

Cross-domain plasmid mediated AMR transmission

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BACKGROUND: why investigate inter-host domain antimicrobial resistance (AMR) dissemination

(Farm) animals are potential reservoirs of clinically-relevant AMR

AMR transfer from animals to humans complicates treatment & containment

i-4-1-Health project

Aim: study AMR in Dutch-Belgian cross-border region

Isolates: **Humans** (hospitals, nursing homes, day-cares) & livestock (**broilers**, **pigs**)

i-4-1-Health results:

Illumina whole genome sequencing (WGS) of 474 ESBL- & CPE-resistant isolates

Only sporadic cross-host domain transmission (one case)

i-4-1-Health follow-up:

Are plasmids driving cross-host domain AMR transmission?

METHODS: isolates & data generation

Isolates: 483 Enterobacteriaceae (human n=389, broiler n=82 and pig n=12)

Sequencing: Illumina (paired-end) & Oxford Nanopore (ONT, R9.4 flow cells)

Hybrid assembly: Unicycler (v0.50.1)

QC criteria: > 20x Illumina & >10x ONT depth of coverage

OR

<20X Illumina & > 100X ONT depth of coverage

Final inclusion: 355 Isolates (human n = 244, broiler n = 65 and pig n=4)

METHODS: plasmid characterisation

Plasmid sequence inclusion:

- Complete, circular contigs
- Deemed plasmids by MOB_suite (v3.1.4)

Pling (v2.0.0):

- Plasmid cluster assignment

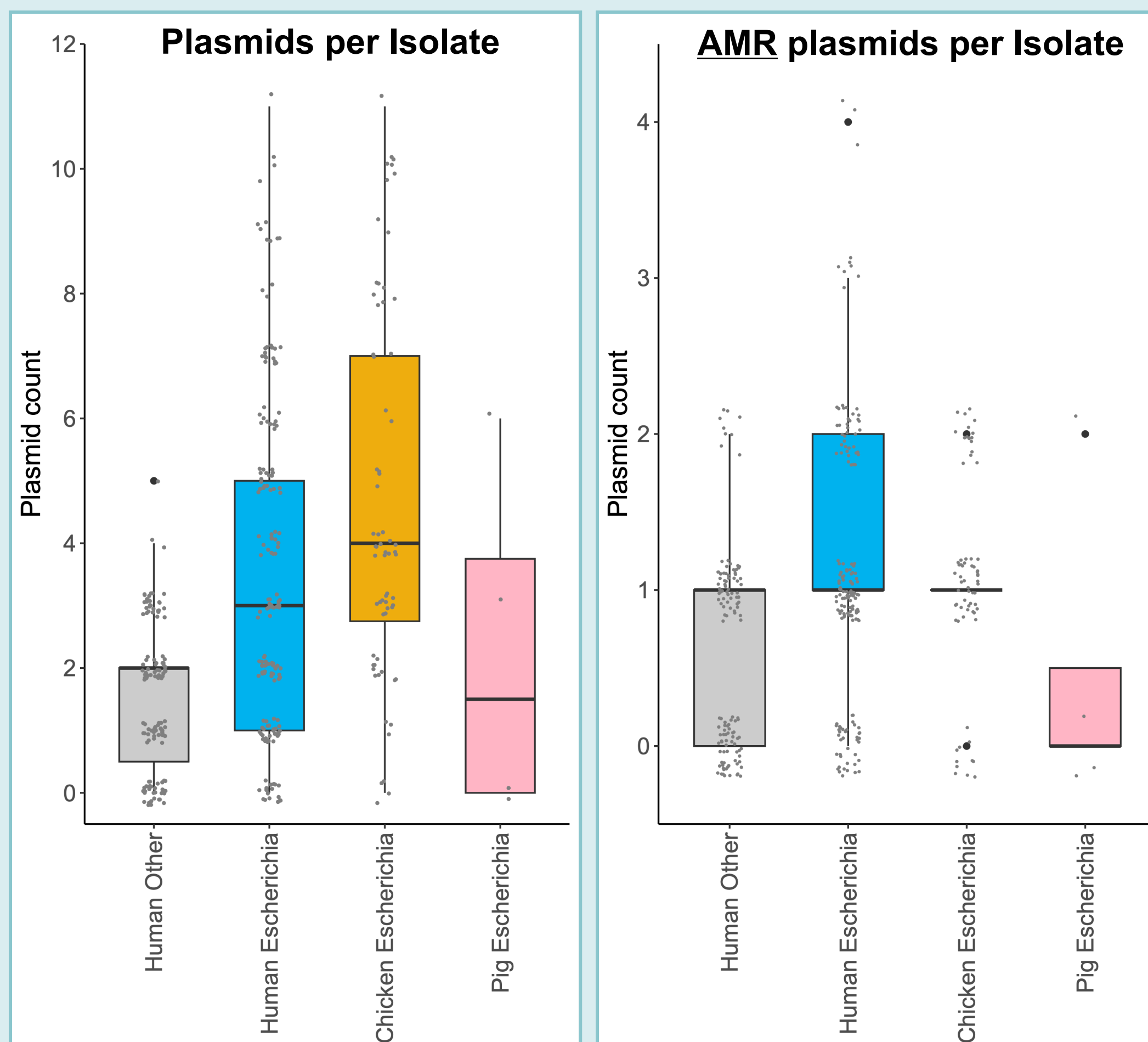
(All bioinformatics tools were used with default settings)

Inclusion:

Total: 1,111 plasmids (332 AMR plasmids)

Host domain	Escherichia	Klebsiella	Enterobacter	Citrobacter	Other	Total
Human	179	53	18	3	3	256
Broiler	74	-	-	-	-	74
Pig	2	-	-	-	-	2
Total	255	53	18	3	3	332

RESULTS: host-domain specific plasmid characteristics



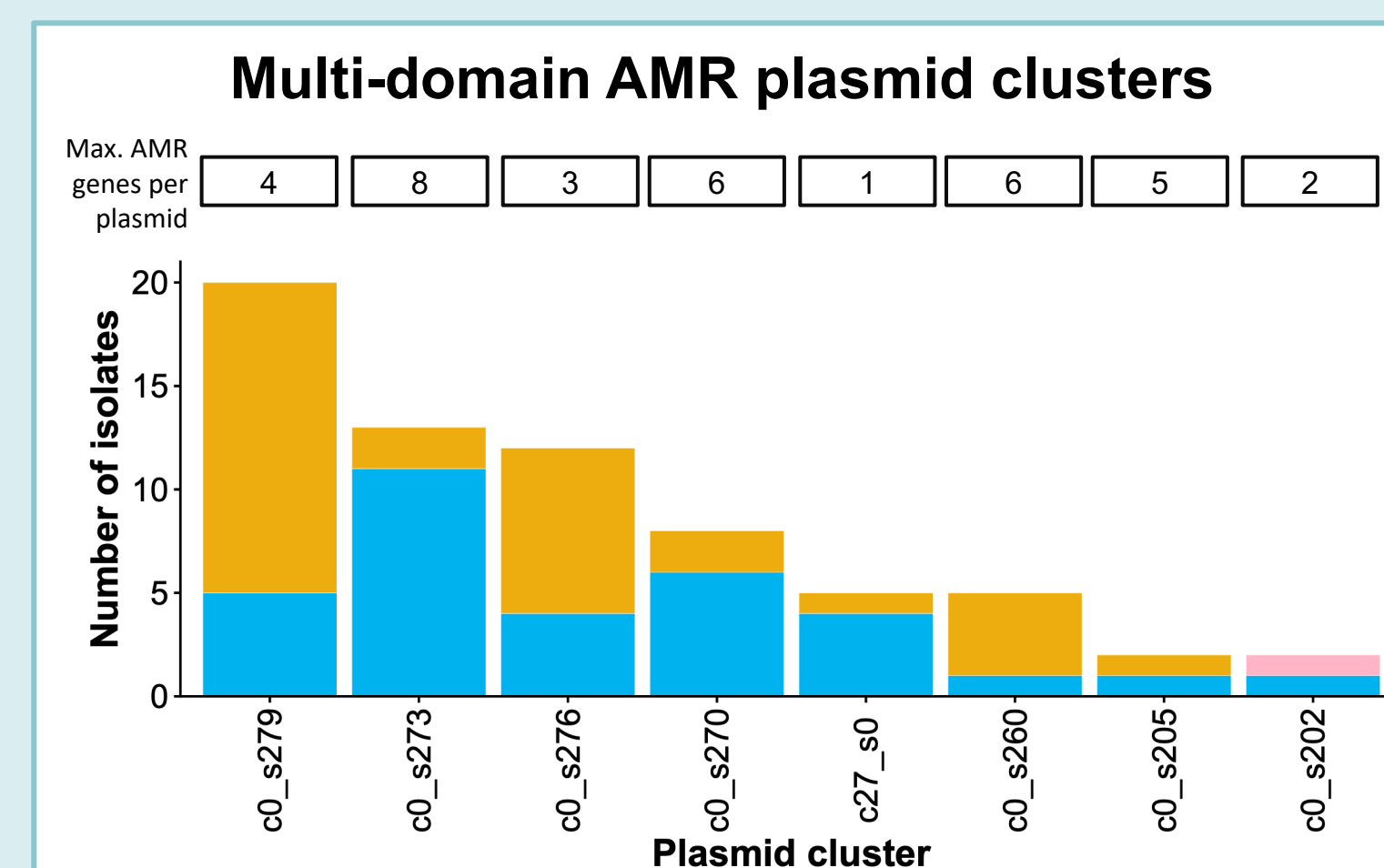
Total number of plasmids per isolate per domain

Total number of AMR gene containing plasmids per isolate per domain

E. coli isolates contain more (AMR) plasmids

Broiler *E. coli* isolates contain the most plasmids

RESULTS: Pling plasmid clusters



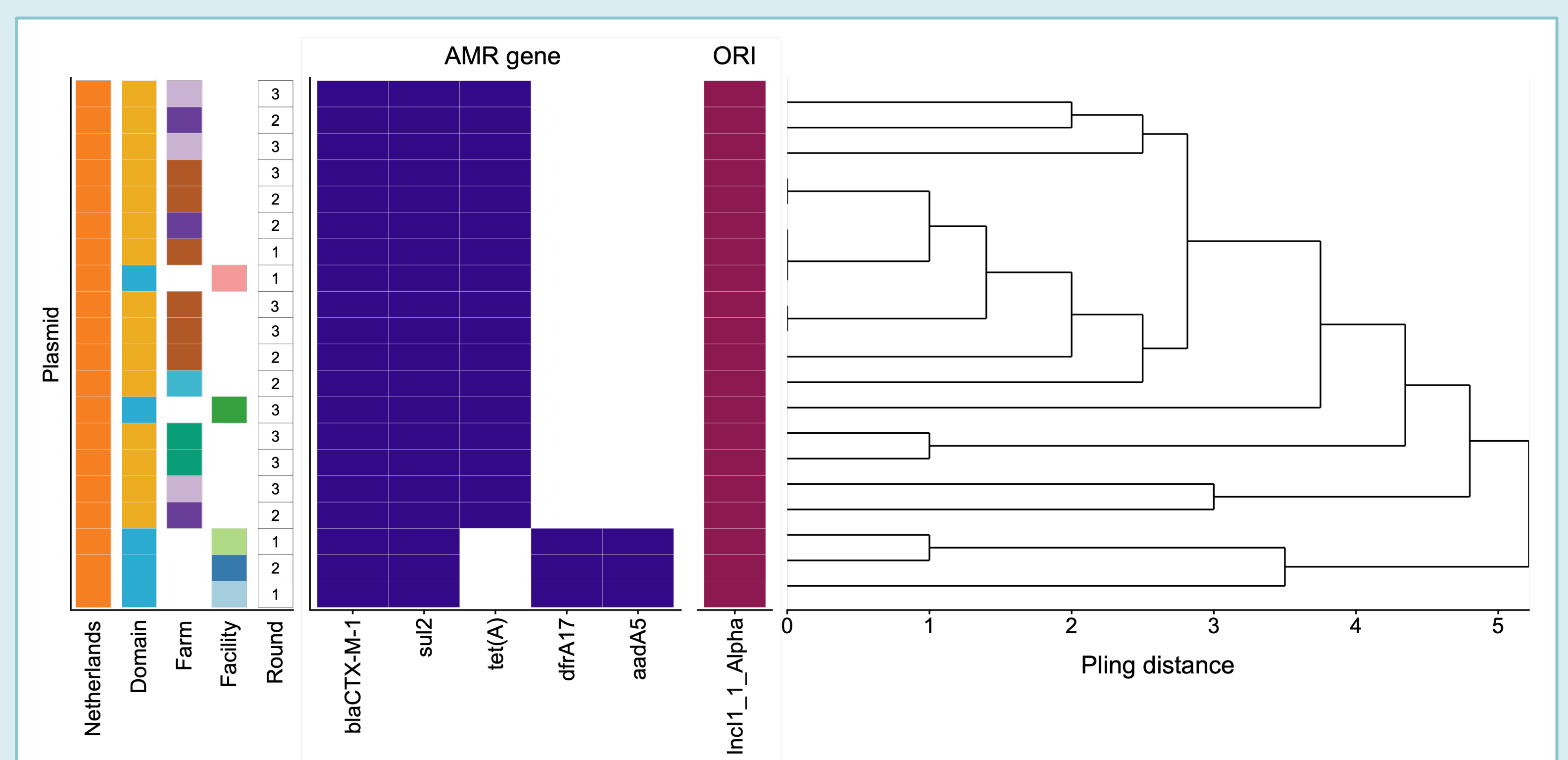
Left: Multi-domain plasmid clusters

Pling AMR-plasmid clusters spanning multiple host-domains.

Below: c0_s279 plasmid cluster characterisation

Metadata, AMR genes, origin of replication and hierarchical clustering of the largest multi-domain AMR-plasmid cluster.

Hierarchical clustering is based on Pling distances



CONCLUSIONS:

- Long-read sequencing demonstrated variable counts between host-domains & isolate species
- Pling identified 8 host-domain-spanning AMR-plasmid clusters
- The largest cluster (n = 20 plasmids), was an IncI1_Alpha plasmid carrying 3-4 resistance genes

Sporadic transmission events may be sufficient to introduce livestock-associated clones / plasmids into the human population

References:

1. De Koster S *et al.*. ESBL-Producing, Carbapenem- and Ciprofloxacin-Resistant *Escherichia coli* in Belgian and Dutch Broiler and Pig Farms: A Cross-Sectional and Cross-Border Study. *Antibiotics* (Basel). 2021 Aug 4;10(8):945. doi: 10.3390/antibiotics10080945. PMID: 34438995; PMCID: PMC8388939.



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