Plasmid-Mediated Antibiotic Resistance Dynamics in Broiler Chickens Revealed by Long-Read Sequencing

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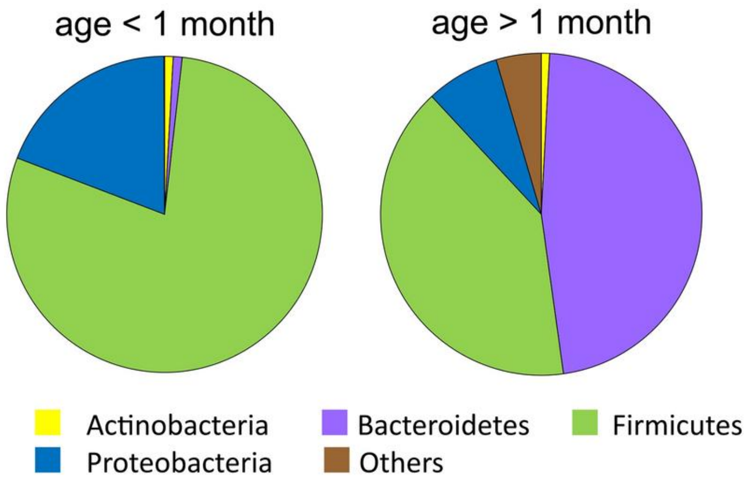
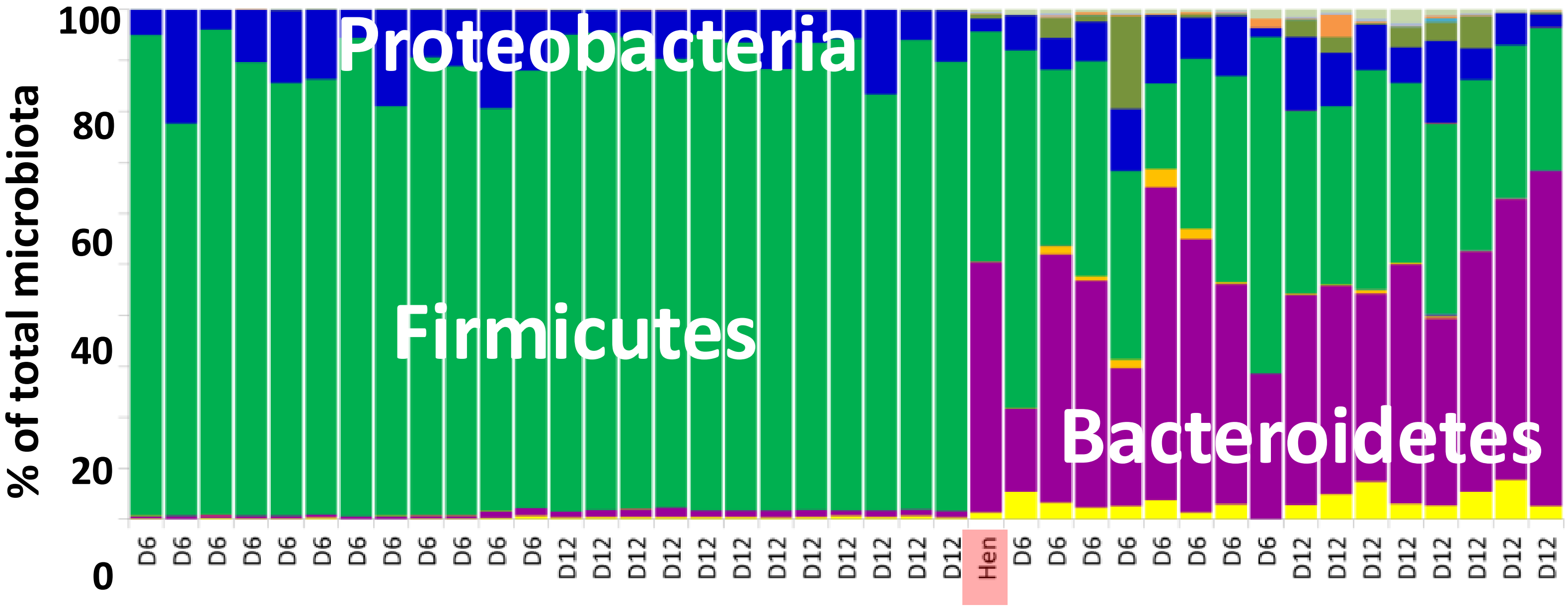
Chicken gut microbiome

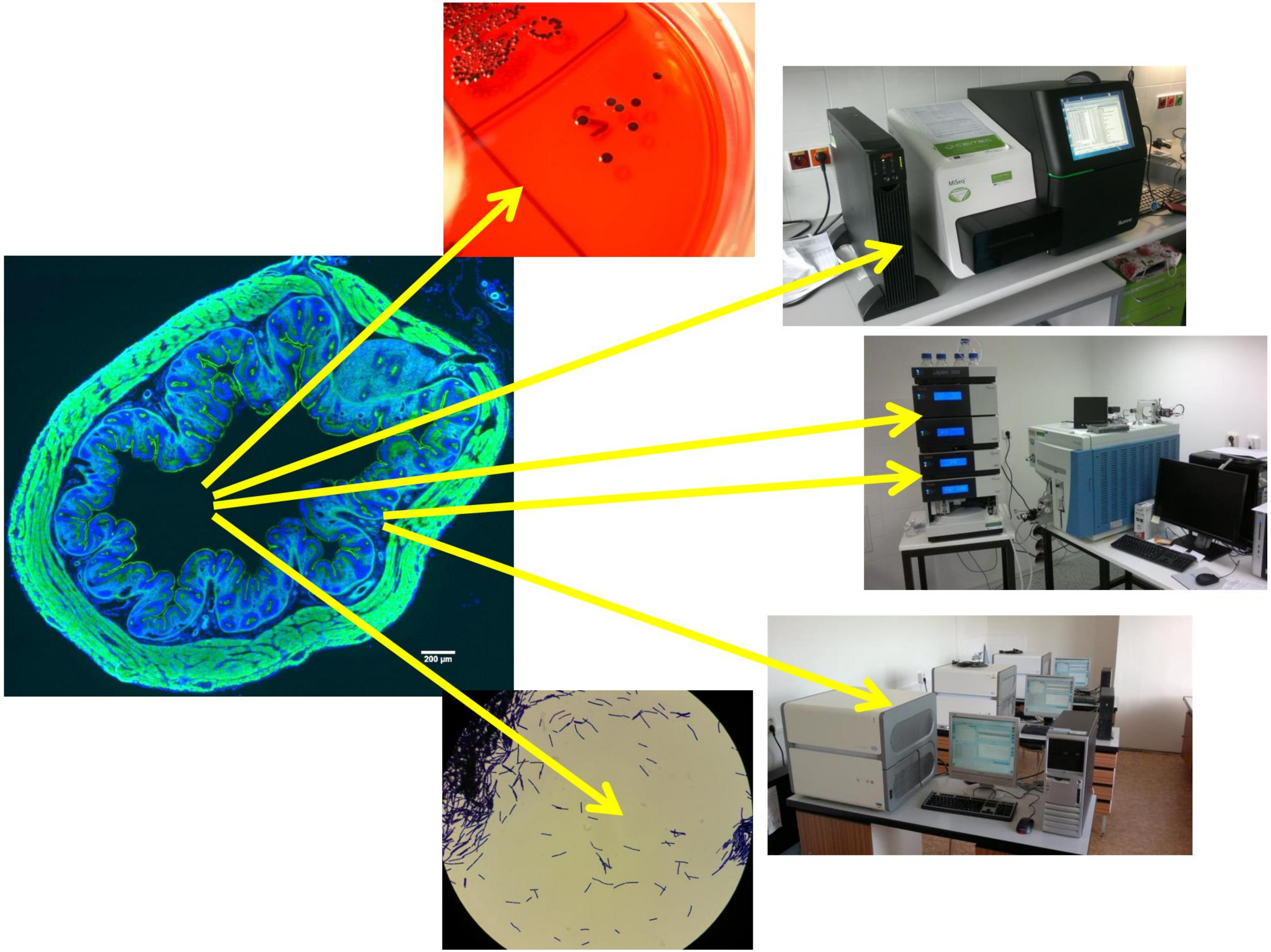
- Antibiotics misuse – chicken **growth promoter** (used until 2006 in EU, 2017 in US)
- **Prophylactic use** later on
- EU **ban** on prophylactic use of **antibiotics** in **farming**, effective in 2022
- Antibiotic resistance genes and their dissemination – threats for human medicine



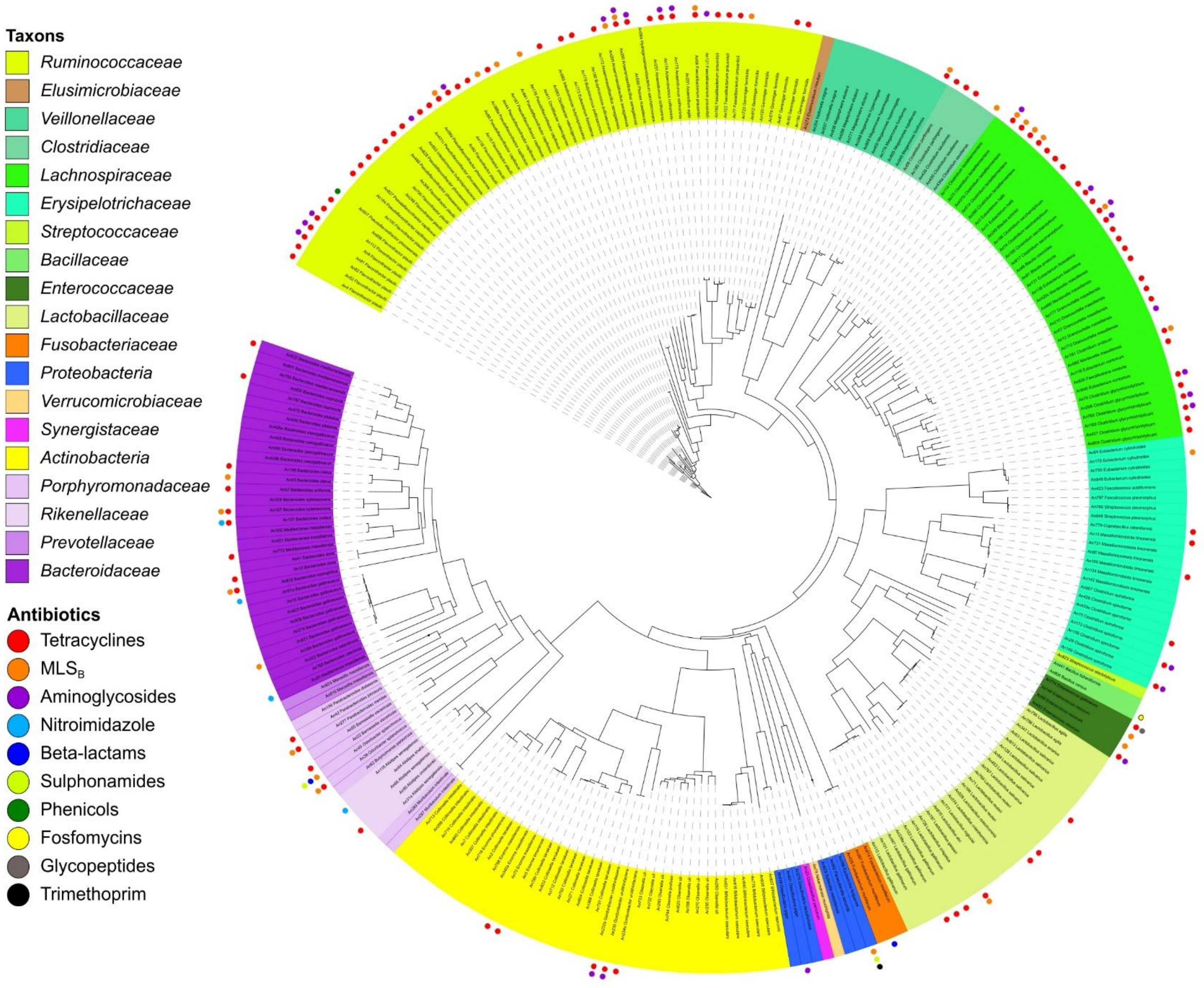
Cecal microbiota of chickens with and without contact with an adult hen



16S rDNA
seq
Qiime2


200 µm
MiSeq

Hunt for beneficial microbiota: antibiotic resistance genes everywhere!



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shot-gun DNA
seq
QC fastp
shovill
assembly
ResFinder db
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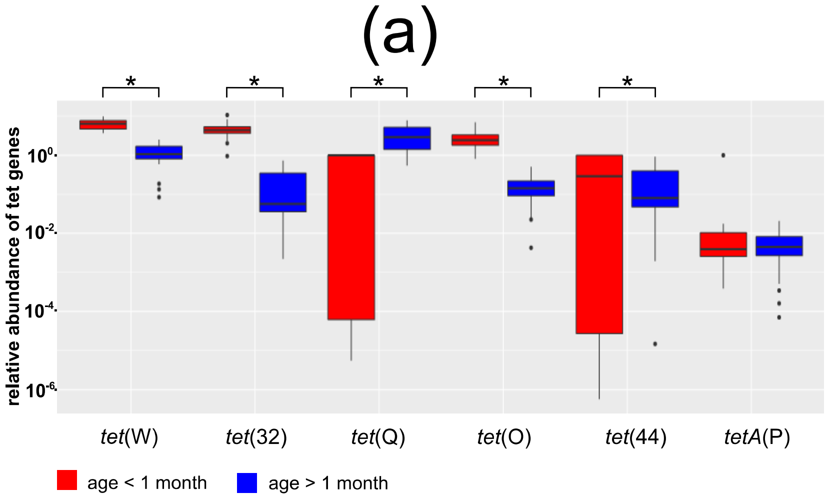
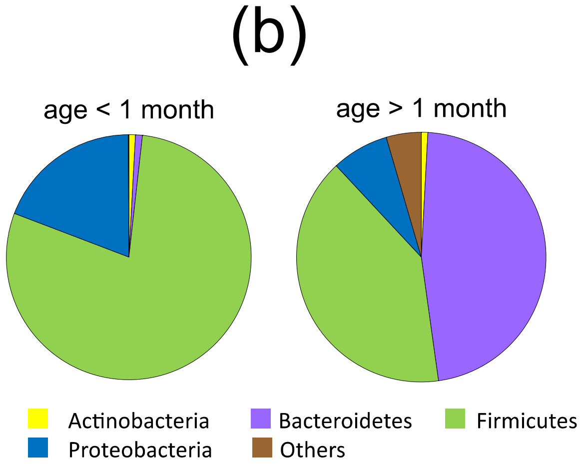
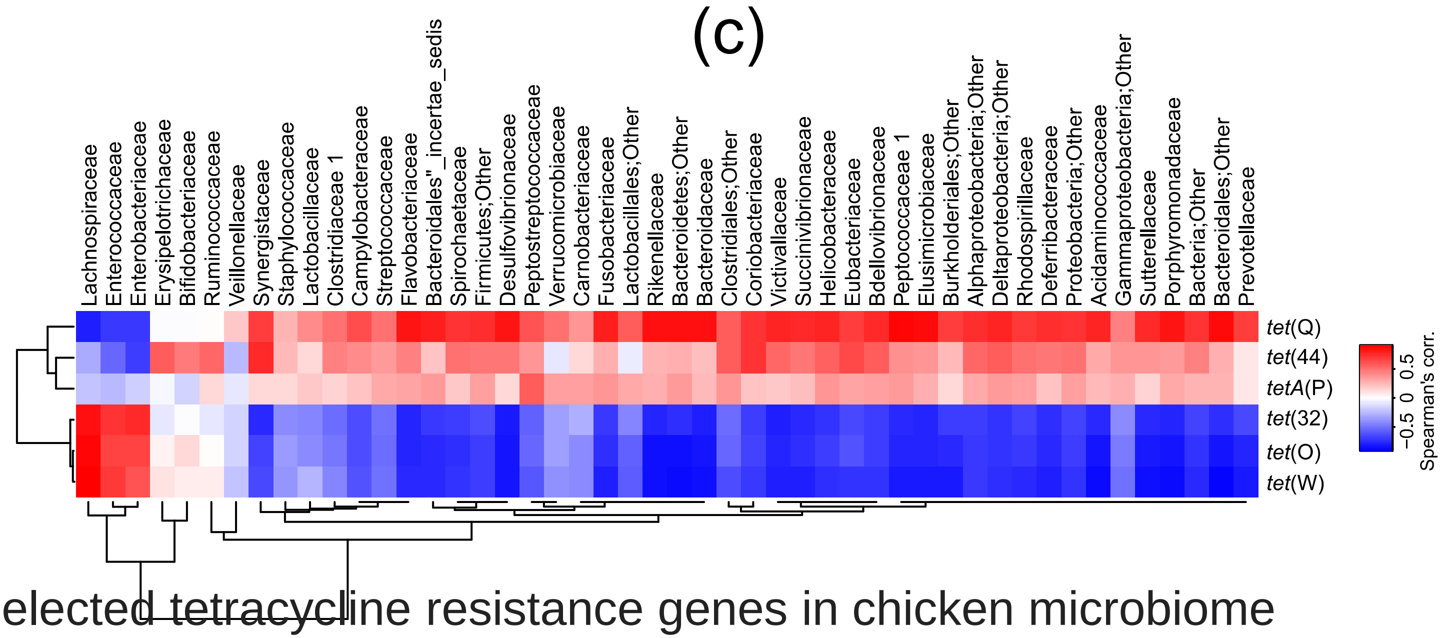




16S rDNA seq
Qiime2
Relative qPCR
Spearman
annotation



(a) Abundance of selected tetracycline resistance genes in chicken microbiome

(b) Microbiota composition in chicken ceaca younger or older than 1 month

(c) Correlation heat map of microbiota composition at family level and frequency of tet genes

Detection of plasmidomes using long-read sequencing

- AIM: to **reconstruct plasmids**
- Metagenomic plasmid DNA extraction – short (10 kb) and long (up to 50 kb) plasmid
- Rolling circle amplification
- MinION sequencing

Plasmidome sequencing:

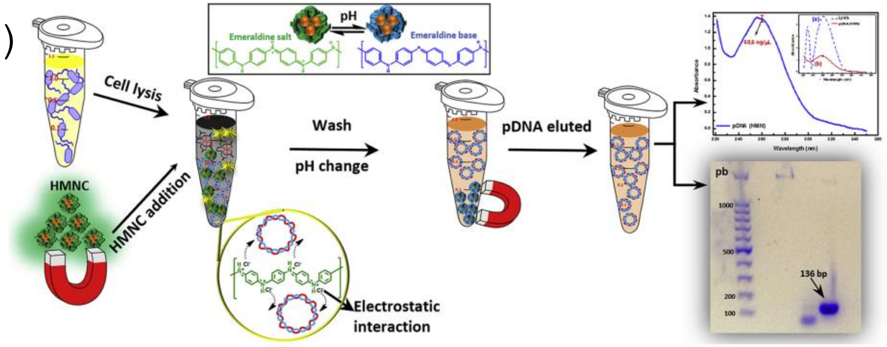
(a) Exogenous plasmid DNA extraction

(b) Depletion of fragmented **chromosomal DNA** – Exonuclease treatment

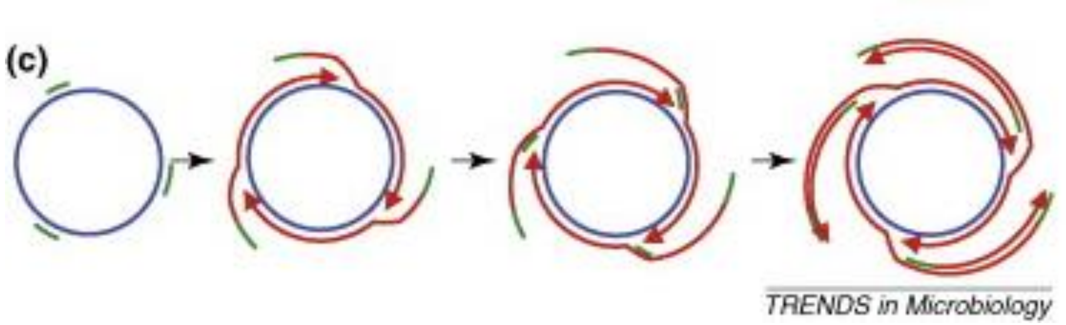
(c) Circular dsDNA amplification via Phi29 polymerase

(d) ONT sequencing

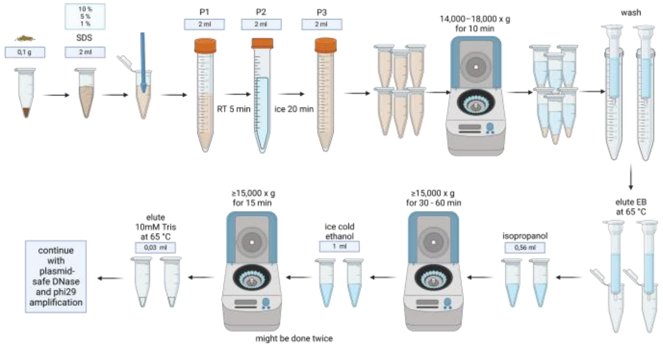
(a)

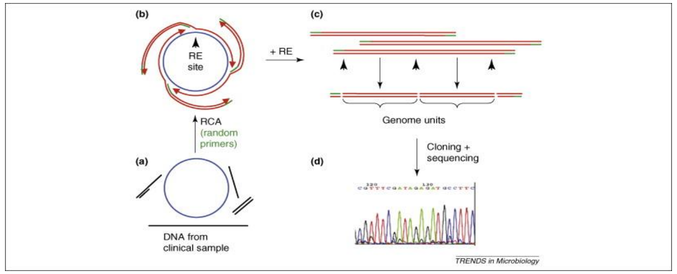


doi:10.1016/j.ab.2019.03.013



doi: 10.1016/j.tim.2009.02.004

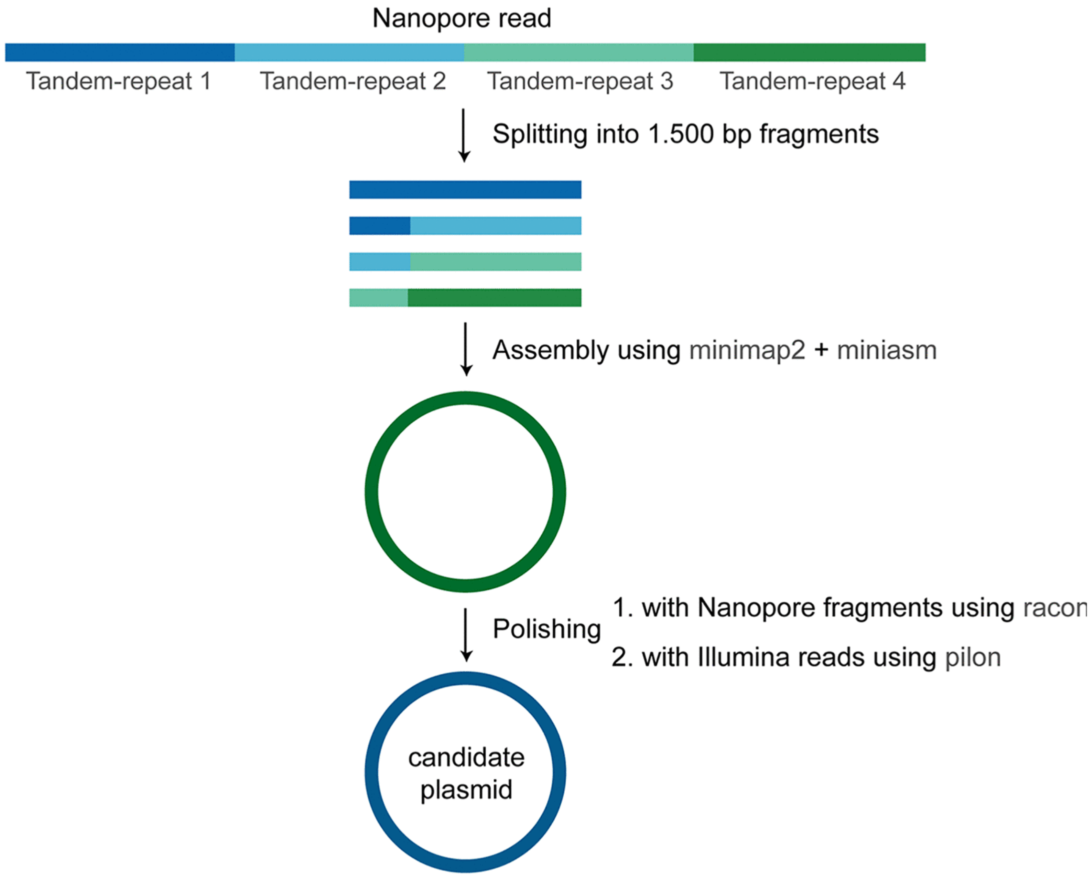




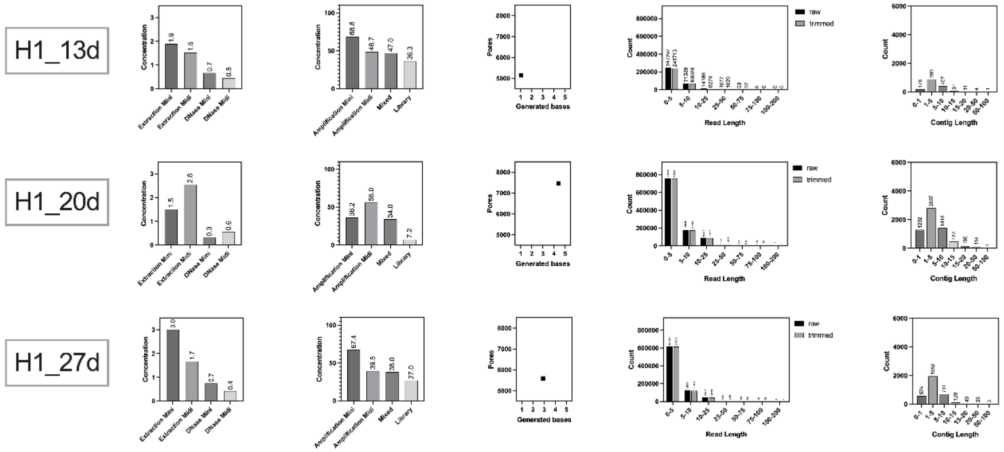
HUGO sequencing project

Plasmidome sequencing:

(d) ONT sequencing and analysis



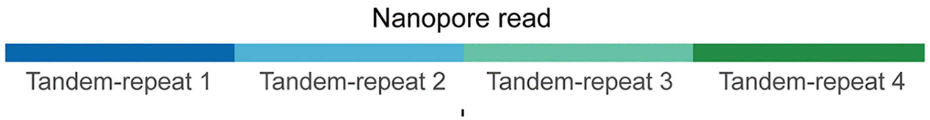
Quality Control



The columns show (from left to right): **DNA concentration** after extraction (ng/µL), **DNA concentration** after library preparation (ng/µL), number of generated bases (**Gb**), **read length** distribution (raw vs. trimmed), and **contig length** distribution after assembly

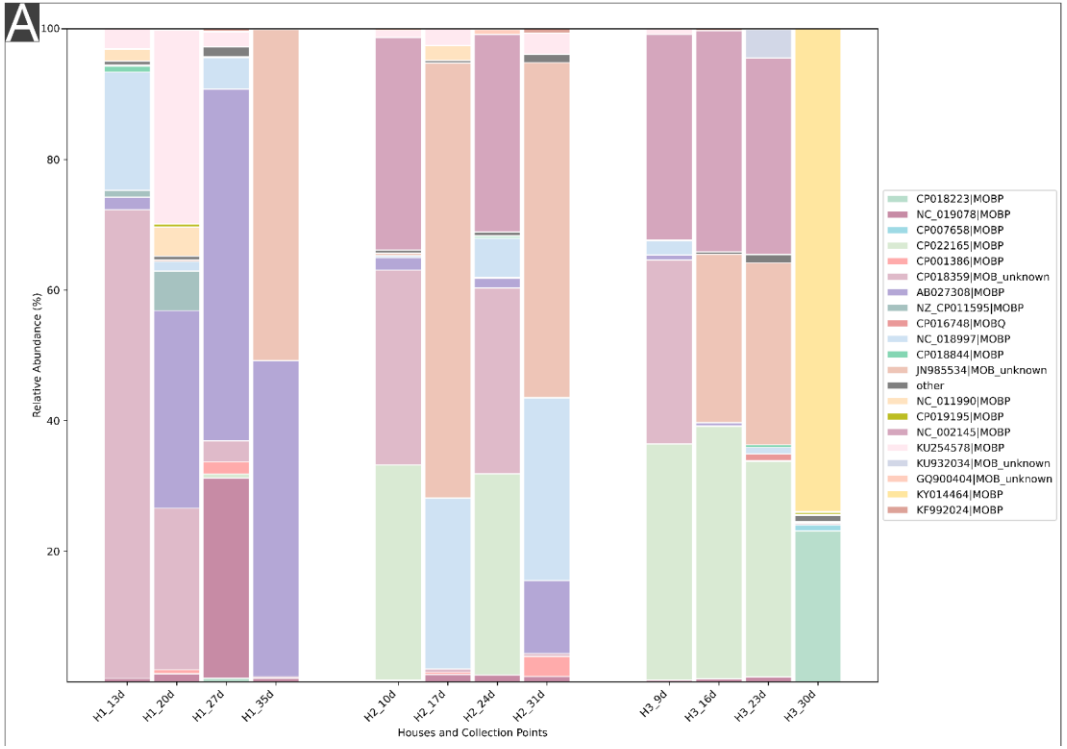
DOI: 10.1128/mSystems.00283-21

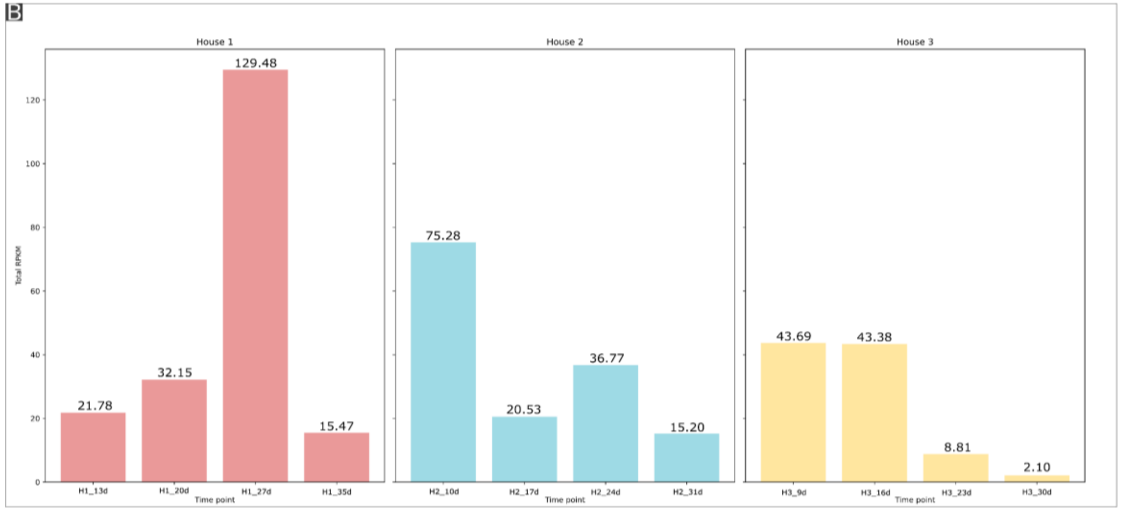
Plasmidome sequencing:
(d) ONT sequencing and analysis

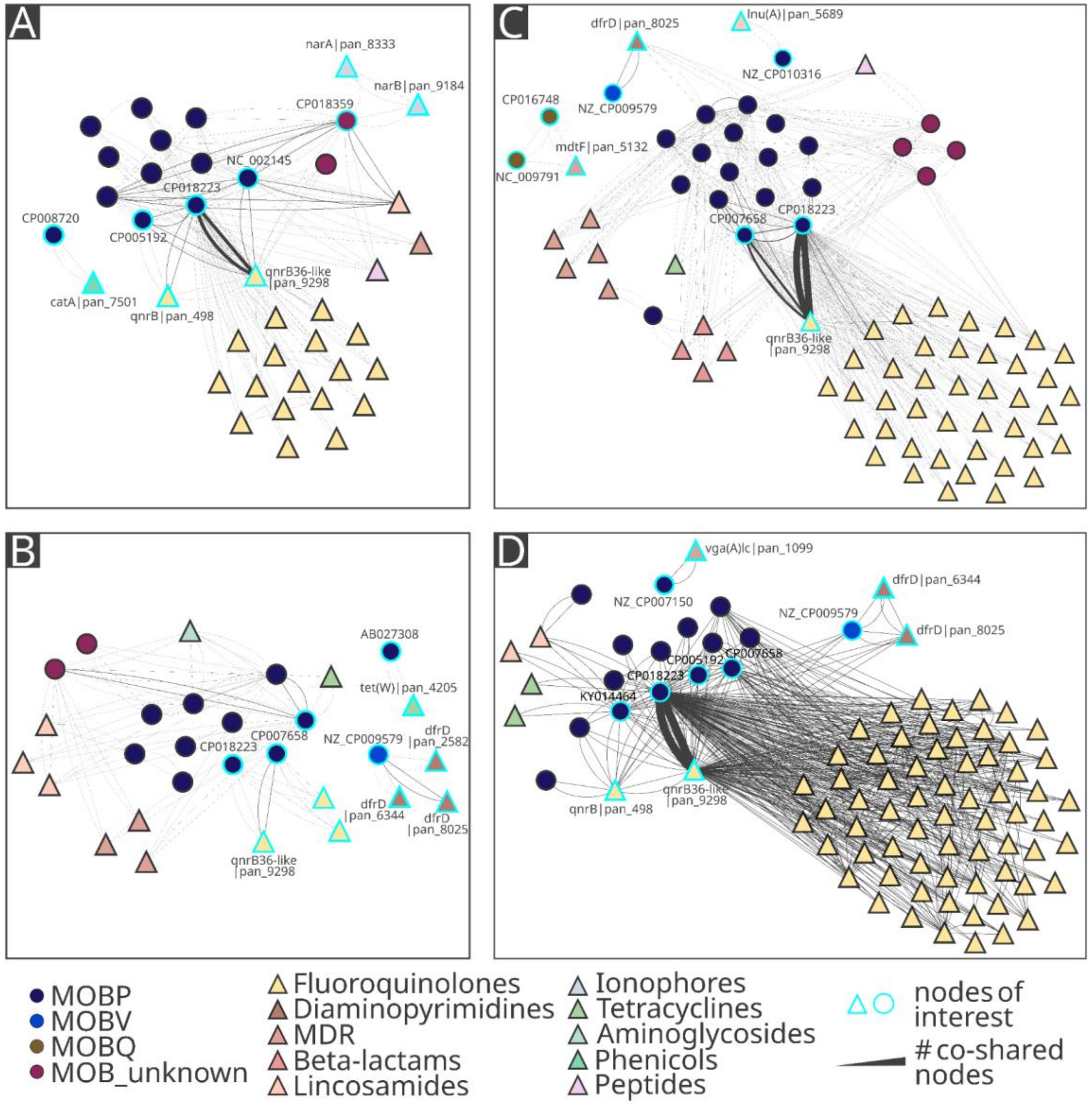


Abundance and prevalence of targeted genes:
KMA tool v1.4.15 with option '-bcNano'
databases PanRes v1.0.1,
MGE v1.0.2 and
MOB-suite v3.1.9

Further Analysis:
threshold for coverage and identity was set to 80% and 90%, respectively.
relative abundance was calculated and normalized using the **RPKM** (Reads Per Kilobase Million) value



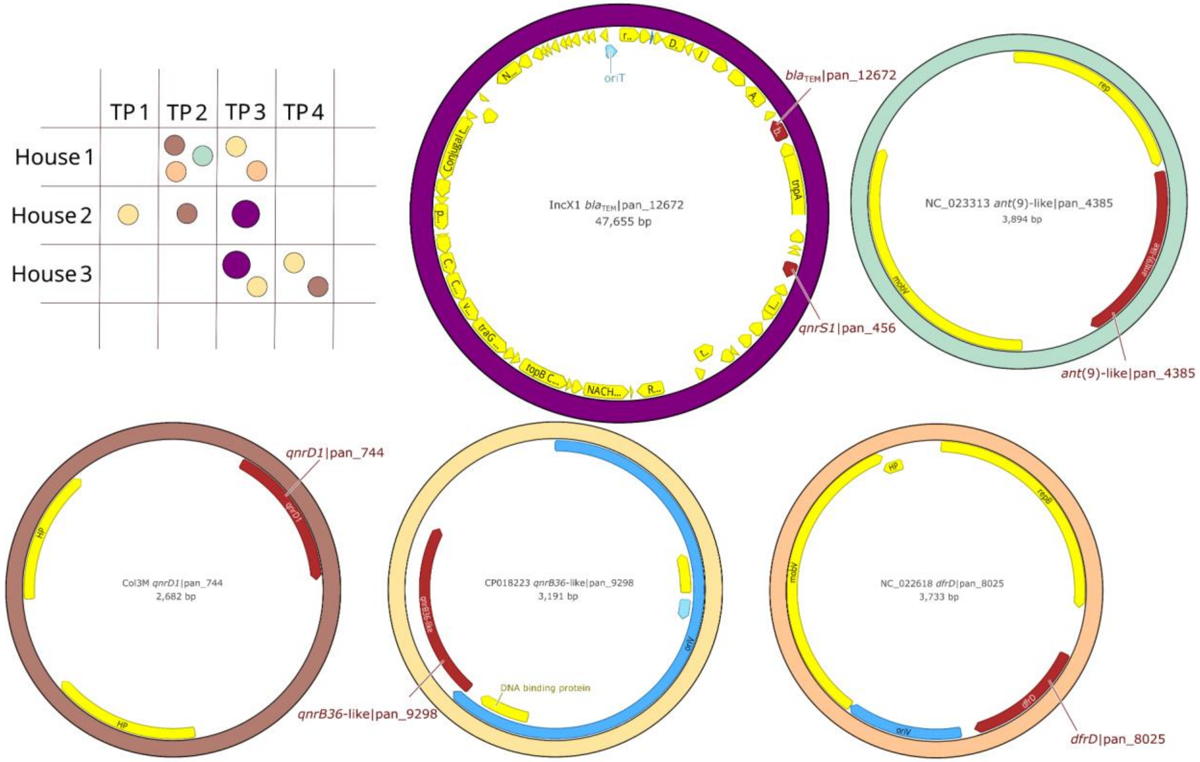




Co-occurrence network of antibiotic resistance genes and plasmids at different time points

binary presence-absence matrix was constructed for each read (plasmid trait vs ARG trait)

Plasmid *de novo* reconstruction



Read carrying ARGs were assembled using Flye v2.9.5 with '--meta' option
error-correct by Racon v1.5.0
Plasmid type analyzed against MOB-suite amd PLSDB
Annotated with Bakta v1.10.3

Conclusion

- **Targeted plasmidome sequencing** uncovers hidden resistance dynamics (e.g., MOBP plasmid-borne fluoroquinolone resistance).
- Combining with shot-gun metagenomic sequencing strengthens resistome surveillance and HGT risk assessment.
- Better understanding of gene mobility in complex/agricultural ecosystems.
- Highlights the need for continued improvements in:
  - Wet lab workflows – for more efficient plasmid isolation and sequencing.
  - Dry lab analysis – for better plasmid classification, assembly, and resistance gene annotation.

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