

Comprehensive whole genomic analysis of *Escherichia coli* and *Salmonella* spp. isolates in neonatal diarrhea among calves from Latin American Industrial Dairy Farms

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INTRODUCTION

Neonatal calf diarrhea is a multifactorial enteric disease and one of the major causes of economic losses during the rearing stage, being responsible for around 57% of the mortality of pre-weaning calves. Most frequent bacteria are *Escherichia coli* and *Salmonella* spp. In Latin American Industrial Dairy Farms 20% of calves die from diarrhea caused by these pathogens. According to local diagnostics, 67% of bacteria are multi-resistant to antibiotics.

OBJECTIVE

To characterize through Whole Genome Sequencing (WGS) the serotypes, virulence factors and antimicrobial resistance mechanisms of a collection of *E. coli* and *Salmonella* spp. isolates obtained from neonatal diarrhea samples from dairy calves in Latin American dairy farms.

MATERIALS AND METHODS

- ▶ More than 90 dairy farms from productive regions of Argentina, Brazil, Chile, and Mexico were enrolled for the study.
- ▶ Rectal swabs were collected from calves (n=722) aging up to 4-weeks old and with presence of diarrhea (fecal consistency score 2 or 3). Swabs were enriched, and the presence of *E. coli* pathotypes was determined by qPCR. In parallel, enrichments were inoculated in *Salmonella* plus CHROM - agar to isolate *Salmonella* spp. Colonies were reisolated three consecutive times and subsequently tested by qPCR.
- ▶ DNA from *Salmonella* spp. and *E. coli* strains was extracted, libraries prepared, and sequencing performed through Illumina platform.
- ▶ Genomic sequences were then analyzed using a custom workflow pipeline developed at PhageLab SpA. The resulting genomes were subjected to gene annotation, identification of potential virulence factors, and antibiotic/disinfectants resistance genes.

There is a high *E. coli* diversity (20-49 serovars) and low *Salmonella* spp. prevalence (9%) across the different dairy farms in Latin America. All isolates had pathogenic mechanisms to generate diarrhea in calves.

86% of *E. coli* isolates and 100% of *Salmonella* isolates were resistant or multi-resistant to antimicrobials.



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RESULTS

- ▶ 604 samples were obtained from dairy calves with symptoms of diarrhea (table 1). From those samples:
- ▶ 160 were obtained from Argentina, 199 from Brazil, 71 from Chile and 174 from Mexico.
- ▶ Table 1 describes the isolation process and selection for WGS.
- ▶ In all the countries, the frequency of *E. coli* was very high in comparison to *Salmonella* spp. (figure 1).

E. coli

- ▶ 99 different serotypes were identified in the 236 *E. coli* isolates (figure 2): Argentina (49), Brazil (48), Chile (20) and Mexico (20).
- ▶ At least 12 pathogenicity islands (PAIs) were identified among all the *E. coli* isolates.
- ▶ All the *E. coli* isolates have genes related to antimicrobial resistance.

Salmonella spp

- ▶ Given that the prevalence of *Salmonella* spp. was small (9% of the total), the number of different identified serovars was seven. Typhimurium (n=9) and Newport (n=7) were the most frequent serovars founded (figure 5).
- ▶ At least 13 *Salmonella* Pathogenicity Islands (SPIs) were identified among all the *Salmonella* spp. isolates (figure 6).
- ▶ All the *Salmonella* isolates have genes related to antimicrobial resistance.

TABLE 1. Isolation process and selection for WGS

Bacteria	Country	First Screening	Isolated colonies	Selected and sequenced colonies	QC pass samples
<i>E. coli</i>	Argentina	85	850	100	86
	Brazil	61	599	75	71
	Chile	27	270	43	39
	Mexico	39	390	53	40
<i>Salmonella</i> spp.	Argentina	55	22	6	5
	Brazil	65	15	4	3
	Chile	46	25	6	6
	Mexico	55	19	10	9
TOTAL					259

FIGURE 1. Distribution of sequenced isolates per origin.

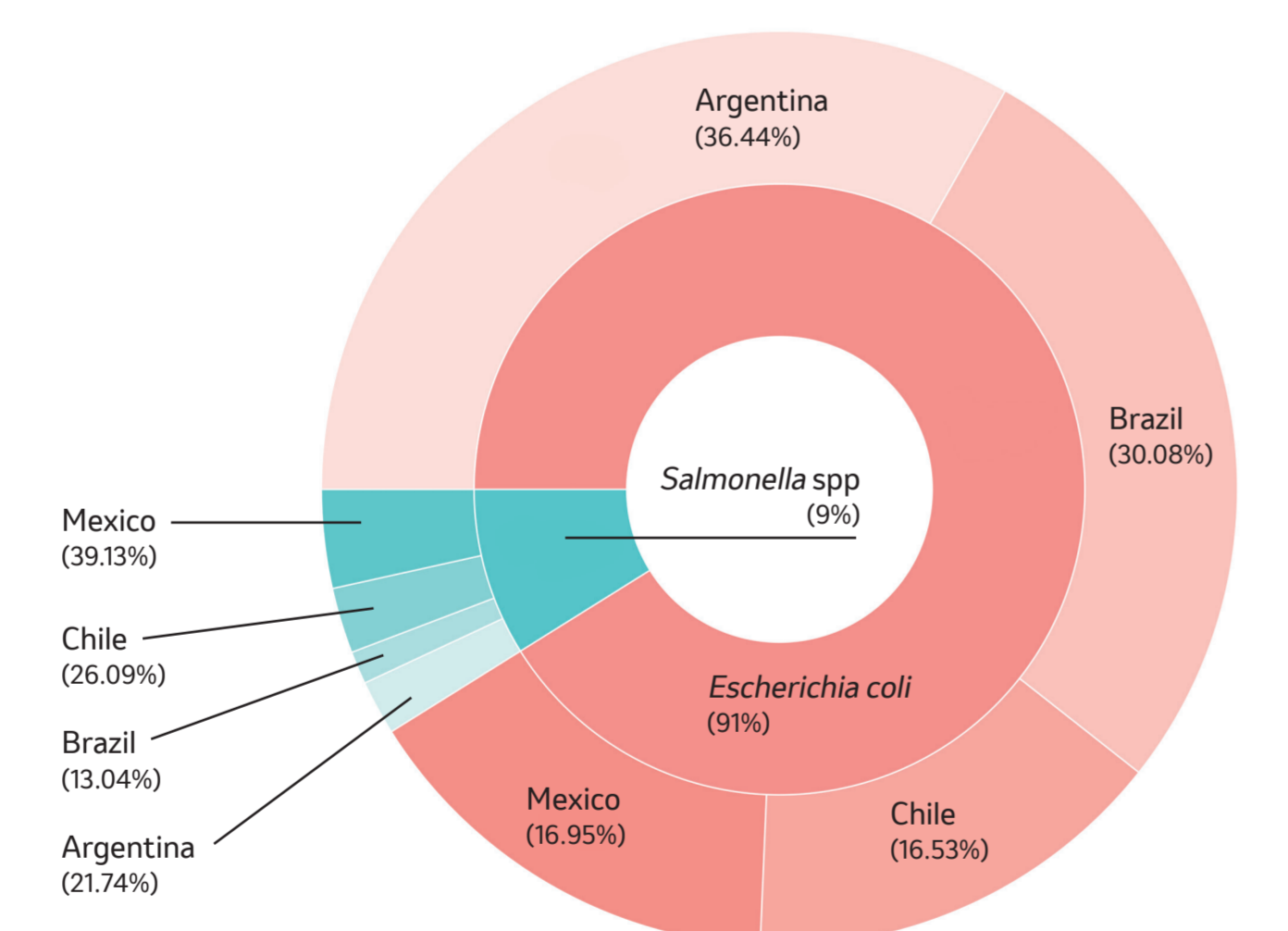


FIGURE 2. Distribution of identified serotypes per origin.

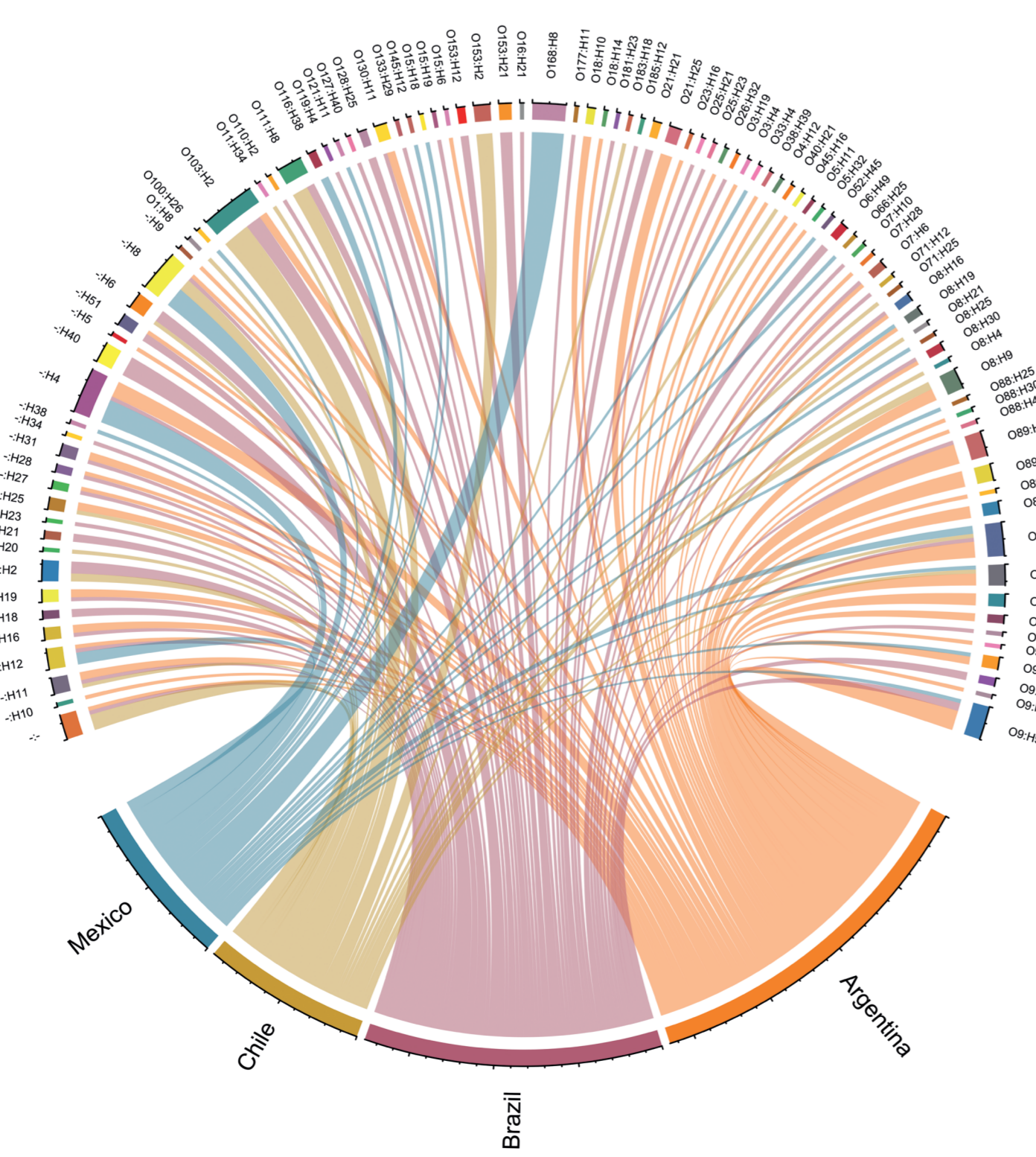


FIGURE 3. Presence and absence of PAIs among all *E. coli* isolates.

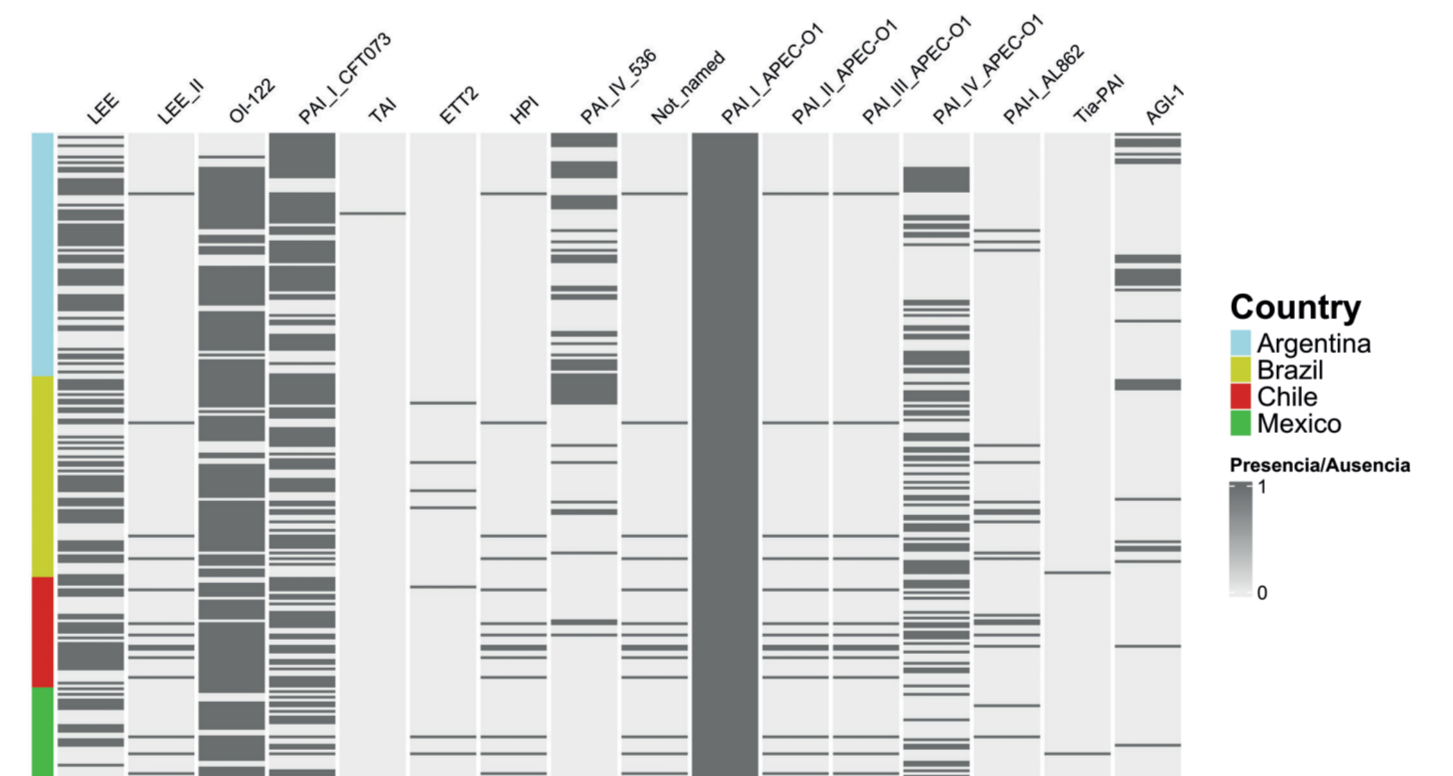


FIGURE 4. Presence of families of antimicrobial resistances genes among all *E. coli* isolates. FPA: Folate Pathway Antagonist. QAC: Quaternary Ammonium compounds.

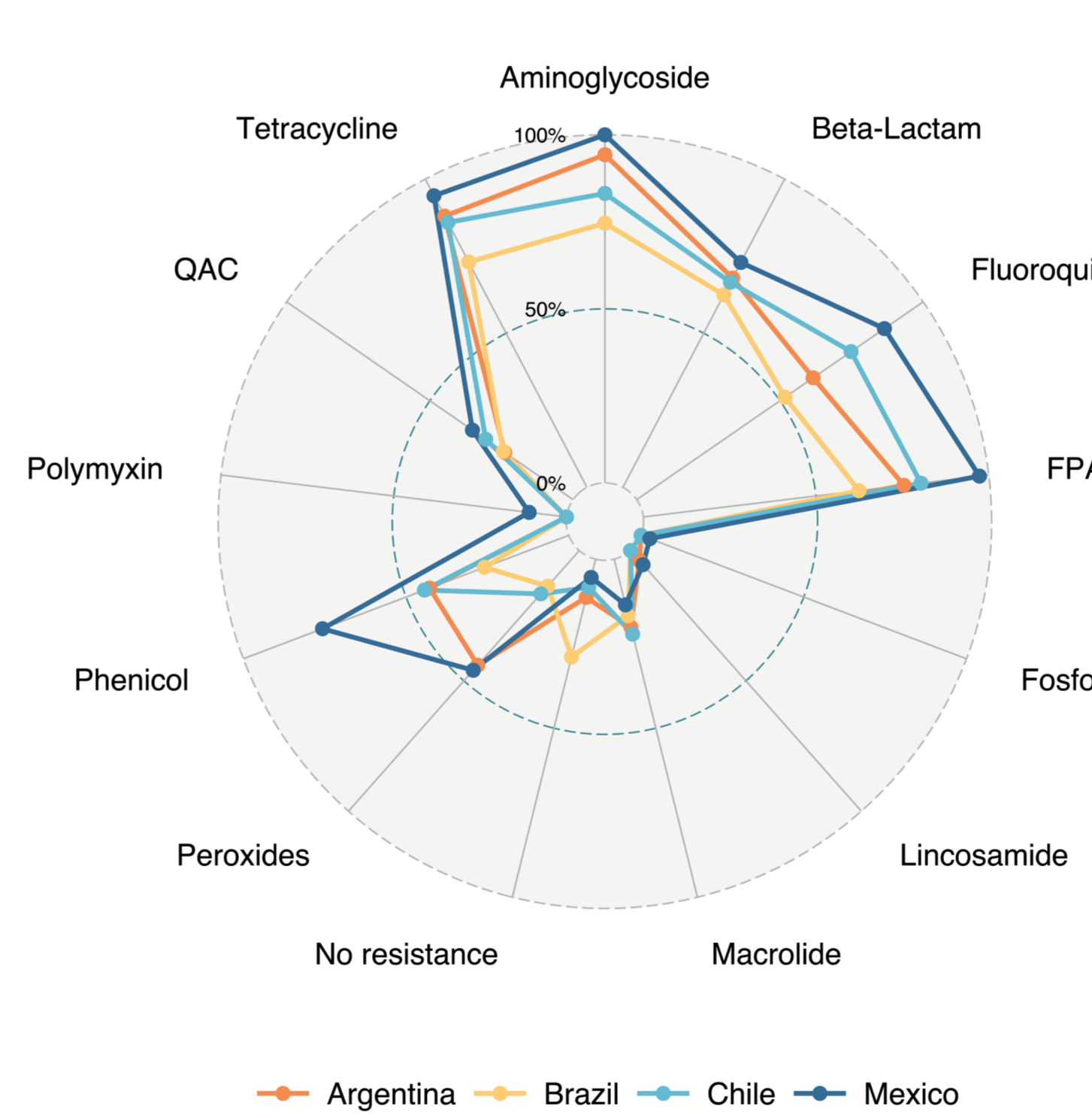


FIGURE 5. Distribution of serovars identified per origin.

Country	Cerro	Dublin	1,1,4,[5],12:d-	Newport	Senftenberg	Typhimurium	Urbana
Argentina	2	1	0	0	0	2	0
Brazil	0	1	1	0	0	1	0
Chile	0	0	0	0	0	6	0
Mexico	0	0	0	7	1	0	1

FIGURE 6. Presence and absence of SPIs among all *Salmonella* isolates.

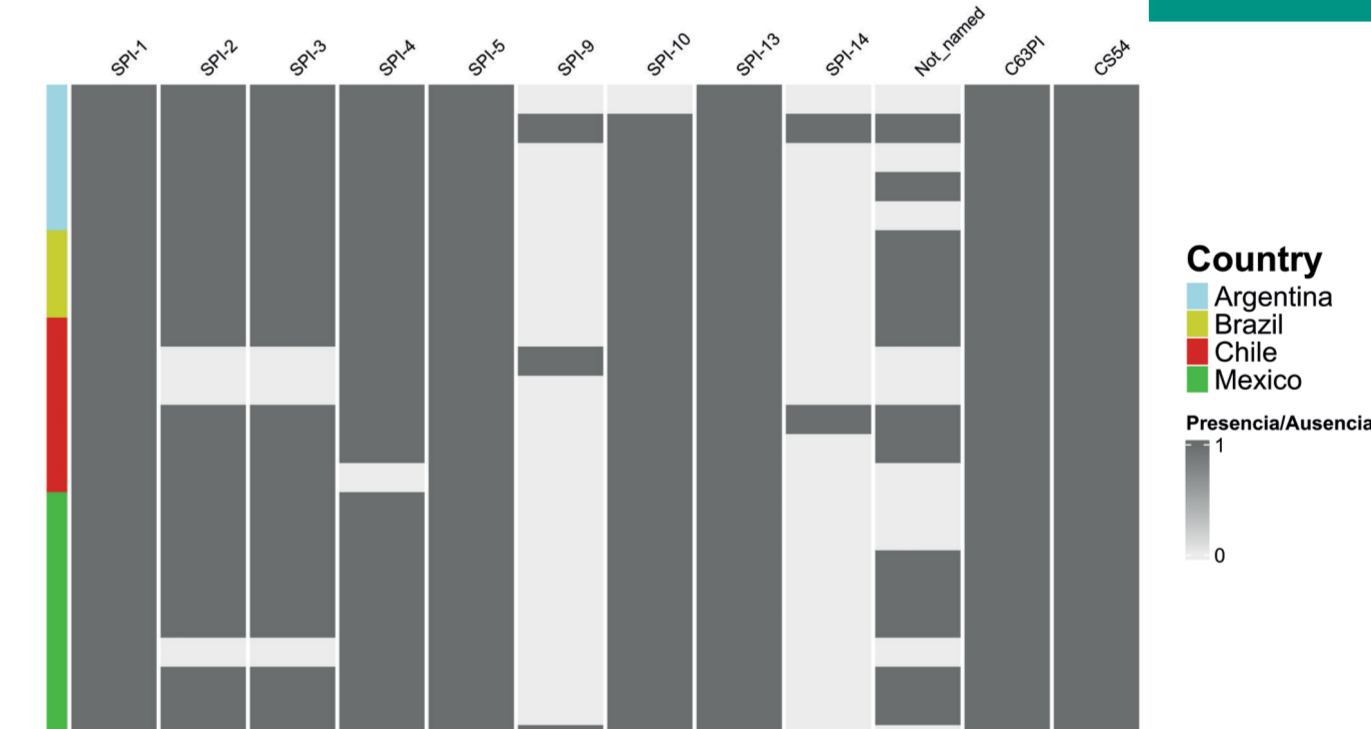
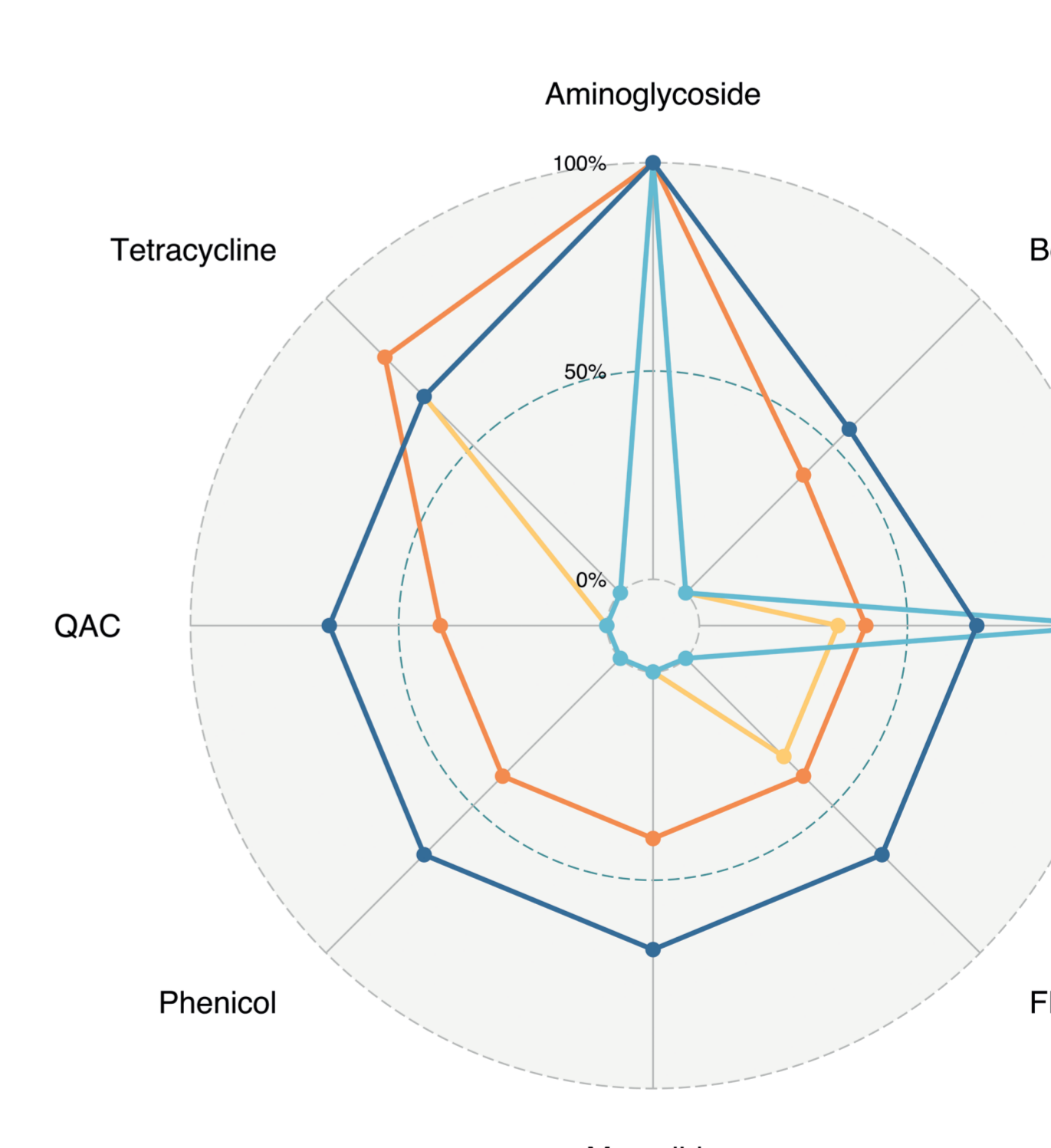


FIGURE 7. Presence of families of antimicrobial resistances genes among all *Salmonella* spp. isolates. FPA: Folate Pathway Antagonist. QAC: Quaternary Ammonium compounds.



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Abstract number: 1448, May 22nd