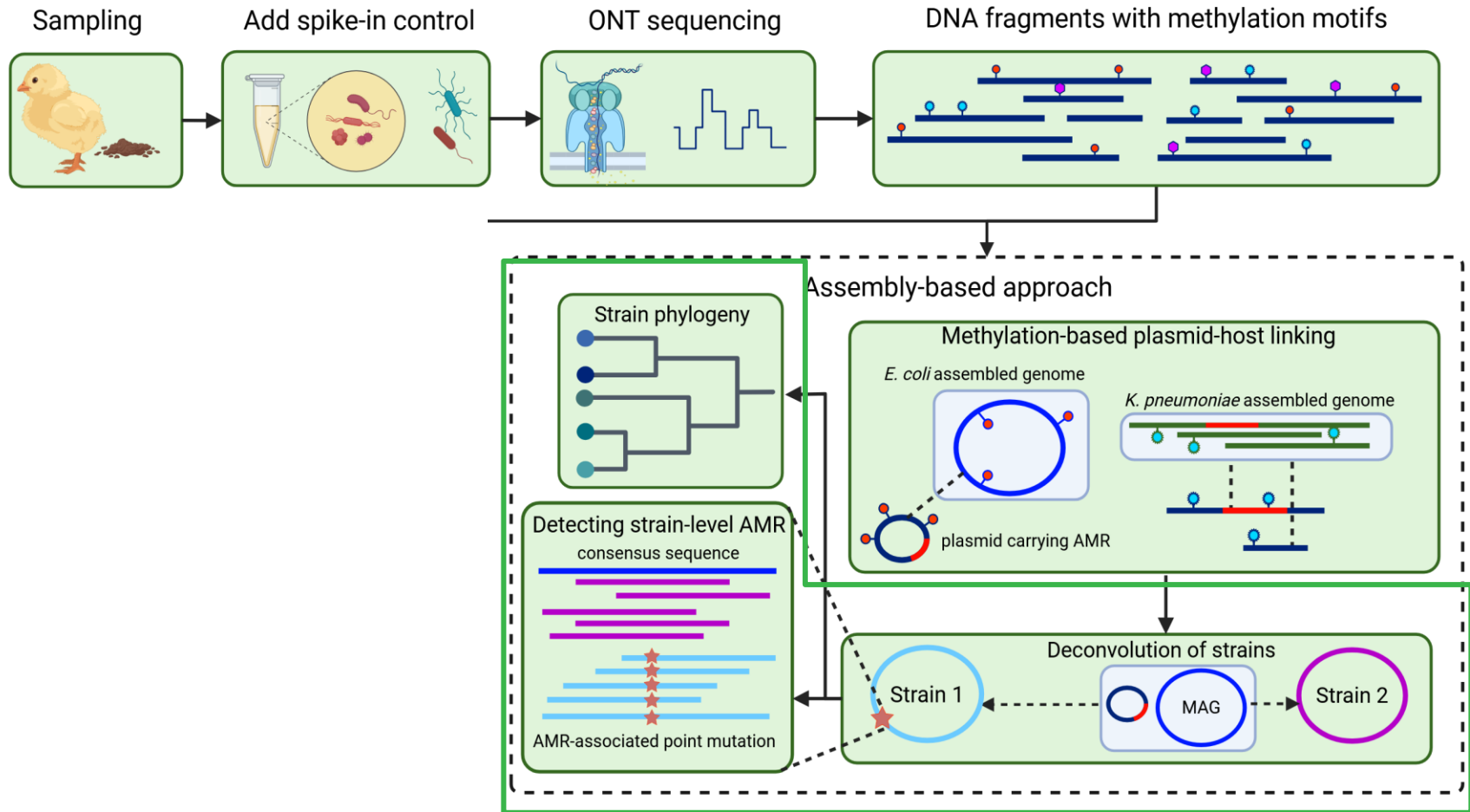
Contigs

Methylation motifs

Bloemen et al., under review

Use Cases – Animal Feces

Proxy for (on-farm) AMR monitoring



Use Cases – Animal Feces

Proxy for (on-farm) AMR monitoring

Tree scale: 0.1

DA1 DA2 LAP-2 TEM-1 (F) (G) 2 B19 S7 A SNP E SNP 2 (A) A1 A14 smid

Context: AMR in food chain via food-producing animals; rapid detection; AMR on plasmids

Challenge: complex matrix, host/background DNA, linking AMR to bacterial host & plasmid; data interpretation

Solution: spike-in control; optimized DNA extraction; ONT-methylation; adapted bioinformatics methods

0.91 --- Farm A Isolate 2 ILMN
0.1 --- Farm A Isolate 2 ONT
0.11 --- Farm A Isolate 1 ONT



Strain-level analysis uncovers AMR-related SNP, and confirms plasmid-host association

Conclusions

Metagenomics as a tool for integrated AMR gene surveillance

- OH applications in food/feed
- Resistome detected – untargeted, culture independent
- Genetic context of AMR genes
- Promising for host-AMR gene linking

Importance of method development/validation for all steps

- Use of spike-ins, negative and positive controls

but needs some technical refinement

- Hi-C sequencing
- Use of AMR probe-capture methods



Points of attention & Challenges ahead

- Sample complexity and variety
- Time
- Cost
- Sensitivity and specificity
- Bioinformatics analysis
- Regulatory considerations

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Bioit team

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Dr. Bavo Verhaegen

Dr. Tom Van Nieuwenhuysen

Céline De Sterck, Donia Baccari, Astrid Huwaert

Sciensano – Bacterial Diseases (NRC *Salmonella*)

Dr. Wesley Mattheus



FAVV-AFSCA

Brigitte Pochet, Vera Cantaert

Agentschap zorg & gezondheid

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Hammami



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Contact

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sigrid.dekeersmaecker@sciensano.be

Thank you for your attention!
Questions?



*“No one can whistle a symphony.
It takes an orchestra to play it.”
H.E. Luccock.*