

Characterization of *Escherichia coli* Isolated from Poultry in the Chobe region of Botswana by molecular methods

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Introduction & Background

Botswana is a diverse middle-income country located in a dryland environment in Sub-Saharan Africa. Diarrheal illness incidence is high in rural dryland environments, including the Chobe region of Botswana, where peaks are often associated with high rainfall events. Antibiotic resistance (ABR) is also a growing problem in the region. Contributions of poultry to diarrheal illness and ABR in the region are not well understood.

Purpose: Characterize the virulence potential and ABR of *E. coli* isolated from chicken purchased from local vendors in Botswana using molecular techniques, including whole genome sequencing

Methods

Poultry was purchased from two vendors in Botswana and processed within 4 hrs

Buffered peptone rinse enriched into brilliant green bile broth for 24 hour

Brilliant green bile broth enrichments plated onto Eosin Methylene Blue Agar. Characteristic colonies with a green sheen were selected and streaked for isolation four times.

Isolated colonies Shipped to Virginia Tech

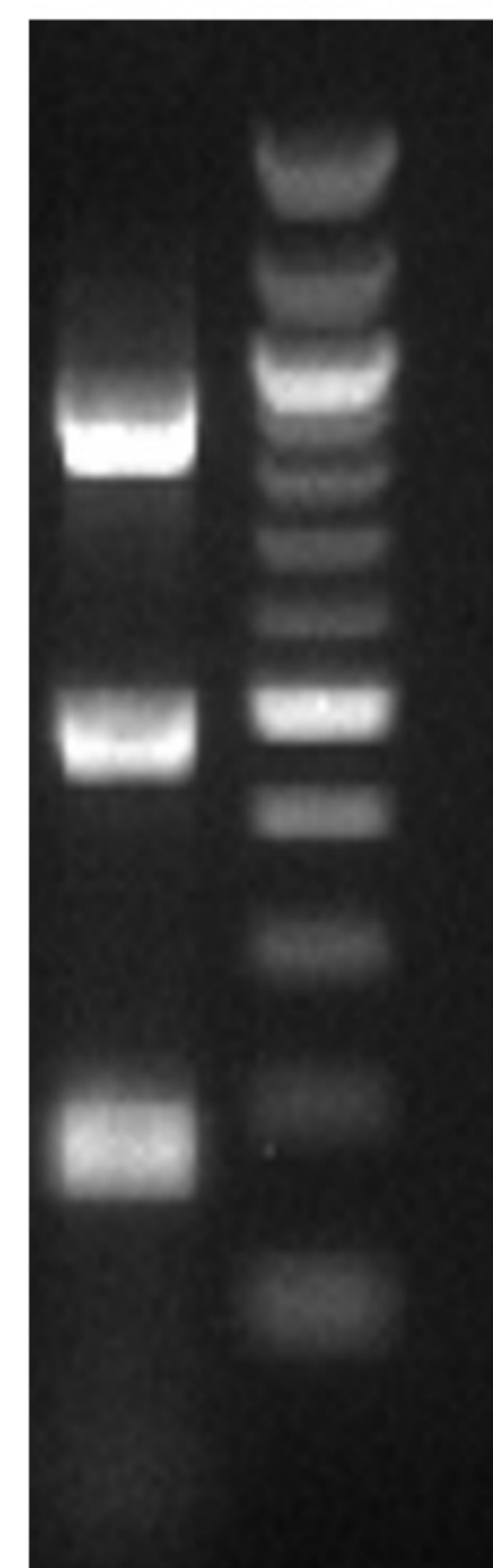
Kirby-Bauer Antibiotic Disc Diffusion

PCR Screening

CLSI methods and Breakpoints: used for screening of *E. coli* isolates.

- Ampicillin
- Amoxicillin
- Azithromycin
- Chloramphenicol
- Ciprofloxacin
- Cefotaxime
- Doxycycline
- Gentamicin
- Meropenem
- Streptomycin
- Tetracycline
- Trimethoprim/Sulfamethoxazole

E. coli genus 903bp
Eae EHEC 482bp
Est1b ETEC 171bp



- DNA extracted using Biobasic kit.
- A mPCR protocol was developed for *E. coli* for detection of *E. coli* genus (*phoA*), *eae* present in all EPEC, EHEC, and *est1b* present in ETEC..

DNA from ABR *E. coli* Shipped to University of Regina for Whole Genome Sequencing via Illumina MiSeq. WGS analysis using BV-BRC.

References

Clinical and Laboratory Standards Institute. M100 Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Clinical and Laboratory Standards Institute. Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2018.

Results and Discussion

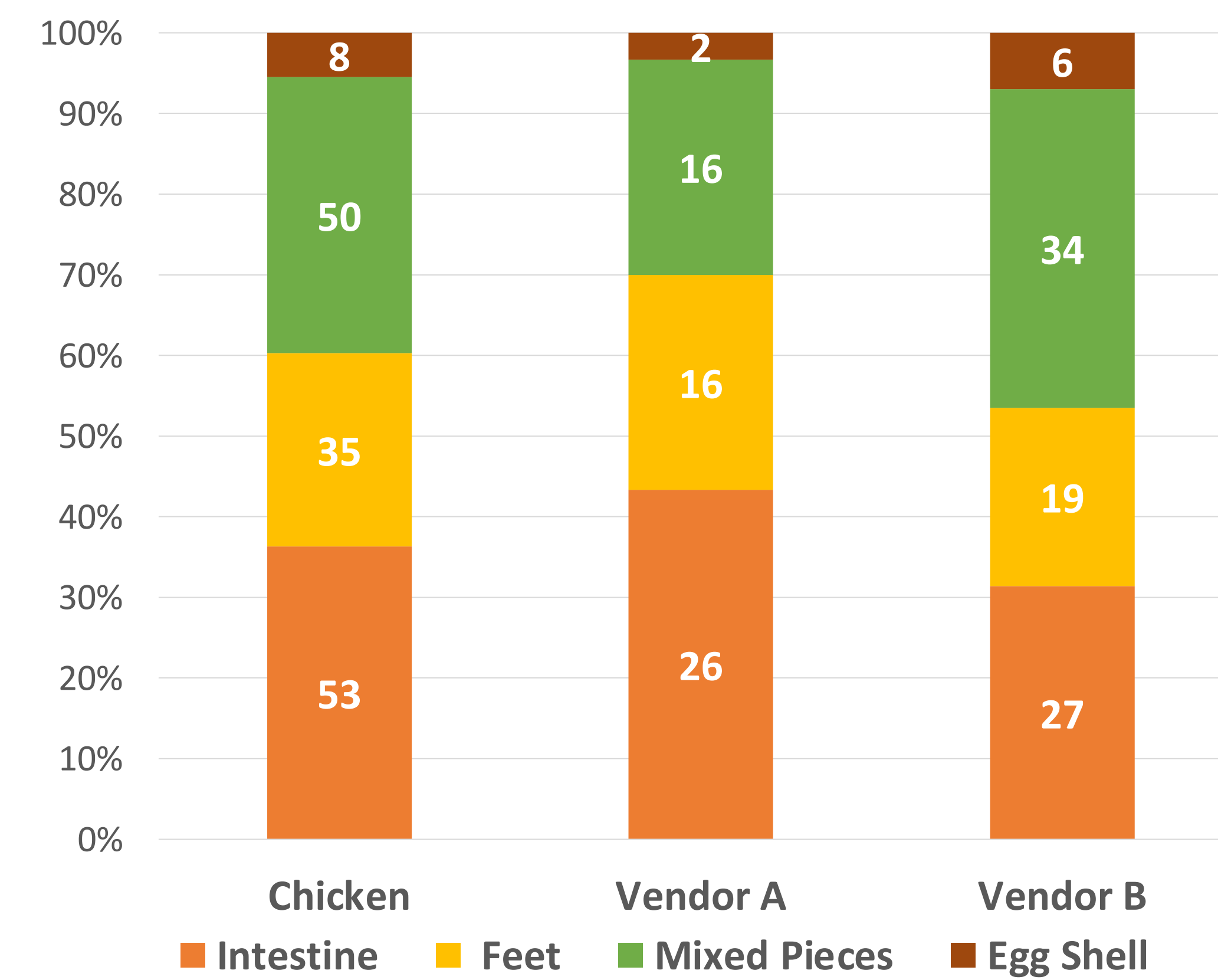
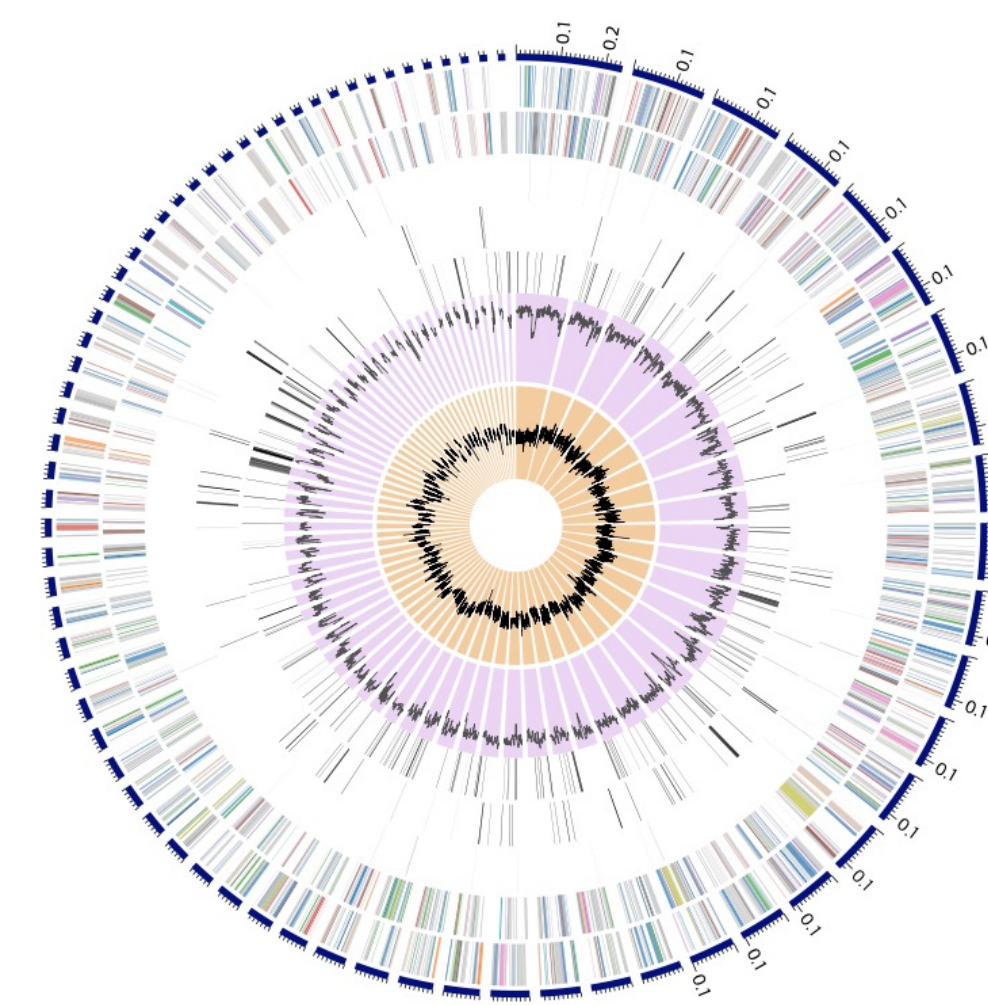


Figure 1: Distribution of *E. coli* isolated from different parts from different and vendors. Number is *phoA* confirmed isolates per category. Y-axis shows the percentage of isolates per category.

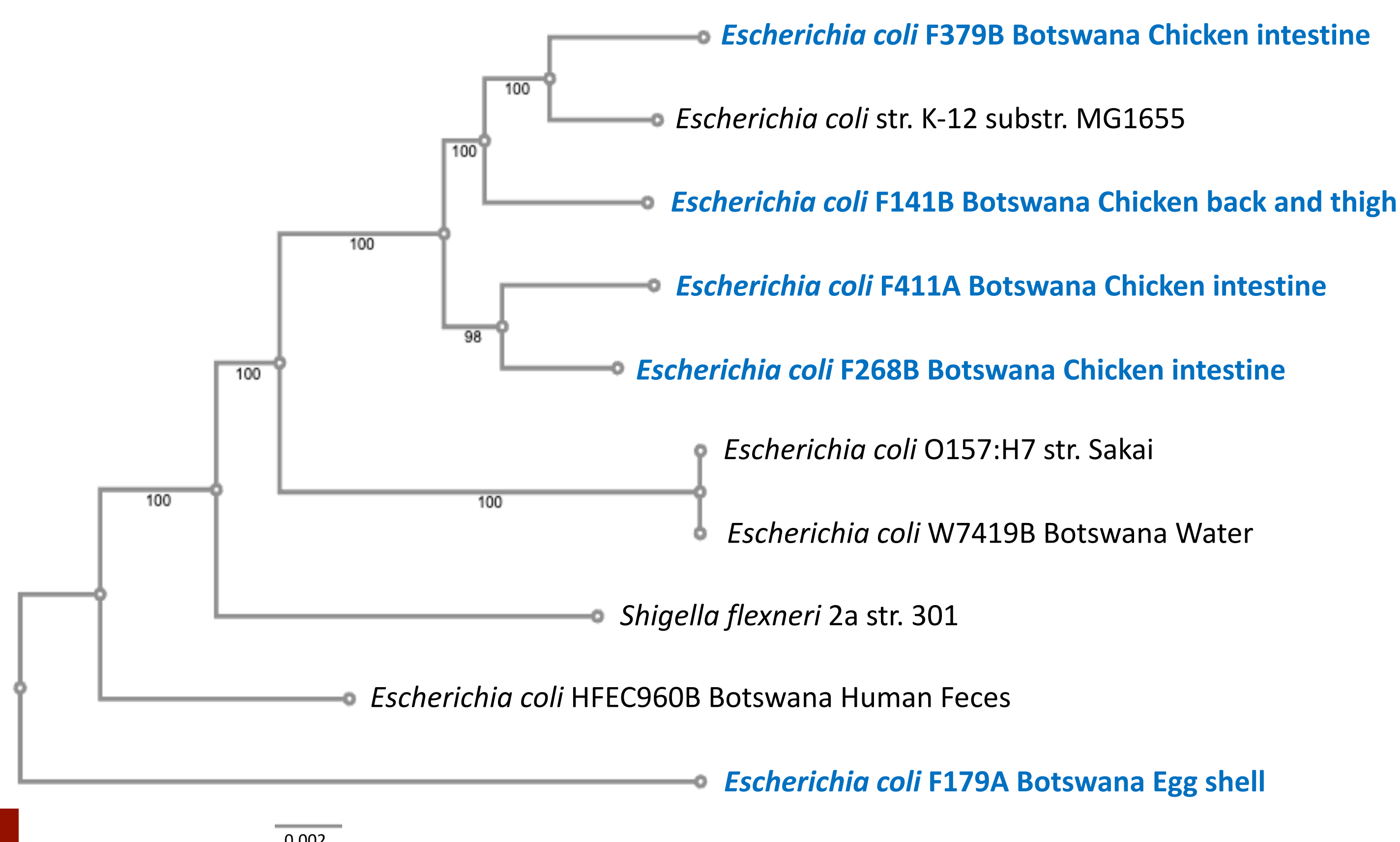
- *E. coli* was isolated from 62% (38/61) of chicken samples. (Fig 1.)
- Only one sample contained *E. coli* that were potentially diarrheagenic via amplification of *eae*.



Class	Virulence Factor Genes	Function
Colonization	<i>eae</i>	attaching and effacing of enterotype
	<i>tir</i>	translocated intimin receptor
	<i>liiA</i>	initial attachment to the enterotype
	<i>fimH</i>	type I fimbriae
Fitness	<i>sdhA</i>	quorum sensing signaling
	<i>fyuA</i>	ferric yersiniabactin uptake
	<i>espA</i>	translocator structures of T3SS, <i>E. coli</i> common pili
Effectors	<i>espB</i>	translocator structures of T3SS, phagocytosis
	<i>espD</i>	translocator structures of T3SS
	<i>espF</i>	mitochondrial death, tight junction disruption, immune evasion, host cell death
	<i>ipaH</i>	phagocytosis inhibition

Figure 3: Circular genome visualization of the genome annotations in F411A (potential diarrheagenic *E. coli*).

- A total of 270 virulence factor genes were annotated in the genome of F411A strain including genes conferring functions related to colonization, fitness, and effector proteins suggesting it is Enteropathogenic.



- Multidrug-resistant *E. coli* isolates from chicken were sequenced and compared to common type strains and strains isolated from water and human in Botswana.

Figure 3: Phylogenetic tree of multidrug-resistant *E. coli* genomes.

Percent Resistance of Chicken Isolates by Antibiotic Type and Part

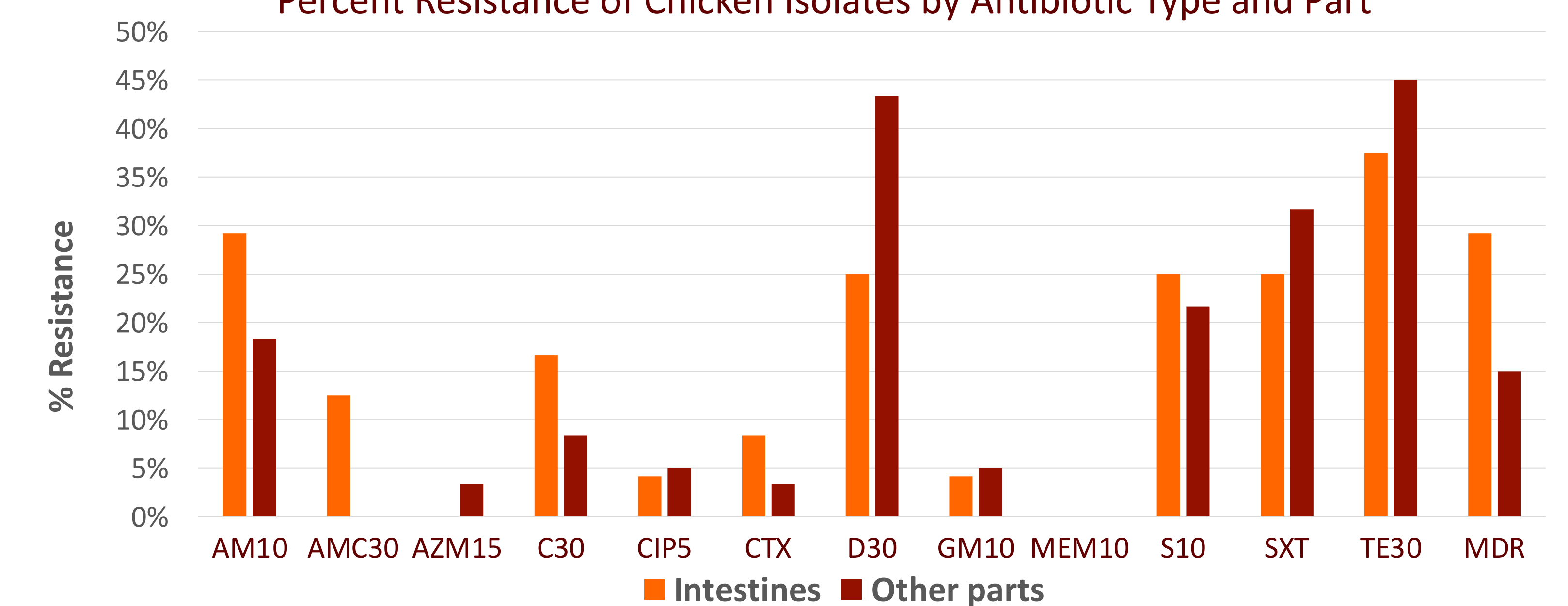


Figure 2: Results of antibiotic resistance testing for chicken isolates tested. Two isolates per sample tested. Number of isolates characterized by their zones of inhibition as resistant in the form of percent resistance from the total.

- Resistance to antibiotics (Figure 2)
 - Resistance to antibiotics more common in *E. coli* isolates from intestines
 - Resistance most common to tetracycline, doxycycline, ampicillin and trimethoprim/sulfamethoxazole
- A total of 36 isolates showed multidrug resistance
- Multidrug-resistant *E. coli* isolate = resistance shown against at least 3 different antibiotics were more commonly isolated from chicken intestines, but the feet were also frequent sources (15%).

Antimicrobial Resistance Genes

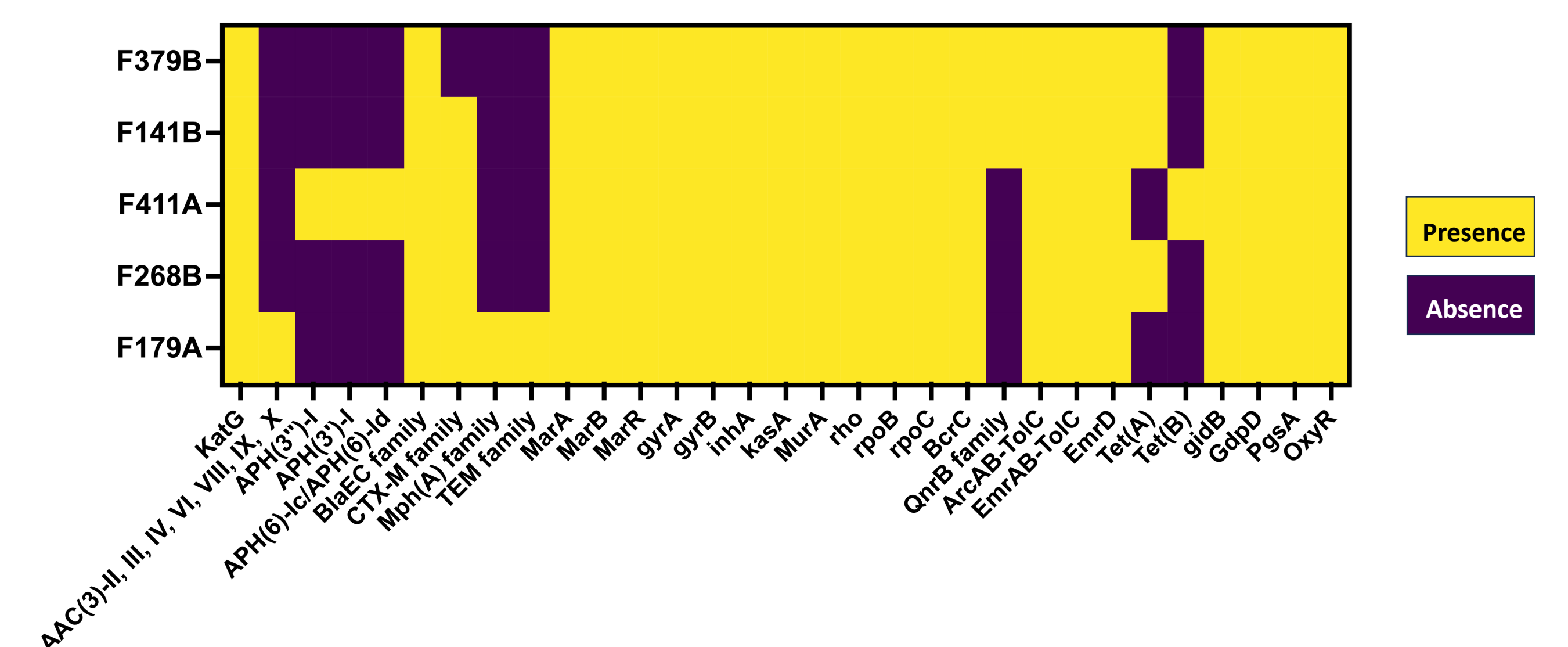


Figure 4: Antimicrobial resistance genes annotated in the genomes of multidrug-resistant isolates from chicken.

- A total of 88 antimicrobial resistance genes, including those conferring resistance to aminoglycosides, β -lactams, and tetracyclines, were annotated in the genome of F411A strain.
- Non-pathogenic *E. coli* strains also possessed a similar number of antimicrobial resistance genes as F411A. In particular, quinolone resistance genes were annotated in the F379B and F141B.

Table 1: Antimicrobial resistance genes annotated in the genomes and corresponding AMR mechanisms

AMR Mechanisms	Genes
Antibiotic activation enzyme	KatG
Antibiotic inactivation enzyme	APH(3)-I, APH(3)-I, APH(6)-Ic/APH(6)-Ic, AAC(3)-II, III, IV, VI, VII, IX, X, BlaEC family, CTX-M family, Mph(A) family, TEM family
Antibiotic resistance gene cluster, cassette, or operon	MarA, MarB, MarR
Antibiotic target in susceptible species	gyrA, gyrB, inhA, kasA, MurA, rho, rpoB, rpoC
Antibiotic target protection protein	BorC, QnrB family
Efflux pump conferring antibiotic resistance	AcrAB-TolC, AcrAD-TolC, EmrAB-TolC, EmrD
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA
Regulator modulating expression of antibiotic resistance genes	AcrAB-TolC, EmrAB-TolC, OxyR

Summary

- *E. coli* were frequently present in chicken available for sale in the Chobe region of Botswana.
- The isolates were similar but belonged to different MLST clades indicating multiple sources of contamination.
- Future studies will compare isolates to human and wildlife isolates to determine contributions of chicken to circulation of ABR *E. coli* in the Chobe region of Botswana.
- The emergence of multi-drug resistant *E. coli* isolates, posing a significant public health concern, underscores the need for continuous surveillance in poultry industry in Botswana.

Acknowledgements

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