Detection of Antibiotic Resistance and Virulence Genes in *Salmonella* Strains Isolated From Retail in Chile.

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Abstract

Salmonella enterica is a major cause of foodborne illness, resulting in over 90 million cases and 150,000 deaths annually worldwide. Found in water and foods like meat and milk, it generally causes gastroenteritis, requiring antibiotic treatment only in severe cases. However, increasing antibiotic resistance in this species presents a significant public health challenge globally.

Objective: To genetically characterize *Salmonella* strains isolated from retail food in Ñuble and Maule regions of Chile.

Methodology: A total of 225 raw chicken meat samples (175 from Maule and 50 from Ñuble) were analyzed under the *Salmonella* surveillance program using sampling criteria n=5, c=0. Isolates were identified by the VIDAS system, confirmed by MALDI-TOF, and sequenced on the MiSeq. Classic MLST, core genome MLST, and *Salmonella* In Silico Typing Resource (SISTR v1.1.3) were performed. Antibiotic resistance genes (ARG) were detected using AMRFinderPlus and virulence genes (VG) by AMRfinderPlus templates (MBioSEQ Ridom Typer v11.1).

Results: Nine *Salmonella* strains were isolated, of which eight were *S*. Infantis ST32 (CC31; 6,7,14: r:1,5) and one was *S.* Bredeney ST897 (CC33; 1,4,12,27: l, v:1,7). Three clusters of closely related strains were observed with 0- 2 allele differences, and three unrelated strains were identified. IncFIB plasmids were common in *S*. Infantis and Col_rep_cluster in *S.* Bredeney. *S*. Infantis strains presented the ARG *aac(3)-IVa, aph (3')-Ia, aph(4)-Ia, aadA1, mdsA/mdsB, fosA3, floR, qacEdelta1, gyrA_D87Y, sul1, tet(A) and dfrA14. bla* $_{CTX\text{-}M\text{-}65}$ (ESBL) was detected in five plasmids IncFIB (p310.8_510443-25; p311.3_510444-25; p313.5_510447-25; p314.1_510448-25 and p311.8_513238-25). In *S.* Bredeney, the ARG detected were *aac(3)-IId, aph(3')-Ia, aadA2/aph(3'')- Ib/aph(6)-Id, blaTEM-1, mdsA/mdsB, lnu(G), floR, qnrB19, sul2, tet(A)/tet(B)*, and *dfrA12*. A total 147 VG were detected, highlighting exotoxin, adherence, and effector delivery systems.

Conclusion: The analyzed *Salmonella* strains exhibited various ARG and VG, underscoring the need for continuous genomic surveillance to mitigate public health risks in Chile.

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Introduction

Salmonella enterica is a leading cause of foodborne illness worldwide, responsible for over 90 million cases and 150,000 deaths annually. Commonly transmitted through contaminated food products such as poultry and dairy, it typically causes self-limiting gastroenteritis. Because of this the global rise in antibiotic-resistant *Salmonella* strains represents a growing public health concern.

In Chile incidence of non-typhoidal *Salmonella* invasive disease is relatively low, yet it represents about 45% of foodborne illness.

During 2025 more than 16 salmonelosis outbreaks were reported between Maule and Ñuble regions.

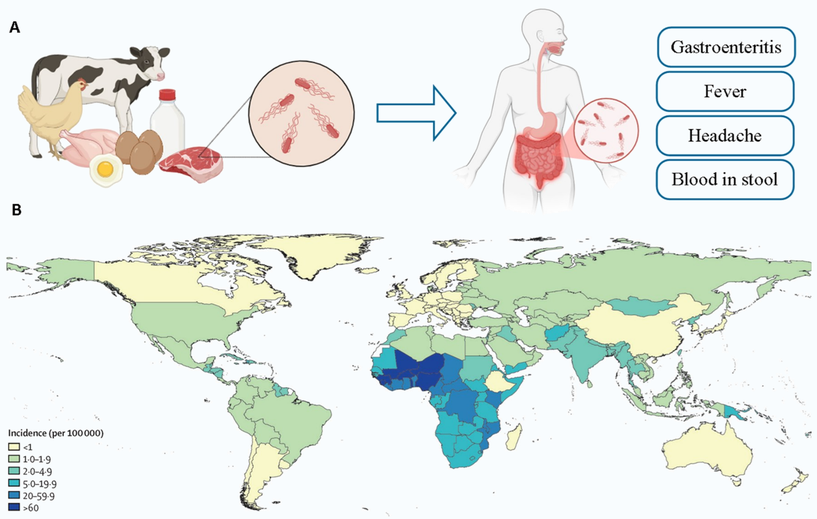

***Figure 1**. **A** Salmonella infection and salmonelosis main simptoms. **B** Non-typhoidal salmonella invasive disease incidence rate (per 100.000) by country in 2017 (modified from Jeffrey D. et al, 2017)*

Materials & Methods

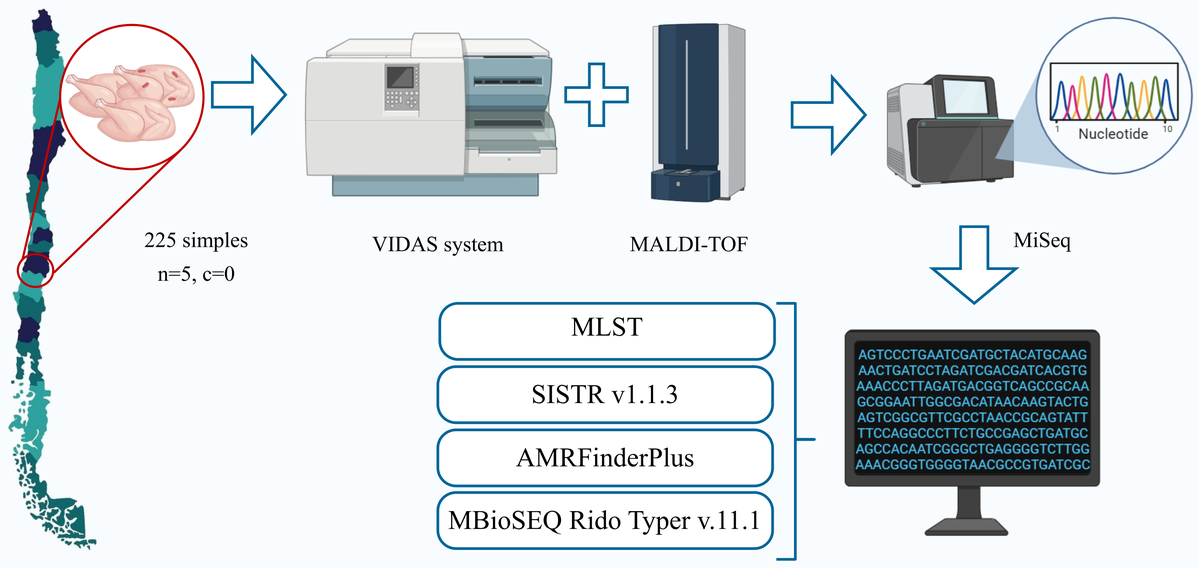

***Figure 2.** Graphical methodology. 225 Samples of chicken meat were obtained from Maule and Ñuble retail stores. Samples were anayzed under Salmonella surveillance program, using sample criteria n=5, c=0, meaning five samples of each lot were tested, and if one of the samples is contaminated, the whole lot is rejected. Bacteria isolated was identified using VIDAS system and confirmed by MALDI-TOF. Bacteria were then sequenced and bioinformatic analyses were performed. Strains were characterized by MLST and typed and subtyped by Salmonella In Silico Typing Resource (SISTR v1.1.3). Antibiotic resistance genes (ARG) were detected by AMRFinderPlus and virulence genes (VR) were identified by AMRfinderPlus templates (MBioSEQ Rido Typer v11.1).*

Results

Nine *Salmonella* strains were isolated, of which eight were *S.* Infantis ST32 (CC31; 6,7,14: r:1,5) and one was *S.* Bredeney ST897 (CC33; 1,4,12,27: l, v:1,7). Three clusters of closely related strains were observed with 0-2 allele differences, and three unrelated strains were identified.

***Table 1**. Characterization of Salmonella strains according to origin and sample type*

Sample ID	ST	Complex Type	CC	Country of Isolation	Region	Source	Serovar	Antigenic Formula
510442-25	897	27992	33	Chile	Maule	Raw chicken meat	Bredeney	1,4,12,27:l,v:1,7
510443-25	32	27993	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
510444-25	32	27993	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
510445-25	32	27994	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
510446-25	32	27994	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
510447-25	32	2966	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
510448-25	32	2966	31	Chile	Maule	Raw chicken meat	Infantis	6,7,14:r:1,5
513239-25	32	29861	31	Chile	Ñuble	Raw chicken meat	Infantis	6,7,14:r:1,5
513238-25	32	29860	31	Chile	Ñuble	Raw chicken meat	Infantis	6,7,14:r:1,5

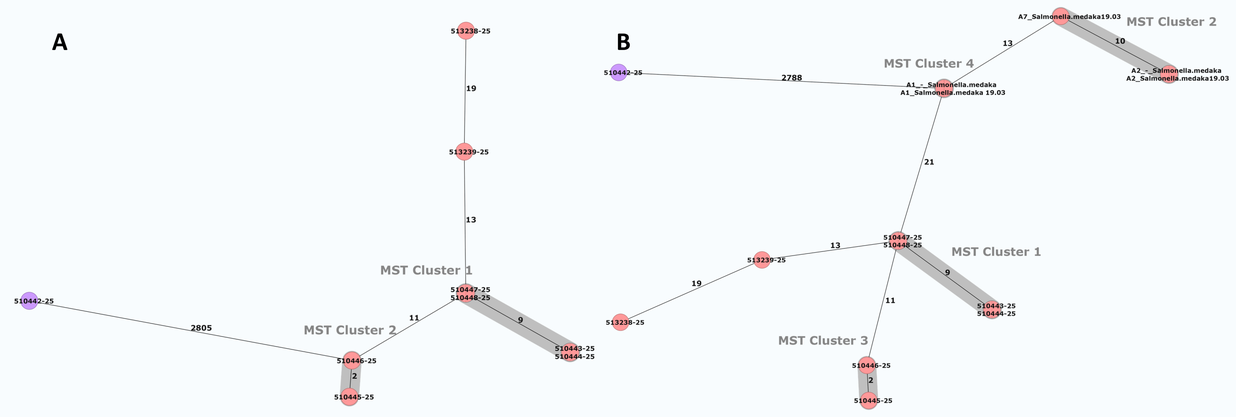

***Figure 3. A.** Minimum spanning tree (MST) of the nine isolates analyzed in this study. **B.** MST including an additional 11 clinical Salmonella strains. Isolates are shown as colored circles according to their sequence types (STs) defined by the seven-locus MLST scheme. Black numbers on the connecting lines indicate the number of allelic differences between isolates based on the cgMLST scheme, which comprises 3,002 target genes for Salmonella. Isolates within a cluster threshold of ≤10 alleles are shaded grey to indicate clusters.*

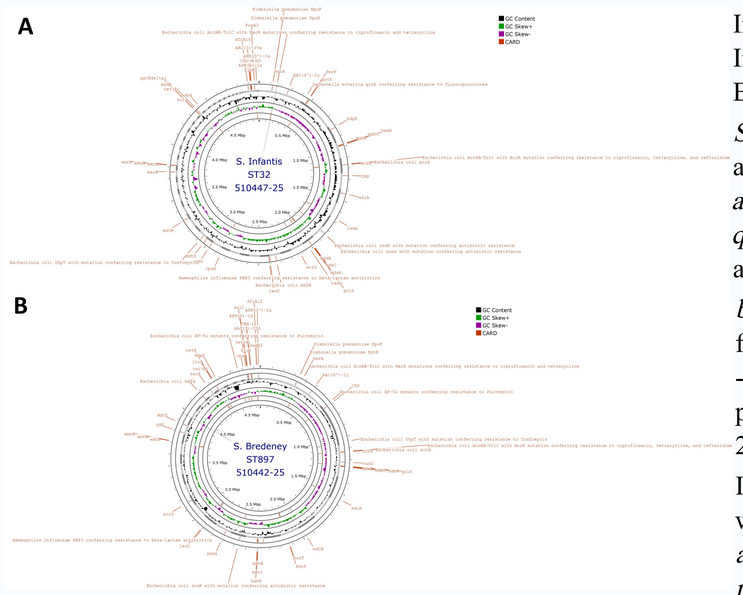

***Figure 4**. Circular genoma of **A** .S. infantis **B**. S. bredeney. The rings represent the coding genes, the external anotation show antibiotic resistance genes and mutations.*

IncFIB plasmids were common in *S.* Infantis and Col_rep_cluster in S. Bredeney.

S. Infantis strains presented the ARG *aac(3)-IVa, aph(3')-Ia, aph(4)-Ia, aadA1, mdsA/mdsB, fosA3, floR, qacEdelta1, gyrA_D87Y, sul1, tet(A)* and *dfrA14.*

bla $_{CTX\text{-}M\text{-}65}$ (ESBL) was detected in five plasmids IncFIB (p310.8_510443-25; p311.3_510444-25; p313.5_510447-25; p314.1_510448-25 and p311.8_513238-25).

In *S.* Bredeney, the ARG detected were *aac(3)-IId, aph(3')-Ia, aadA2/aph(3'')- Ib/aph(6)-Id, blaTEM-1, mdsA/mdsB, lnu(G), floR, qnrB19, sul2, tet(A)/tet(B),* and *dfrA12.*

A total 147 VG were detected, highlighting exotoxin, adherence, and effector delivery systems.

***Table 2**. Characterization of Salmonella strains according to origin and sample type Presence of antibiotic resistance genes in sequenced Salmonella .*

ID	ST	Serovar	Apramycin	Gentamicin	Hygromycin	Kanamycin	Streptomycin	Tobramycin	Beta-lactam	Cephalosporin	Fosfomycin	Lincosamide	Chloramphenicol	Florfenicol	Quaternary Ammonium	Quinolone	Sulfonamide	Tetracycline	Trimethoprim
510442-25	897	Bredeney	ND	aac(3)-IId	ND	aph(3')-Ia	aadA2 / aph(3'')-Ib / aph(6)-Id	ND	blaTEM-1	ND	ND	lnu(G)	floR	floR		qnrB19	sul2	tet(A) / tet(B)	dfrA12
510443-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	aph(3')-Ia	aadA1	aac(3)-IVa	ND	blaCTX-M-65 (ESBL)	ND	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
510444-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	aph(3')-Ia	aadA1	aac(3)-IVa	ND	blaCTX-M-65 (ESBL)	ND	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
510445-25	32	Infantis	ND	ND	ND	aph(3')-Ia	aadA1	ND	ND	ND	ND	ND	ND	ND	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
510446-25	32	Infantis	ND	ND	ND	ND	aadA1	ND	ND	ND	ND	ND	ND	ND	qacEdelta1	gyrA_D87Y	sul1	tet(A)	ND
510447-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	aph(3')-Ia	aadA1	aac(3)-IVa	ND	blaCTX-M-65 (ESBL)	fosA3	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
510448-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	aph(3')-Ia	aadA1	aac(3)-IVa	ND	blaCTX-M-65 (ESBL)	fosA3	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
513239-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	ND	aadA1	aac(3)-IVa	ND		ND	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14
513238-25	32	Infantis	aac(3)-IVa	aac(3)-IVa	aph(4)-Ia	aph(3')-Ia	aadA1	aac(3)-IVa	ND	blaCTX-M-65 (ESBL)	ND	ND	floR	floR	qacEdelta1	gyrA_D87Y	sul1	tet(A)	dfrA14

ND: Not determinated

Conclusion

The *Salmonella* strains obtained from retail chicken meat exhibited various virulence and antibiotic resistance genes, these finds support the need for continuous genomic surveillance to mitigate public health risks in Chile.

Bibliography

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