Long-read shotgun metagenomics as a One Health tool to characterize antimicrobial resistance in food-producing environments

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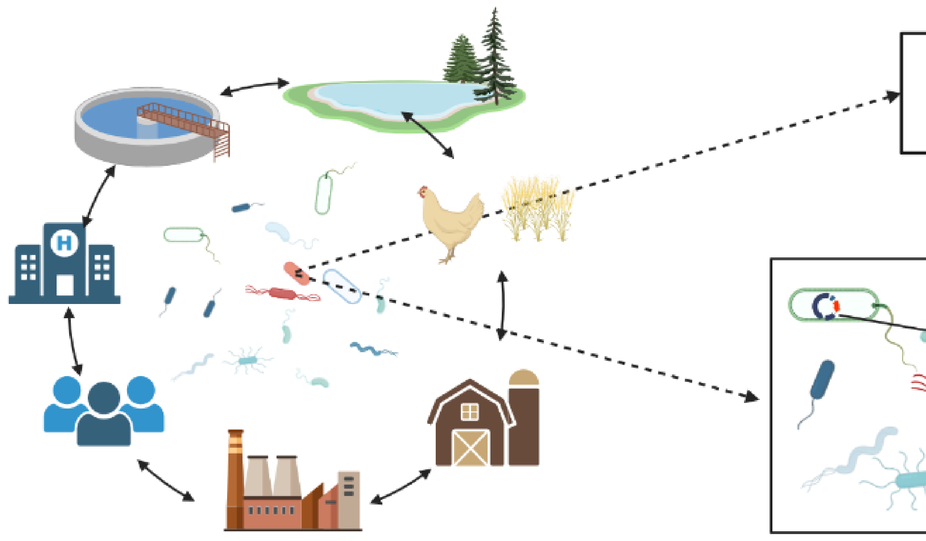
Background

- Food-producing environments: important source of antimicrobial resistance (AMR)
- Current methods detecting AMR: targeted, requiring *a priori* knowledge and/or culturing
- **Shotgun metagenomics**: identify all genetic material in sample → efficient, rapid and comprehensive diagnostics
- Before application, we need to **develop and validate** metagenomic approaches **from sampling and DNA extraction to sequencing and bioinformatics analysis**

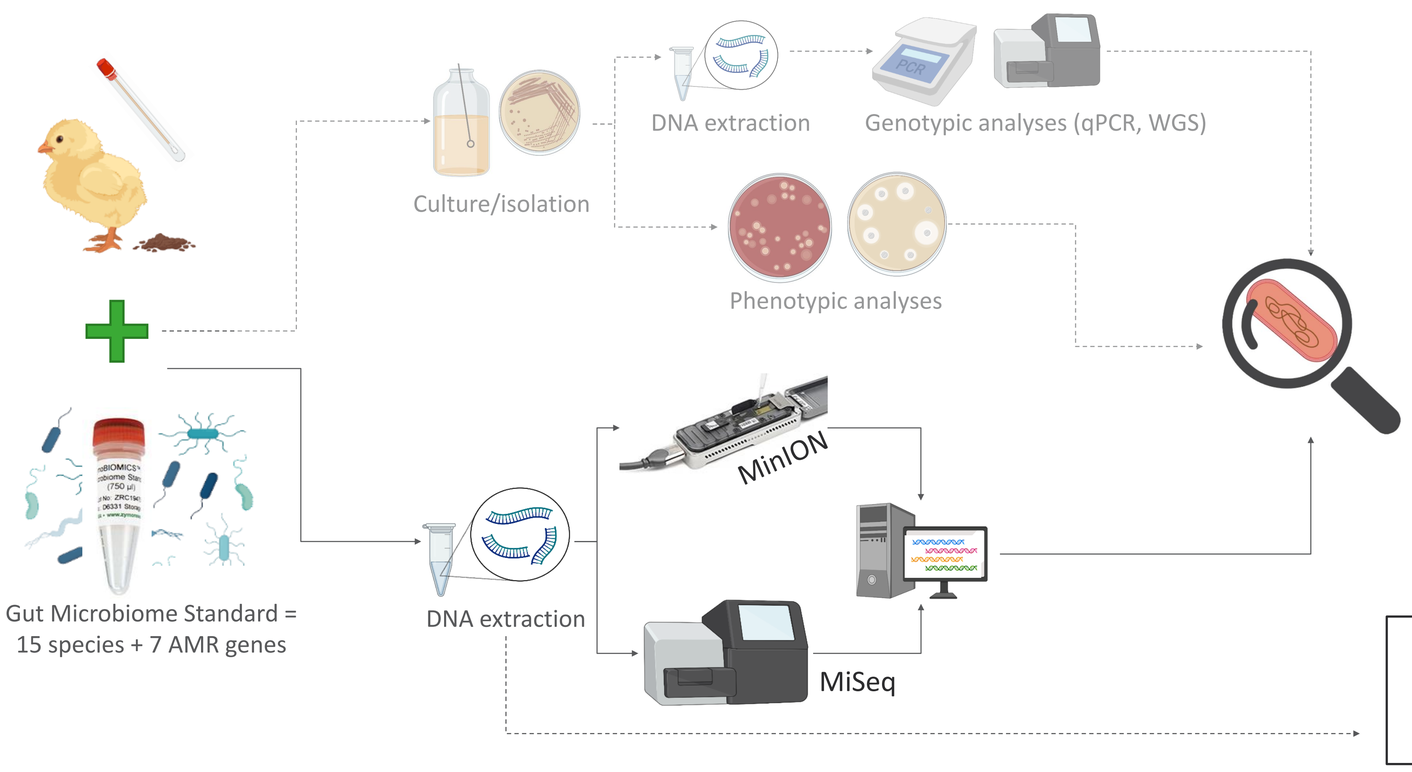


Conventional testing
- A priori knowledge
- Limited picture
- Culture dependent
- Time-consuming

Metagenomics
- Open approach
- Comprehensive
- Culture independent
- Rapid

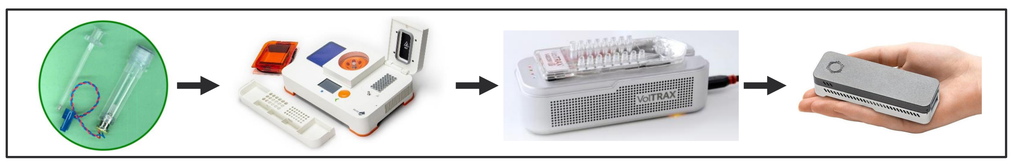
- **Nanopore sequencing**: long sequencing reads in real-time on portable device → better detection of microbial genes and scaffold them to their host chromosomes in complex metagenomics samples, improving taxonomic classification and identification of AMR genes.

Methods



- Metagenomic sequencing is faster than many current diagnostic method by bypassing culturing or isolation steps
- As a benchmark: chicken fecal samples spiked with a microbial standard, containing several AMR genes
- Nanopore long-read sequencing (MinION) was compared to short-read sequencing (MiSeq Illumina)
- DNA extraction and sequencing performed on portable devices, allowing for on-site metagenomics
- Bioinformatics analyses (KMA-based) to identify species and to link them to their AMR genes

Portable DNA extraction and sequencing



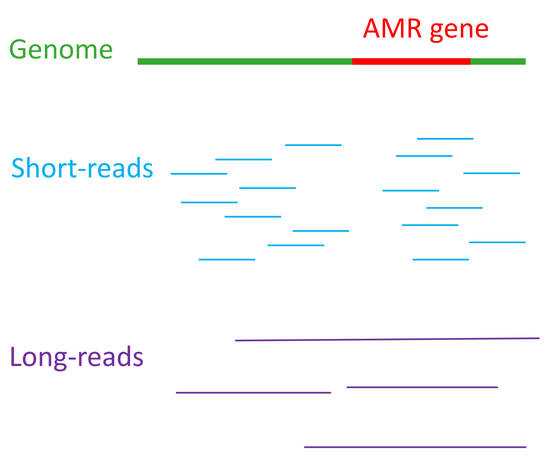
Results

Species	Relat. Abund.	Gram	ONT	Illumina
Escherichia coli	14%	-	+	+
Faecalibacterium prausnitzii	14%	+	+/-	+/-
Veillonella rogosae	14%	-	+	+
Roseburia hominis	14%	+/-	+	+
Bacteroides fragilis	14%	-	+	+
Prevotella corporis	6%	-	+	+
Bifidobacterium adolescentis	6%	+	-	-
Fusobacterium nucleatum	6%	-	+	+/-
Lactobacillus fermentum	6%	+	+	+
Clostridioides/dium difficile	1.50%	+	+	+/-
Akkermansia muciniphila	1.50%	-	+	+
Methanobrevibacter smithii	0.10%	+	-	-
Salmonella enterica	0.01%	-	-	-
Enterococcus faecalis	0.001%	+	-	-
Clostridium perfringens	0.0001%	+	-	-

+: detected with high KMA mapping scores; +/-: detection with low KMA mapping scores; -: not detected or trace amount

Species	Theoretical abundance (%)	AMR gene identification: *tet(Q)*	*mdf(A)*	*tet(W)*	*cepA*	*erm(B)*	*aac(6')-Iaa*	*lsa(A)*
Escherichia coli	14		+					
Faecalibacterium prausnitzii	14			+[B]				
Bacteroides fragilis	14	+[B]			+			
Prevotella corporis	6	+[B]						
Clostridioides difficile	1,5					+[B]		
Salmonella enterica	0,01						-	
Enterococcus faecalis	0,001							n/a

Grey: Expected AMR presence; [B]: also present in fecal background; n/a: not analyzed as species was not detected
+: detected with high KMA mapping scores; +/-: detection with low KMA mapping scores; -: not detected or trace amount



- Both short-read and long-read metagenomic sequencing identified the spiked species and the AMR genes, except for low abundance species
- Nanopore long reads allowed to attribute genes to a host species by providing additional genomic context

Conclusion

- **Proof-of-concept** for **simultaneous identification of bacterial species and their AMR genes** in metagenomics samples using long-read shotgun sequencing delivered, achieving a higher taxonomic resolution and by identifying AMR genes and linking them to their hosts
- **Perspective**: technology can help to **elucidate AMR transmission and exchange along food chain microbiome**; explore how to fully transfer this technology to a **fast, easy and direct use on-site**, opening up opportunities for **AMR monitoring and diagnostics** in food chain environments and beyond

REFERENCES

- De Keersmaecker et al, FARMED deliverable D-JRP12-1.1, https://doi.org/10.5281/zenodo.7429361

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