

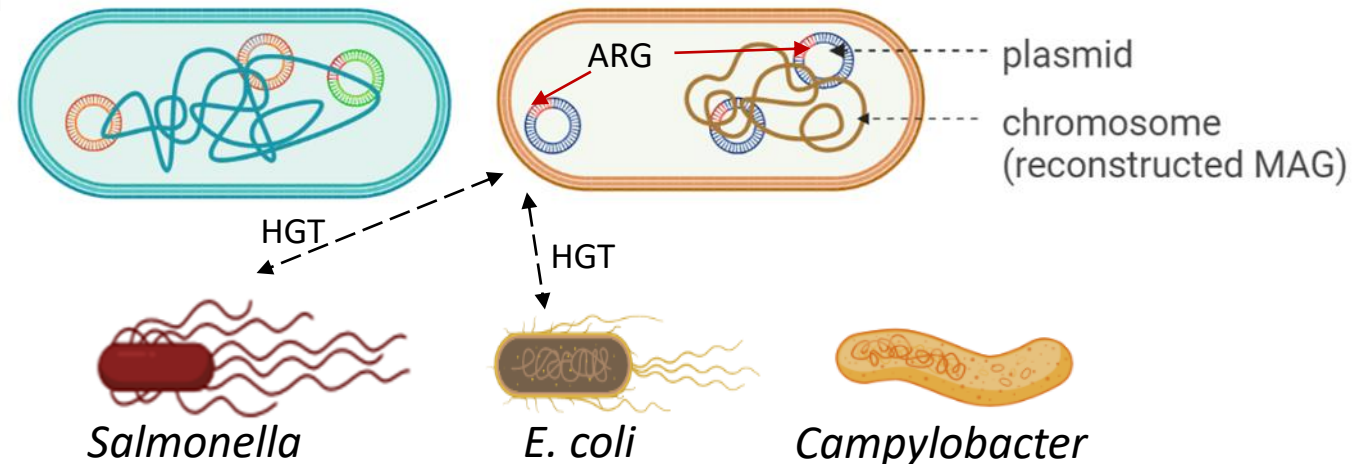
The use of Nanopore sequencing to identify plasmid-borne and chromosomally integrated antimicrobial resistance genes from chicken microbiomes in Gujarat

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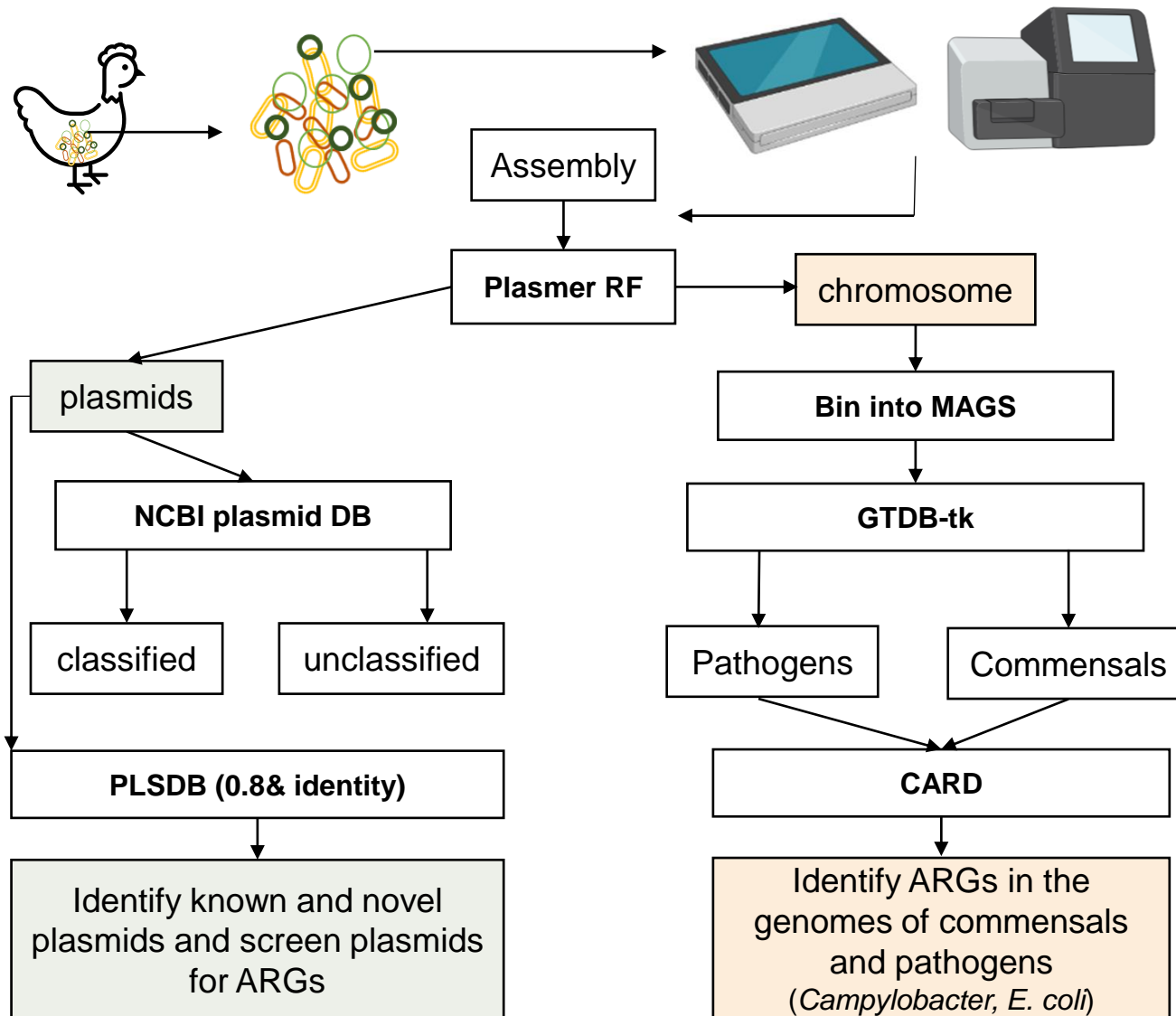
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Key Question: Are there ARGs in plasmids and zoonotic pathogens?

- Antimicrobial resistance genes (ARGs) can be present on plasmids or integrated into the bacterial chromosome.
- ARGs can be transferred from commensal bacteria to pathogens via horizontal gene transfer (HGT).
- The presence of ARGs in zoonotic pathogens is of particular concern.
- We can identify plasmids and construct metagenome-assembled genomes (MAGs) from Nanopore shotgun metagenomes to look for ARGs.



Methods



Results

A total of 3881 putative plasmids were detected, of which 479 had hits to known plasmids in the NCBI Plasmid Database (PLSDB), suggesting significant plasmid novelty in these samples.



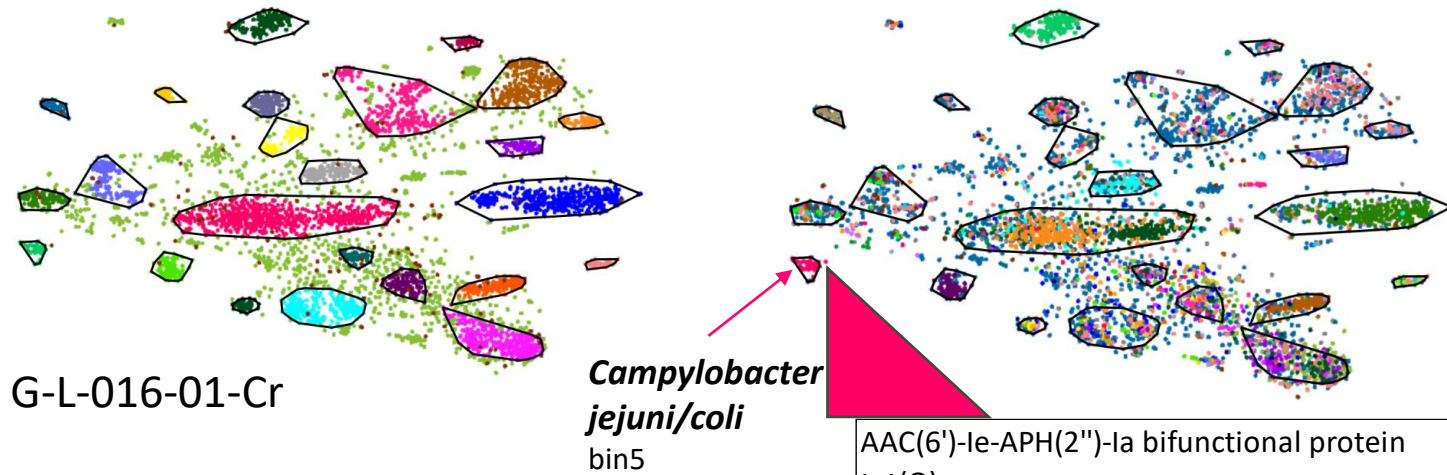
Global distribution of plasmid sources (based on PLSDB)

Table of ARGs detected in plasmids

Gene symbol	Sequence name
aadA1	ANT(3'')-Ia family aminoglycoside nucleotidyltransferase AadA1
aph(3')-Ia	aminoglycoside O-phosphotransferase APH(3')-Ia
qnrS	QnrS family quinolone resistance pentapeptide repeat protein
tet(A)	tetracycline efflux MFS transporter Tet(A)
aph(2'')-Ig	aminoglycoside O-phosphotransferase APH(2'')-Ig
lnu(C)	lincosamide nucleotidyltransferase Lnu(C)
aadA2	ANT(3'')-Ia family aminoglycoside nucleotidyltransferase AadA2
sul3	sulfonamide-resistant dihydropteroate synthase Sul3
dfrD	trimethoprim-resistant dihydrofolate reductase DfrD
tet(W)	tetracycline resistance ribosomal protection protein Tet(W)
vat(E)	streptogramin A O-acetyltransferase Vat(E)
blaTEM	TEM family class A beta-lactamase
sat2	streptothricin N-acetyltransferase Sat2
aac(6')-Ie/aph(2'')-Ia	bifunctional aminoglycoside N-acetyltransferase AAC(6')-Ie/aminogl
erm(B)	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(B)

Results

Contigs were binned into MAGs and taxonomically identified.



G-L-016-01-Cr

Campylobacter jejuni/coli
bin5

Escherichia coli
bin2

- Escherichia coli* EF-Tu mutants conferring resistance to Pulvomycin
- Escherichia coli* gyrA conferring resistance to fluoroquinolones
- Escherichia coli* soxR with mutation conferring antibiotic resistance
- Escherichia coli* soxS with mutation conferring antibiotic resistance

- Klebsiella pneumoniae* KpnE
- acrD
- AcrE
- AcrF
- APH(3')-Ia
- bacA
- emrK
- emrY
- eptA
- eptB
- evgA
- evgS
- gadW
- gadX
- H-NS
- kdpE
- leuO
- marA
- mdtE
- mdtF
- mdtG
- mdtH
- mdtM
- mdtO
- mdtP
- poxtA
- qacJ
- QnrS15
- rsmA
- tet(A)
- tet(C)
- ToIC
- ugd
- YojI

Pathogenic MAGs were screened for ARGs



- AAC(6')-Ie-APH(2'')-Ia bifunctional protein
- tet(O)
- OXA-580
- OXA-610
- cmeC
- cmeB
- cmeA
- cmeR
- vanW gene in vanI cluster

Discussion

Using Nanopore sequencing we were able to identify over 3000 complete circular plasmids in the chicken caecal microbiome, several of which contained ARGs. By assembling the contigs into MAGs, we identified several chromosomally integrated ARGs in *Campylobacter* and *Escherichia coli*. This suggests significant risk to public health from ARGs in chicken from India.