Metagenome-assembled-genomes (MAGs) from the chicken caecum to identify the bacterial hosts of antimicrobial genes

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Which bacterial species harbour antimicrobial resistance genes?

- **Construct metagenome-assembled genomes (MAGs) to identify bacterial hosts of chromosomally integrated antimicrobial resistance genes (ARGs)**
- **Construct plasmids from metagenome sequencing to identify ARG-plasmids (plasmidome)**
- **Identify ARGs present in pathogenic bacteria (extremely high risk to public health)**
- **Identify reservoir of ARGs in caecal commensal bacteria**

Introduction

Antimicrobial resistance (**AMR**) poses an enormous threat to global public health. Unfortunately, antimicrobial resistance genes (**ARGs**) do not obey political, geographical or species borders and the propensity of new ARGs to develop and disseminate among humans, animals, and the environment necessitates the use of a **One Health** approach for its study and management. The poultry industry in Asia is at high risk for the emergence of AMR because the widespread use of antibiotics (Figure 1).
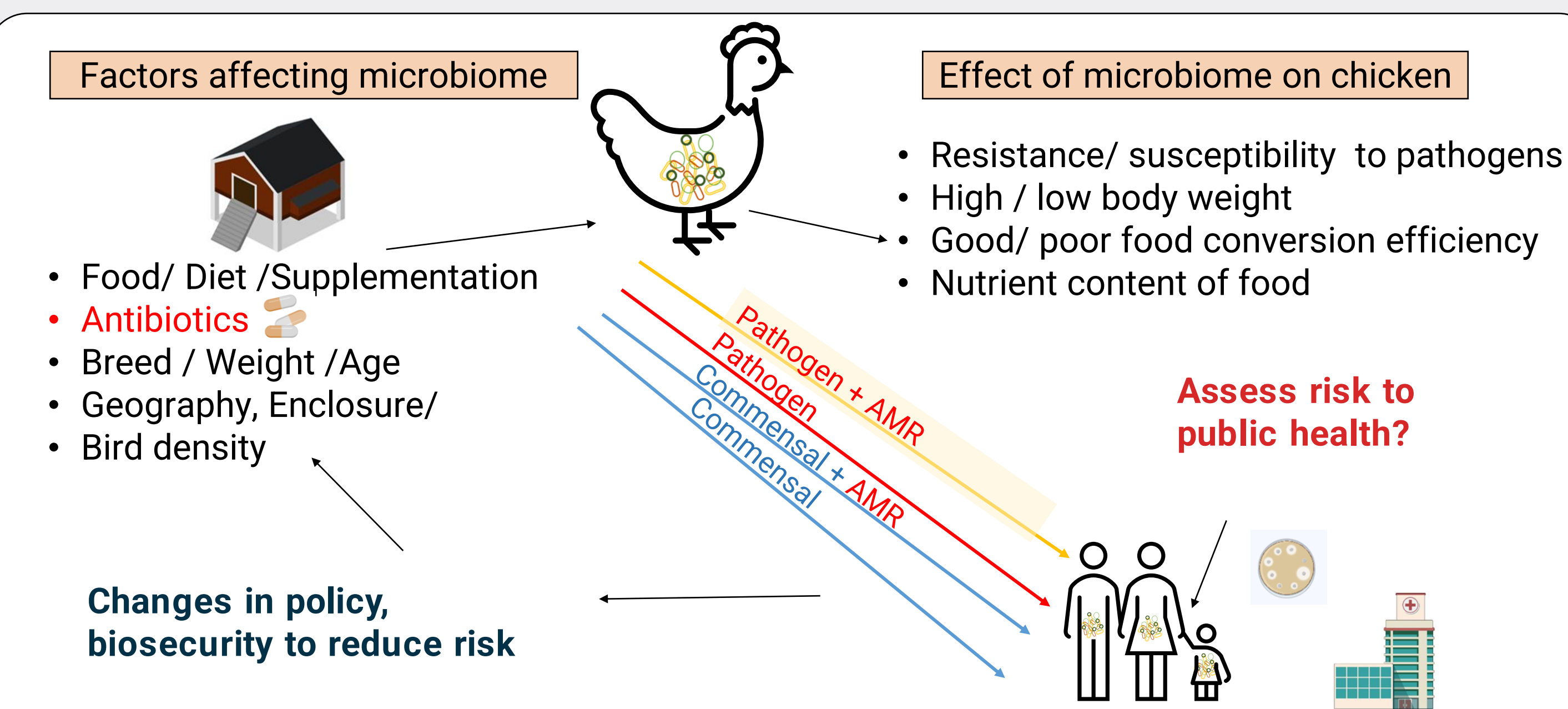

Factors affecting microbiome
- Food/ Diet /Supplementation
- *Antibiotics*
- Breed / Weight /Age
- Geography, Enclosure/
- Bird density

Effect of microbiome on chicken
- Resistance/ susceptibility to pathogens
- High / low body weight
- Good/ poor food conversion efficiency
- Nutrient content of food

Pathogen + AMR
Pathogen
Commensal + AMR
Commensal

Assess risk to public health?

Changes in policy, biosecurity to reduce risk

Figure 1: Identify factors that decrease public health risk (AMR and pathogen abundance) and optimise bird productivity and health.

Bacteria acquire and spread AMR via the horizontal gene transfer (HGT) of ARGs on mobile genetic elements. ARGs can either be in plasmids or integrated into bacterial genomes. The attainment of AMR in zoonotic pathogens such as *Campylobacter*, *Salmonella* and *Escherichia coli* is of specific concern.
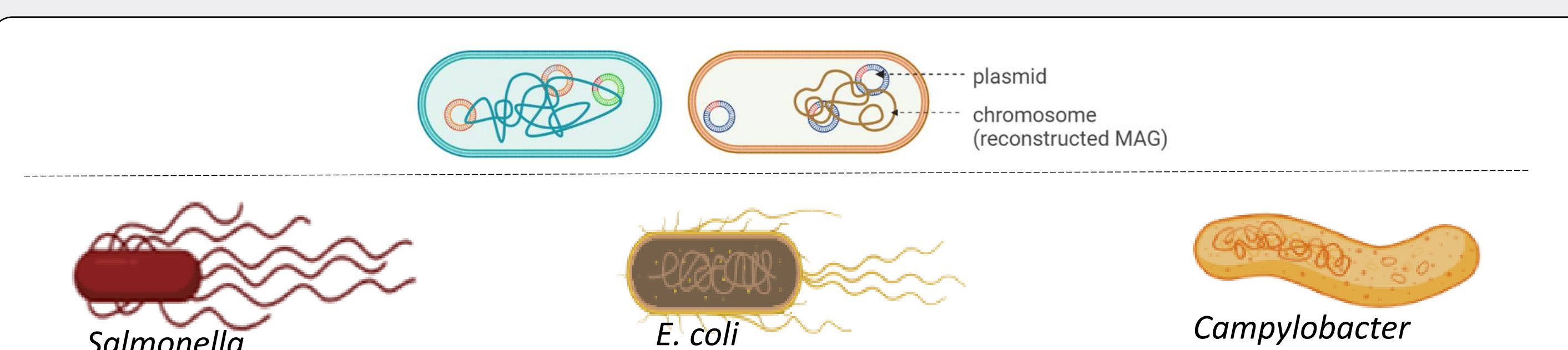

plasmid
chromosome (reconstructed MAG)

Salmonella   *E. coli*   *Campylobacter*

Figure 2: ARGs can be present on plasmids or integrated into the bacterial chromosome. The presence of ARGs in zoonotic pathogens is of particular concern. ARGs can be transferred from commensal bacteria to pathogens via horizontal gene transfer.

Method

To investigate the plasmidome and bacterial hosts of ARGs in chickens, the caecal microbiomes of 15 chickens from farms and markets in Gujarat will be sequenced using long read Oxford Nanopore sequencing of unamplified DNA and shotgun Illumina sequencing to create metagenomic-assembled genomes (MAGs). The hybrid assemblies will be compared against a plasmid database to identify contigs that are plasmids. The use of long-read sequencing should capture chromosomally integrated ARGs in MAGs and allow them to be associated with a bacterial host (Figure 3).
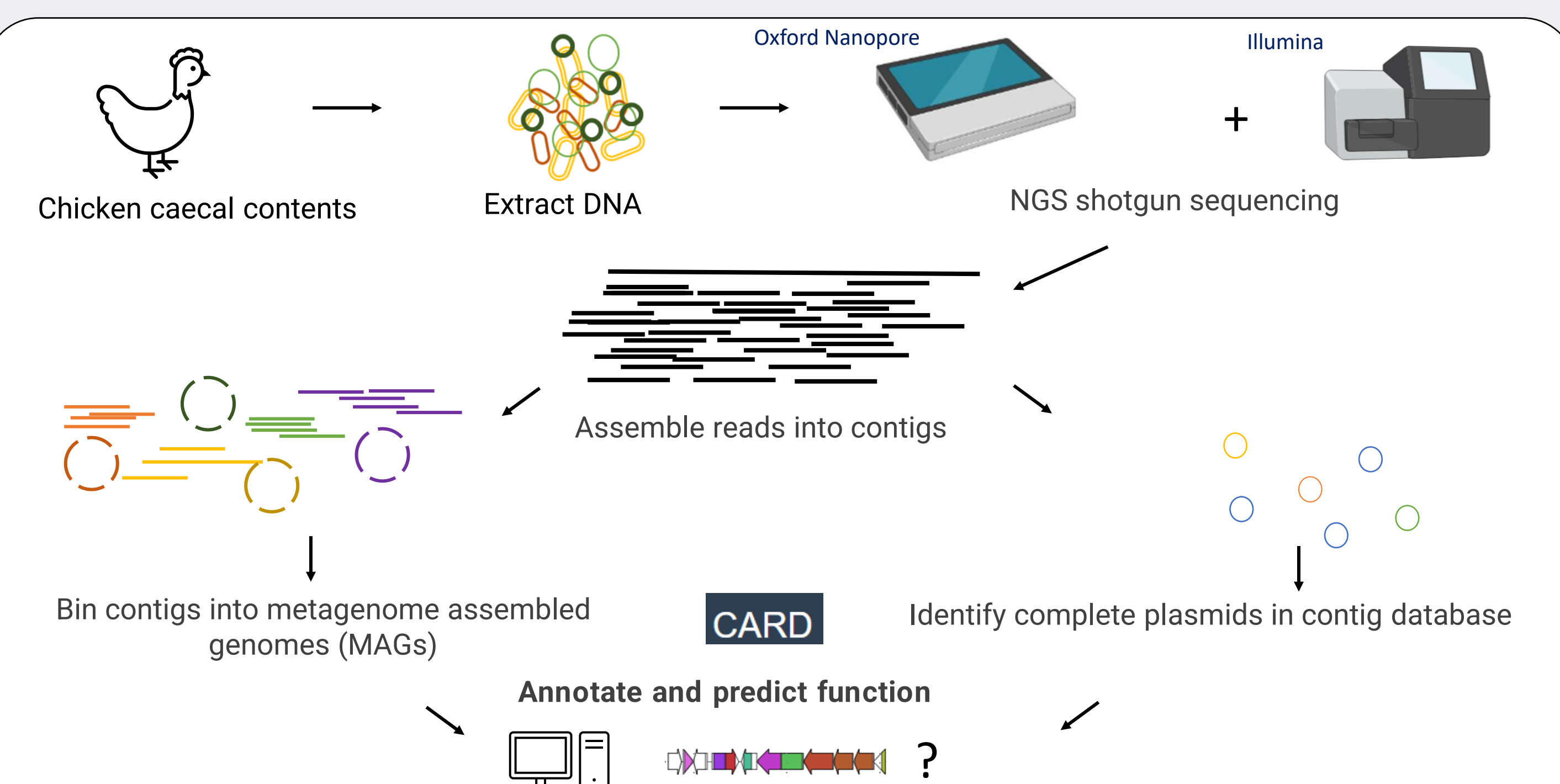

Chicken caecal contents   Extract DNA   Oxford Nanopore   Illumina   NGS shotgun sequencing

Assemble reads into contigs

Bin contigs into metagenome assembled genomes (MAGs)   CARD   Identify complete plasmids in contig database

Annotate and predict function

Figure 3: We will construct MAGs and a plasmid catalogue. We aim to identify the bacterial hosts of high-risk AMR plasmids.

Results

Preliminary analysis has identified high variability in the abundance of bacterial genera in caecal contents of different chickens. *Salmonella*, *Campylobacter* and *Escherichia coli* are present at low abundance (< 0.5%) in most birds.
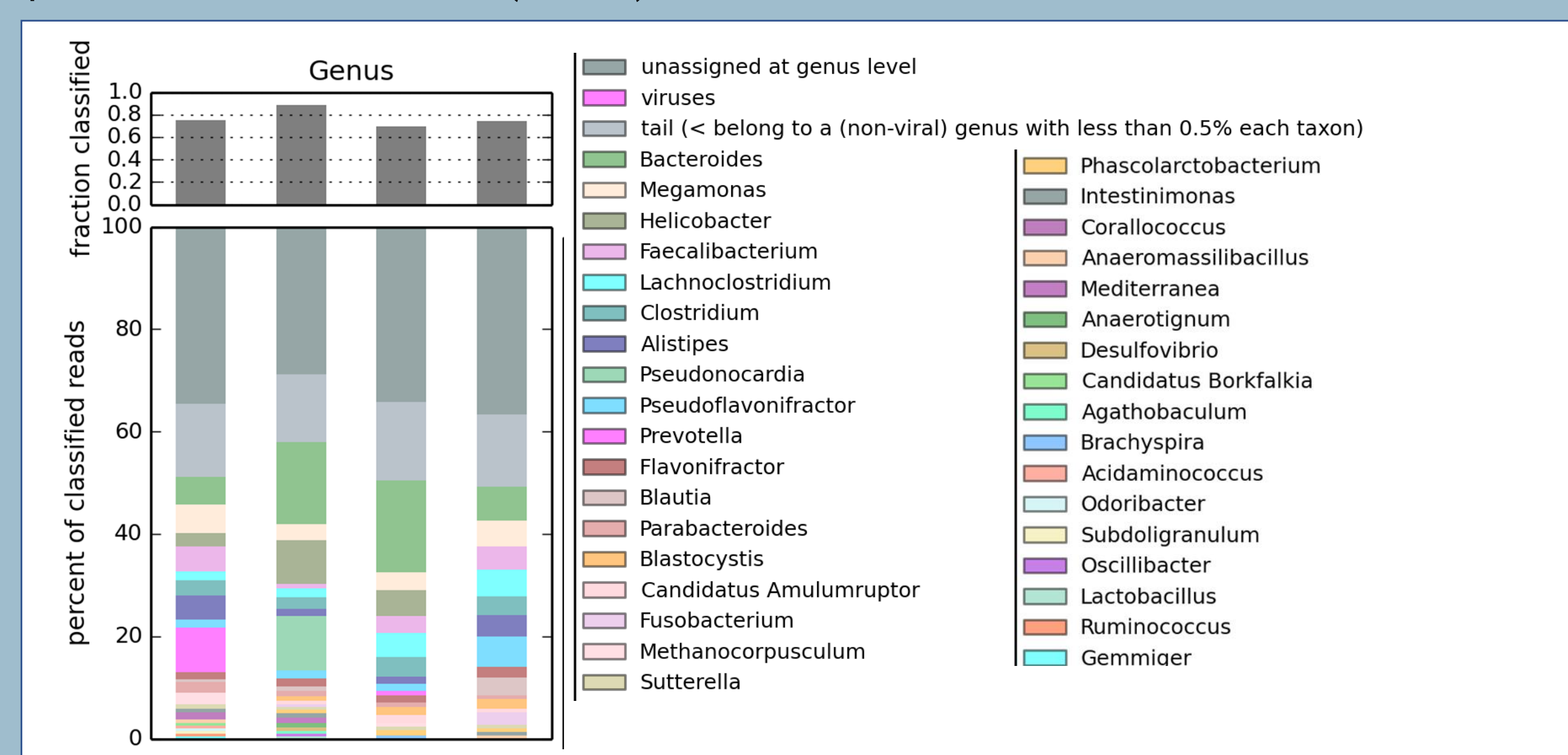

A pilot co-assembly of four samples from 4 minION flow cells resulted in an assembly of 300Mb. Contigs were binned into 72 putative MAGs, with 23 MAGs representing known species (>95% ANI) and 49 representing incomplete or novel species/ genera.

User Genome	Phylum	Class	Order	Family	Genus	Species	FastANI Reference	FastANI ANI
bin.084.fa	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola dorei	GCF_013009555.1	99.13
bin.045.fa	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola plebeius_A	GCF_003437535.1	96.98
bin.061.fa	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Prevotella	Prevotella sp900554045	GCA_900554045.1	97.49
bin.049.fa	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Amulumruptor	Amulumruptor caecigallinarius	GCA_001941205.1	96.67
bin.047.fa	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes dispar	GCF_006542685.1	97.47
bin.074.fa	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes sp000434235	GCA_000434235.1	97.69
bin.074.fa	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidales	Tannerellaceae	Parabacteroides	Parabacteroides johnsonii	GCF_000156495.1	98.04
bin.026.fa	Campylobacterota	Campylobacteria	Campylobacterales	Helicobacteraceae	Helicobacter_D	Helicobacter_D pullorum	GCF_008801855.1	97.6
bin.018.fa	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus crispatus	GCF_002088015.1	97.26
bin.069.fa	Firmicutes_A	Clostridia	Lachnospirales	Anaerotigaceae	Anaerotignum	Anaerotignum lactatifermentans	GCF_900142265.1	97.13
bin.027.fa	Firmicutes_A	Clostridia	Lachnospirales	Anaerotigaceae	UMGS1670	UMGS1670 sp902406135	GCA_902406135.1	96.78
bin.079.fa	Firmicutes_A	Clostridia	Lachnospirales	Lachnospiraceae	Blautia	Blautia sp002161285	GCF_002161285.1	97.52
bin.032.fa	Firmicutes_A	Clostridia	Lachnospirales	Lachnospiraceae	Mediterraneibacter	Mediterraneibacter sp900555215	GCA_900555215.1	95.57
bin.035.fa	Firmicutes_A	Clostridia	Lachnospirales	Lachnospiraceae	UBA7182	UBA7182 sp002160135	GCA_002160135.1	97.75
bin.075.fa	Firmicutes_A	Clostridia	Oscillospirales	Oscillospiraceae	UBA9475	UBA9475 sp002161675	GCA_002161675.1	95.49
bin.086.fa	Firmicutes_A	Clostridia_A	Christensenellales	CAG-314	CAG-314	CAG-314 sp000433775	GCA_000433775.1	97.38
bin.036.fa	Firmicutes_A	Clostridia_A	Christensenellales	CAG-917	UMGS1688	UMGS1688 sp900545885	GCA_900545885.1	96.95
bin.004.fa	Firmicutes_A	Clostridia_A	Christensenellales	CAG-917	UMGS1688	UMGS1688 sp900554085	GCA_900554085.1	98.62
bin.093.fa	Firmicutes_C	Negativicutes	Acidaminococcales	Acidaminococcaceae	Phascolarctobacterium	Phascolarctobacterium sp000436095	GCF_010669225.1	96.89
bin.024.fa	Firmicutes_C	Negativicutes	Selenomonadales	Selenomonadaceae	Megamonas	Megamonas funiformis	GCF_010669225.1	97.83
bin.014.fa	Firmicutes_A	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium_A	Fusobacterium_A sp900555485	GCA_900555485.1	98.06
bin.011.fa	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	CAG-521	CAG-521 sp002329575	GCA_002329575.1	98.16
bin.087.fa	Synergistota	Synergistia	Synergistales	Synergistaceae	An23	An23 sp900544635	GCA_900544635.1	96.28

Results

From 216 ARGs identified the most abundant confer resistance to tetracycline, macrolide, aminoglycoside and lincosamide antibiotics classes.
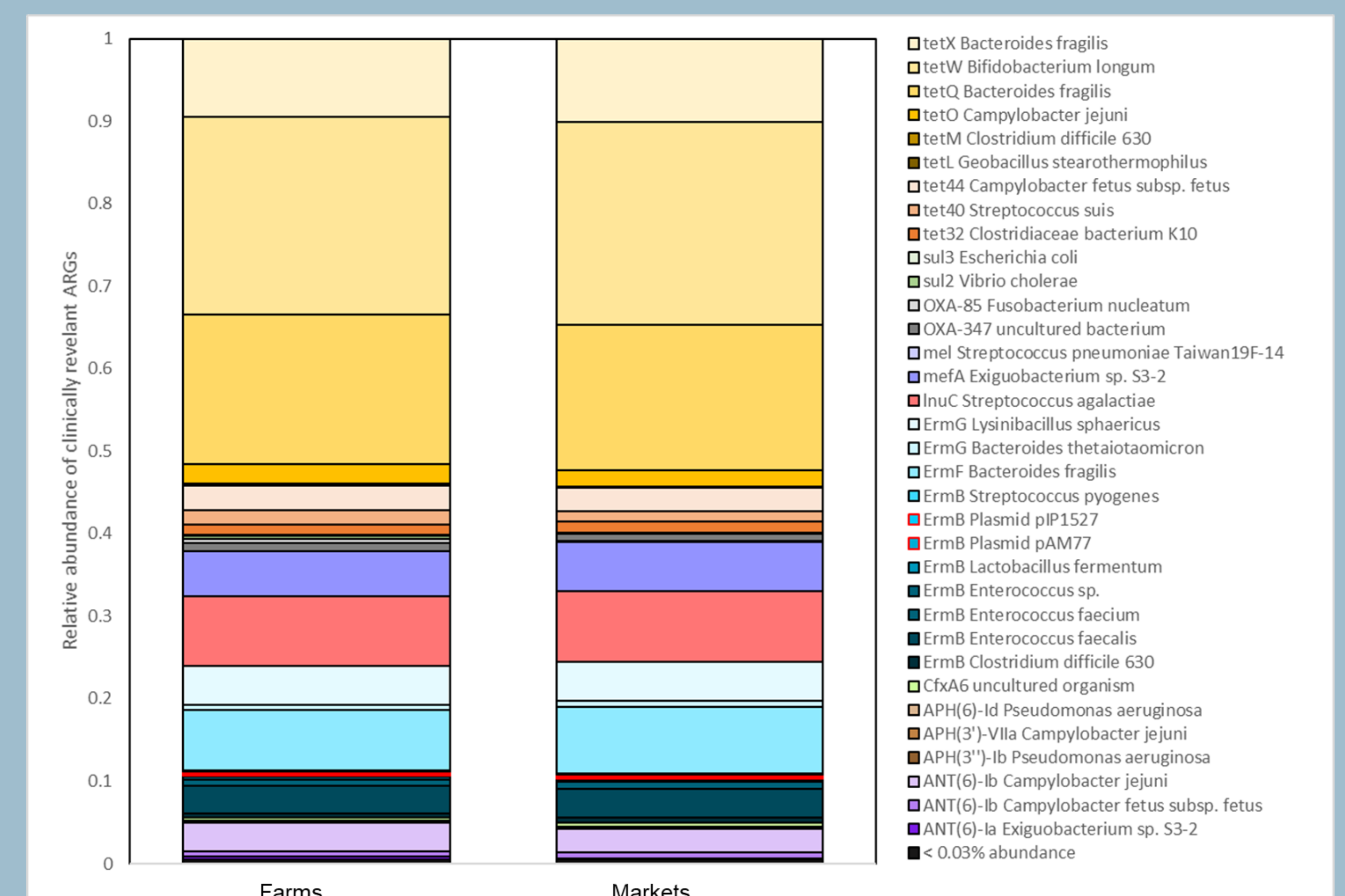
Future work

The entire dataset will be co-assembled to create a comprehensive catalogue of MAGs and plasmids.
- Each MAG will be submitted to CARD to detect chromosomally integrated ARGs.
- The contigs will be searched for complete plasmids in order to describe the plasmidome of these samples.

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