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Virulence profiles, antimicrobial resistance, and biofilm formation in *Salmonella enterica* isolated from retail chicken meat in Jalisco, Mexico



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Abstract

Poultry meat is a source of foodborne illnesses caused by *Salmonella*. This study aimed to determine the virulotype, antimicrobial resistance, and biofilm formation of *Salmonella* isolated from raw chicken marketed in Jalisco, Mexico. Virulence genes were analyzed by PCR, and antimicrobial susceptibility was assessed by the Kirby-Bauer method for 195 *Salmonella enterica* isolates; biofilm formation was investigated for three isolates on stainless steel at 9, 14, and 25 °C. All isolates carried a conserved set of chromosomal virulence genes. The *sspH1* gene was detected in 55 % of the isolates, and *sopE* in 23 %; however, *pefA* and *spvC* were absent, suggesting genetic variability in pathogenic potential. The Vb virulotype was more prevalent and associated with supermarket samples and the warm season ($P < 0.05$). Seventy-nine percent of the isolates showed resistance to tetracycline, 78 % to nalidixic acid, 76 % to chloramphenicol, 27 % to third-generation cephalosporins, and 12 % to ciprofloxacin; 79 % were multidrug-resistant. Multidrug resistance was associated with specific virulotypes and with the point of sale. Biofilm formation was dependent on strain and temperature ($P < 0.05$), but not on native microbiota. Chicken meat marketed in supermarkets and markets is a source of *Salmonella* with conserved

chromosomal virulence genes, high multidrug resistance, and resistance to critical antimicrobials. These results are useful for quantitative microbiological risk assessment models that estimate the persistence and exposure associated with the pathogen in poultry products.

Keywords: Virulotype; Antibiotics; Antimicrobial multiresistance; Biofilms; Intraspecies variability; Salmonella

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Introduction

Poultry meat is the most produced globally, with about 40 % of global meat production in 2023¹. Poultry meat consumption has increased in recent decades due to population growth, urbanization, and rising incomes in developing countries, as well as its affordability, low fat content, and few religious and cultural barriers². The poultry industry is of great relevance in Mexico, which, in 2023, ranked as the world's seventh-largest producer of poultry meat, with a production of 3.8 million tonnes and a *per capita* consumption of 37.3 kg³. Production is projected to increase in 2025, driven by higher private investment in the sector and more favorable input prices, which will improve productivity throughout the chain⁴. Despite their economic importance, poultry meat and its derivatives are a major source of foodborne illnesses and vehicles for pathogens such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Staphylococcus aureus*. Poultry is the main reservoir of *Salmonella*, and outbreaks due to consumption of poultry products have increased, as have cases and hospitalizations associated with this pathogen. In the United States, between 1997 and 2023, 217 outbreaks of *Salmonella* due to chicken meat consumption were recorded, with 5,951 cases, 854 hospitalizations, and 6 deaths⁵. In Mexico, there is no official public information on the number and impact of outbreaks associated with *Salmonella* and poultry products, but its high prevalence in chicken meat suggests that it plays a relevant role in the epidemiology of salmonellosis in the country. Morbidity data indicate that, in the last 5 yr (2020-2024), there was an annual average of 42,799 cases of salmonellosis, mainly among people aged 25 to 44 yr and with a greater impact in the warm months⁶; for its part, the prevalence of *Salmonella* in chicken meat ranged from 18 to 63 % in Aguascalientes, Jalisco, Durango, Mexico City, and Querétaro⁷⁻¹⁰. A great diversity of serotypes and antimicrobial resistance profiles has been documented among the isolates; nevertheless, few studies have reported attributes such as virulence determinants and biofilm formation. The purpose of this study was to determine the virulotype, antimicrobial resistance profile, and biofilm formation of *Salmonella* isolates from raw chicken marketed at retail in Jalisco, Mexico.

Material and methods

Salmonella isolates

An analysis was conducted on 195 *Salmonella enterica* isolates recovered in a previous study¹⁰ from raw chicken meat (breast) from retail establishments in three municipalities of Jalisco (Guadalajara, Tlaquepaque, and Zapopan), which represent some of the most densely populated areas of the state. Sampling was conducted from July 2019 to February 2020. *Salmonella* was detected using the molecular detection system (Neogen), and isolation and identification were carried out using the U.S. Food and Drug Administration method¹¹. Of the 195 isolates, 23 (12 %) were obtained from

supermarkets, 60 (31 %) from establishments in a wholesale market, and 112 (57 %) from poulterer's shops; likewise, of the 195 isolates, 125 (64 %) were obtained during the warm season, and 70 (36 %) in the cold season. The virulotype and antimicrobial resistance profile of some of the isolates were reported in a previous study that quantified and evaluated the prevalence of *Salmonella enterica* in raw chicken in Jalisco, Querétaro, and Aguascalientes¹⁰. To expand knowledge of the diversity of *S. enterica* in Jalisco and obtain a more comprehensive view of its behavior, all 195 strains isolated in this state were characterized, the sensitivity to other antibiotics was investigated, and the formation of biofilms on stainless steel of three representative isolates was evaluated.

Virulence gene detection

The presence of 13 virulence genes (*agfA*, *hila*, *invA*, *orgA*, *sifA*, *sipA*, *ssaQ*, *sseF*, *sseL*, *sspH1*, *sopE*, *pefA*, and *spvC*) was evaluated with four previously described multiple PCRs¹⁰. PCR products were separated by electrophoresis on 2 % agarose gels (Sigma-Aldrich, USA) and visualized with a ChemiDoc XRS+ system (Bio-Rad, USA). *Salmonella enterica* serotype Typhimurium ATCC 14028, which has all target genes, except *sopE*, was used as a positive control.

Antimicrobial susceptibility test

It was determined using the disc diffusion method¹² on Mueller-Hinton agar (Difco, Becton Dickinson, USA) against 13 drugs (BBL, USA) of importance in human and veterinary medicine¹³: amikacin (AK, 30 µg), amoxicillin + clavulanic acid (AMC, 20/10 µg), ampicillin (AM, 10 µg), ceftriaxone (CRO, 30 µg), cefuroxime (CXM, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GM, 10 µg), nalidixic acid (NA, 30 µg), kanamycin (K, 30 µg), norfloxacin (NOR, 10 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), and trimethoprim + sulfamethoxazole (SXT, 1.25/23.75 µg). *E. coli* ATCC 25922 was used as a quality control strain. Based on inhibition-diameter measurements, the isolates were classified as susceptible, with intermediate (reduced) susceptibility, or resistant according to the performance standards of the Clinical & Laboratory Standards Institute (Table 1)¹⁴. Multidrug-resistant (AMR) isolates were defined as those resistant to three or more classes of antimicrobials.

Table 1. Criteria for the classification of *Salmonella* isolates as sensitive, intermediate, or resistant to the antimicrobials tested¹⁴

Antimicrobial agent	Inhibition halo measurement (mm)			
	Disk content ((g)	Susceptible	Intermediate	Resistant
Amikacin	30	≥ 17	15 - 16	≤ 14
Amoxicillin + clavulanic acid	20/10	≥ 18	14 - 17	≤ 13
Ampicillin	10	≥ 17	14 - 16	≤ 13
Ceftriaxone	30	≥ 23	20 - 22	≤ 19
Cefuroxime	30	≥ 18	15 - 17	≤ 14
Chloramphenicol	30	≥ 18	13 - 17	≤ 12
Ciprofloxacin	5	≥ 31	21 - 30	≤ 20
Gentamicin	10	≥ 15	13 - 14	≤ 12
Nalidixic acid	30	≥ 19	14 - 18	≤ 13
Kanamycin	30	≥ 18	14 - 17	≤ 13
Norfloxacin	10	≥ 17	13 - 16	≤ 12
Streptomycin	10	≥ 15	12 - 14	≤ 11
Tetracycline	30	≥ 15	12 - 14	≤ 11
Trimethoprim + sulfamethoxazole	1.25/23.75	≥ 16	11 - 15	≤ 10

Biofilm formation on stainless steel

Three *Salmonella* strains (435, 451, and 458) were selected to evaluate biofilm formation on stainless steel at 9, 14, and 25 °C in the presence or absence of the native microbiota of raw chicken. These strains, representing the three most prevalent virulotypes, showed different antimicrobial resistance profiles and were recovered from different types of establishments. Rifampicin-resistant mutants were used to facilitate their selective counting. The tests were carried out on sterile 1 cm² food-grade 316 stainless steel squares (coupons) that were immersed in raw chicken extract as a nutrient source.

Preparation of raw chicken extract (RCE) and microbiota concentrate. A raw chicken breast with skin (~150 g) was macerated with distilled water (1:10 w/v) for 1 min in a BagMixer 400P (Interscience, USA). The homogenate was filtered through sterile gauze and distributed into 50 ml aliquots, which were centrifuged at 7,127 ×g for 10 min (Universal 320/320R, Germany). The supernatants were filtered through a 0.45 µm membrane and used as sterile RCEs. Sediments were resuspended in 1 ml of sterile saline (SS), mixed, filtered, and centrifuged at 7,127 ×g for 10 min. The final pellet was resuspended in 30 ml of TSB and used as a "microbiota concentrate". The levels of mesophilic and psychrotrophic bacteria in the microbiota concentrate were ~4 log CFU/ml.

Inoculation of coupons. Each strain was cultured in 3 mL TSB at 35 °C for 18–24 h, washed twice with SS by centrifuging at 15,000 ×g for 3 min, resuspended in 1 ml SS, and adjusted to a 0.5 McFarland's standard (~5×10⁸ CFU/ml). Decimal dilutions were prepared to obtain a suspension with ~1×10⁵ CFU/ml. The coupons were washed with 10 % Hyclin-Plus detergent (Hyclon, Mexico), rinsed, placed in acetone for 30 min, rinsed again, air-dried, and sterilized at 121 °C for 15 min. In each experiment, two groups of coupons (24 coupons/Petri dish) were inoculated: (A) sterile RCE + *Salmonella* suspension (13.5 + 1.5 mL), and (B) sterile RCE + *Salmonella* suspension + microbiota concentrate (13.5 mL + 1.5 ml + 135 µL). The coupons were gently stirred to ensure uniform inoculation. Each strain was studied individually.

Biofilm formation trials. The inoculated coupons were incubated at 9, 14, or 25 °C for 4 h (Climacell 707, MMM Group, Germany). After initial adhesion, each coupon was rinsed twice with 5 mL of SS and vortexed with 1.5 mL of PD for 30 sec. Decimal dilutions were prepared and inoculated with TSA+Rifampicin (200 µg/ml) at 37 °C for 24 h to count *Salmonella*. The coupons were then transferred to tubes containing 2 mL of 1:10-diluted RCE and placed in airtight boxes containing 100 g of potassium sulphate and 30 mL of distilled water to maintain a relative humidity of ~97 %. Incubation continued at 9, 14, or 25 °C. At 24, 48, and 72 h, the procedure was repeated to enumerate the biofilms. Extracellular polymeric substances (EPSs) were quantified in inoculated and incubated coupons in parallel. Each coupon was rinsed twice, stained with 2 mL of 0.1 % violet crystal for 5 min, rinsed, and then the dye was solubilized with 1 mL of 30 % acetic acid. A total of 200 µL was transferred to a 96-well microplate, and absorbance was measured at 595 nm (OD₅₉₅) (Multiskan™ Go, Thermo Scientific, Spain) using 30 % acetic acid as a blank. Three independent replicates were performed per experiment, and the variables were measured in duplicate.

Data analysis

Differences in the frequencies of multidrug resistance and virulotypes by establishment type and season were evaluated using contingency tables and Pearson's χ^2 test ($P < 0.05$). Multivariate ANOVA was performed to assess the effect of temperature and time on biofilm formation and EPS production, and one-way ANOVA was used to examine the effect of time. When significant differences ($P < 0.05$) were detected, Tukey's test was applied. Statgraphics Centurion XVIII software (Statpoint Technologies, Inc., USA) was used.

Results and discussion

Virulence genes

The consistent detection of chromosomal virulence genes (*agfA*, *hilA*, *invA*, *orgA*, *sifA*, *sipA*, *ssaO*, *sseF*, and *sseL*) in the 195 isolates underscores the conserved nature of the main pathogenicity mechanisms in poultry-associated *Salmonella*. These genes are involved in processes of adhesion, invasion, intracellular survival, and immune evasion. The virulence genes encoded by prophages, *sspH1* and *sopE*, were detected in 55 and 23 % of the isolates, respectively, and participate in immune evasion and cell invasion. In contrast, the absence of plasmid-encoded virulence genes, *pefA* and *spvC*, commonly associated with systemic infections, suggests a lower invasive potential in these strains.

Several studies have reported the prevalence of virulence genes in *Salmonella* strains; however, comparisons are complex due to differences in the target genes evaluated. In accordance with the findings of this study, *invA* was detected in 94 to 100 % of the isolates analyzed in Brazil, South Africa, and China^{15,16,17}; by contrast, *hilA* was present in all isolates from Brazil¹⁵ but only in 9 % of those from South Africa¹⁶. *sipA* and *sifA* were found in 87 and 94 % of the strains tested in China and India, respectively^{17,18}. The prophage gene *sopE* was present in almost all isolates from Brazil and Colombia^{15,19}, which differs from the 23 % observed in this study. The plasmid gene *spvC* was reported in 94 % of isolates in China¹⁷, but results of the present work are consistent with the low frequency reported in India¹⁸. These findings reflect the genomic plasticity of *Salmonella*, which plays a central role in its pathogenic potential.

Virulotypes

According to the virulence genes detected, the isolates were classified into four virulotypes: Va, Vb, Vc, and Vd (Table 2). The Va virulotype included strains carrying only chromosomal virulence genes; Vb contained all the chromosomal virulence genes plus the prophage gene *sspH1*; Vc had all the chromosomal genes and both prophage genes (*sspH1* and *sopE*); in contrast, Vd showed all the chromosomal virulence genes and the prophage gene *sopE*. Vb was the most prevalent virulotype (52 %), followed by Va (26 %), Vd (19 %), and Vc (3 %). A significant association was found between the distribution of virulotypes and the type of establishment (χ^2 , $P < 0.05$), indicating that the frequency of virulotypes varies with the origin of the chicken meat.

Table 2: Virulotypes identified in *Salmonella* isolates (n=195) recovered from raw chicken in Jalisco, Mexico

Genes	Virulotype					
	Va	Vb	Vc	Vd		
Chromosomal	<i>agfA</i>	x	x	x	x	
	<i>hilA</i>	x	x	x	x	
	<i>invA</i>	x	x	x	x	
	<i>orgA</i>	x	x	x	x	
	<i>sifA</i>	x	x	x	x	
	<i>sipA</i>	x	x	x	x	
	<i>ssaQ</i>	x	x	x	x	
	<i>sseF</i>	x	x	x	x	
	<i>sseL</i>	x	x	x	x	
	Prophage	<i>sspH1</i>		x		
		<i>sopE</i>			x	x
Plasmid-encoded	<i>pefA</i>					
	<i>spvC</i>					
No. isolates (%)	50 (26)	101 (52)	6 (3.1)	38 (19)		

All isolates from supermarkets (n=23) belonged to the Vb virulotype (Table 3); by contrast, those from the wholesale market (n= 60) and poulterer's shops (n= 112) included all four virulotypes. These results suggest that *Salmonella* isolates from chicken meat sold in supermarkets exhibit lower genetic diversity in terms of virulence genes than those obtained from small establishments. This reduced diversity could be attributed to centralized sourcing practices of large retail chains, which generally use a limited number of suppliers operating under standardized quality control programs. These suppliers are part of vertically integrated poultry companies that process carcasses in federal inspection type (TIF, by its Spanish acronym) establishments, which implement food safety management systems that include good manufacturing practices, sanitation operating procedures, hazard analysis and critical control points (HACCP), and pathogen reduction programs²⁰. In contrast, chicken meat sold at small retail outlets, such as those at the wholesale market or poulterer's shops, typically comes from a large, less-regulated network of suppliers that may include TIF establishments, non-certified plants, and even informal or clandestine slaughter operations.

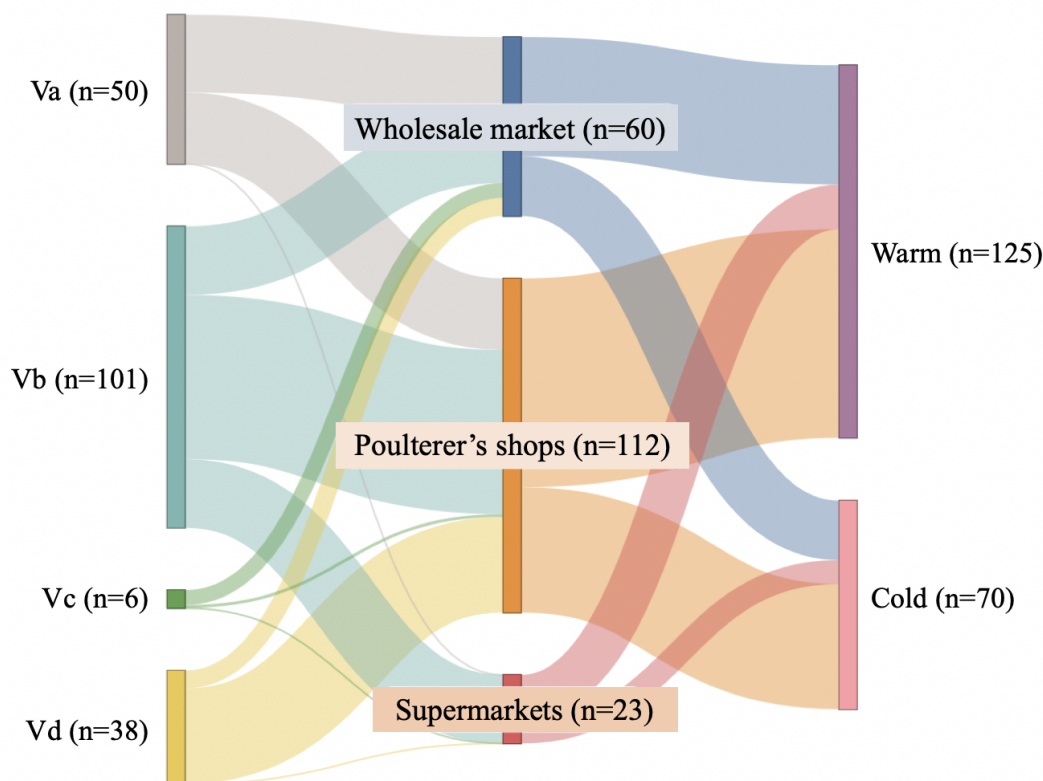
Table 3: Frequency of virulotypes in *Salmonella* isolates (n=195) recovered from raw chicken in Jalisco, Mexico

Virulotype	No. of isolates (%)			
	Supermarket	Wholesale market	Poulterer's shops	Total
Va	0 (0.0)	26 (13)	24 (12)	50 (26)
Vb	23 (12)	23 (12)	55 (28)	101 (52)
Vc	0 (0.0)	5 (2.6)	1 (0.5)	6 (3.1)
Vd	0 (0.0)	6 (3.1)	32 (16)	38 (19)
Total	23 (12)	60 (31)	112 (57)	195 (100)

The findings of the present study coincide with previous studies: among 523 isolates of *S. enterica* analyzed in Europe, 14 virulotypes were detected²¹, whereas seven virulotypes were identified among 153 strains of *Salmonella* recovered from human clinical samples, wastewater, soil, irrigation water, agricultural products, and dairy products in Mexico²²; that same study performed *in silico* virulotyping of 2,963 *Salmonella* genomes originating in Mexico, which yielded 38 virulotypes, and as in these findings, those that included mobile virulence genes were less frequent.

Regarding seasonality, 64 % of the isolates were recovered during the warm season and 36 % during the cold season ($P<0.05$). All virulotypes were more frequent in the warm months (Figure 1), and an association between the distribution of virulotypes and the season was observed (χ^2 , $P<0.05$). Va and Vd were more frequent in the warm season. These findings coincide with previous studies indicating a higher prevalence of *Salmonella* in chicken carcasses during the warm months²³. This trend can be attributed to *Salmonella*'s mesophilic nature and the environmental conditions prevalent in the warm season (high temperatures and higher humidity), which favor the pathogen's survival and proliferation throughout the food chain. National epidemiological data corroborate this trend, as cases of salmonellosis in Mexico increase in the warmest months of the year⁶. The observed seasonal dynamics highlight the importance of strengthening food safety practices and health surveillance during the warm months in order to mitigate the increased risk of *Salmonella* contamination and transmission.

Figure 1. Virulotypes identified in *Salmonella* isolates (n=195) recovered from raw chicken in Jalisco, Mexico

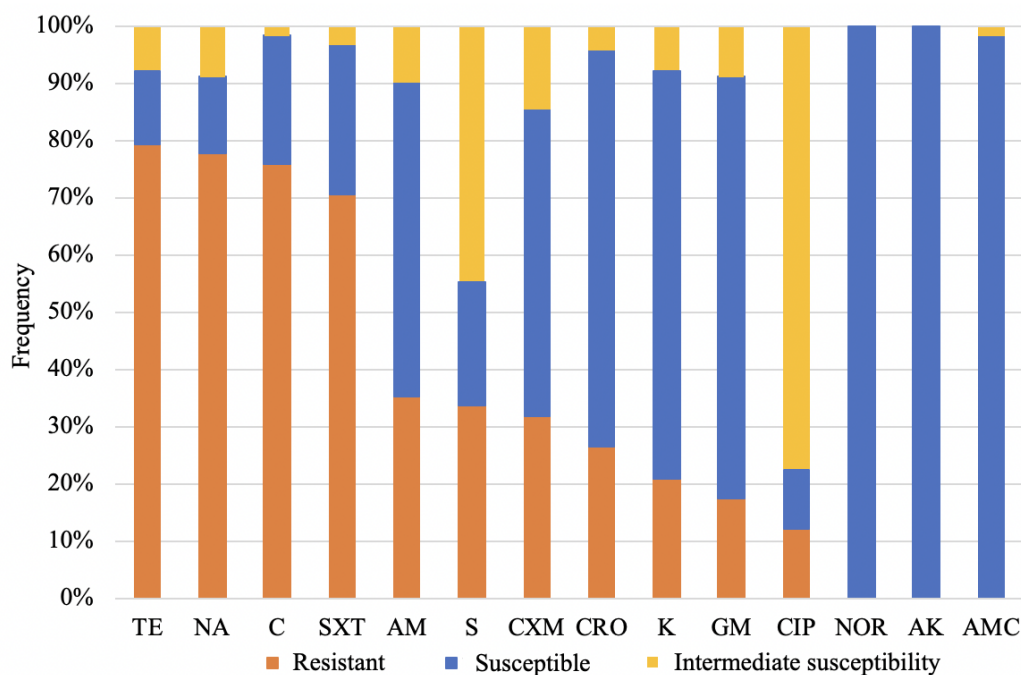


The Sankey diagram shows the distribution of each virulotype by the establishment of origin and season. The number in parentheses indicates the number of isolates of each virulotype, establishment, and season. The width of each band is proportional to the number of isolates, facilitating visual appreciation of their distribution.

Antimicrobial resistance

Of the 195 isolates analyzed, 90 % (n= 175) were resistant to at least one antimicrobial agent. The most frequent resistance was to TE (79 %), followed by NA (78 %), C (76 %), SXT (71 %), AM (35 %), S (34 %), CMX (32 %), CRO (27 %), K (21 %), GM (17 %), and CIP (12 %) (Figure 2). No resistance against AK, NOR, or AMC was detected. This coincides with previous studies from other regions of Mexico that found a higher frequency of resistance to ampicillin (87 %), chloramphenicol (80 %), and tetracycline (78 %) among 41 *Salmonella* strains isolated from chicken meat²⁴; for its part, in another study, 82 % of 139 *Salmonella* isolates were resistant to ampicillin, 82 % to chloramphenicol, 63 % to pefloxacin, 27 % to gentamicin, and only 7 % to ceftriaxone⁸. Similar results have been reported in other countries. In the United States, the most frequent resistance among *Salmonella* isolated from retail chicken was to streptomycin (62 %), tetracyclines (55 %), and nalidixic acid (30 %)²⁵. These trends are consistent with the report that tetracyclines are the most widely used agents in animal health globally, representing 35 % of the total²⁶.

Figure 2. Resistance frequency, intermediate (reduced) susceptibility, and susceptibility against antimicrobials of human and veterinary importance among 195 *Salmonella* isolates recovered from raw chicken



Of concern is the high proportion of isolates resistant to antimicrobials for use in human medicine, including some of highest priority and critical importance (HPCI), such as quinolones and third-generation cephalosporins (Table 4); antimicrobials of high priority and critical importance (HiPCI), such as aminoglycosides; and highly important antimicrobials (HI), such as tetracyclines, amphenicols, penicillins, second-generation cephalosporins, and sulfonamides²⁷. In addition, a significant proportion of isolates were resistant to six classes of critically important veterinary antimicrobial agents (CIVA) and two classes of highly important veterinary agents (HIVA)²⁶.

Table 4. Frequency of antimicrobial resistance in *Salmonella* isolates (n=195) recovered from raw chicken in Jalisco, Mexico

Class	AMI	Resistant isolates		WHO			WOAH	
		No.	%	HPCI	HiPCI	HI	CIVA	HIVA
Cephalosporins (3rd gen)	CRO	52	27	x			x	
	CIP	21	12	x			x	
Quinolones	NOR	0	0	x			x	
	NA	152	78	x			x	x
	AK	0	0		x		x	
Aminoglycosides	GM	34	17		x		x	
	K	41	21		x		x	
	S	66	34		x		x	
Tetracyclines	T	155	79			x	x	
Sulfonamides	STX	138	71			x	x	
Amphenicols	C	148	76			x		
Penicillins	AM	69	35			x	x	
	AMC	0	0			x	x	
Cephalosporins (2nd gen)	CXM	62	32			x		x

AMI= antimicrobials; WHO= World Health Organization; HPCI= highest priority critically important antimicrobials; HiPCI= high priority and critically important antimicrobials; HI= highly important antimicrobials; WOAH= World Organisation for Animal Health; CIVA= critically important veterinary antimicrobials; HIVA= highly important veterinary agents.

Fluoroquinolones are used intensively in poultry production due to their low toxicity and broad spectrum of action. The results show a high frequency of resistance to quinolones, with 78 % of isolates resistant to NA and 12 % to CIP; in addition, 77 % had reduced susceptibility to CIP. The decreased efficacy of ciprofloxacin is concerning, as it is commonly used to treat severe *Salmonella* infections⁴. NA and CIP share a mechanism of action; therefore, NA-resistant isolates tend to show lower susceptibility to CIP due to mutations in the *gyrA* gene²⁸. In the United States, reduced susceptibility to CIP in *Salmonella* strains from poultry products increased from 0 % in 2015 to 40 % in 2020, and is expected to reach 45 % by 2025²⁹, even though the use of fluoroquinolones in poultry is not approved in that country³⁰. In Mexico, quinolones NA, CIP, NOR, and enrofloxacin are allowed in food animals under veterinary prescription; their widespread use could explain the levels of resistance observed. Resistance and reduced susceptibility to CRO were observed in 27 and 5.3 % of the strains, respectively. This frequency is higher than the 7 % reported 16 years ago for *Salmonella* strains from chicken meat in Mexico²⁴. In the United States, 13 % of pathogen isolates recovered from retail chicken were resistant to CRO in 2021, and an increase to 21 % is projected in 2025²⁵.

Antibiotic-resistant nontyphoid *Salmonella* infections are on the rise and pose a serious threat to public health; although most cases require only symptomatic treatment, severe or extraintestinal infections may require antibiotics³¹. Infections with resistant *Salmonella* strains are associated with worse clinical outcomes, with more frequent hospitalization, hospital stay ≥ 3 days, and higher mortality³².

Antimicrobial multiresistance (AMR) was observed in 79 % of the isolates analyzed, which presented 36 different resistance profiles. Thirty-nine percent of the isolates showed resistance to ≥ 6 antimicrobials (Table 5). Two isolates (1 %) of supermarket chicken meat were resistant to 11 antimicrobials from seven different classes. Resistance to ≥ 9 antimicrobials of 6-7 classes was observed in 35 (18 %) isolates, most of them from poulturer's shops, and an association was found between the AMR phenotype and the type of establishment (χ^2 , $P < 0.05$), with a higher frequency of multidrug resistance in strains from poulturer's shops.

Table 5. : Profiles of higher antimicrobial multiresistance of *Salmonella* isolates (n=61) recovered from raw chicken in Jalisco, Mexico

Multidrug resistance (No. of antimicrobials)	No. of isolates	Virulotypes	Origin	Season
NA-CIP-AM-CRO-CXM-C-S-K-GM-TE-SXT (11)	2	Vb	Supermarket	Warm
NA-CIP-AM-CRO-CXM-S-K-GM-TE-SXT (10)	1	Vb	Wholesale market	Warm
NA-AM-CRO-CXM-C-S-K-GM-TE-SXT (10)	19	Vb	Supermarket (3), Poulterer's shop (16)	Cold (13), Warm (6)
NA-CIP-AM-CRO-CXM-C-S-K-GM-TE (10)	2	Vb	Supermarket	Cold
NA-AM-CRO-CXM-C-S-GM-TE-SXT (9)	1	Vb	Wholesale market	Cold
NA-AM-CRO-CXM-C-S-K-TE-SXT (9)	8	Vb	Supermarket (2), Poulterer's shop (6)	Cold (3), Warm (5)
NA-AM-CRO-CXM-C-S-K-GM-TE (9)	2	Vb	Supermarket	Warm
NA-AM-CRO-CXM-K-GM-TE-SXT (8)	2	Vb	Supermarket	Cold
NA-AM-CRO-CXM-C-S-TE-SXT (8)	6	Vb	Poulterer's shop	Cold (5), Warm (1)
NA-AM-CRO-CXM-C-S-GM-TE (8)	1	Vb	Supermarket	Warm
NA-CIP-AM-CRO-CXM-C-S-TE (8)	1	Vb	Wholesale market	Cold
NA-AM-CRO-CXM-C-S-K-TE (8)	2	Vb	Supermarket	Warm
NA-AM-CRO-CXM-C-TE-SXT (7)	2	Vb	Wholesale market (1), Poulterer's shop (1)	Warm
NA-AM-CRO-CXM-C-S-TE (7)	2	Vb	Wholesale market (1), Supermarket (1)	Cold (1), Warm (1)
NA-CIP-CXM-S-K-TE-SXT (7)	1	Vb	Supermarket	Cold
NA-AM-C-S-GM-TE-SXT (7)	1	Vc	Wholesale market	Cold
NA-C-S-K-GM-TE-SXT (7)	1	Vb	Poulterer's shop	Warm
NA-CIP-CXM-C-TE-SXT (6)	3	Va	Wholesale market (1), Poulterer's shop (2)	Cold (1), Warm (2)
NA-AM-C-S-TE-SXT (6)	4	Va (1), Vb (3)	Wholesale market (1), Poulterer's shop (3)	Cold (3), Warm (1)

When stratified by virulotype, the highest frequency of AMR was observed in Vb (n=79), followed by Va (n= 38), Vd (n= 36), and Vc (n= 2). All isolates from supermarkets were of the Vb virulotype; the high resistance in these isolates could reflect the intensive use of antimicrobials, characteristic of intensive poultry production systems. In 2010, poultry production used ~148 mg of antimicrobials/kg of meat produced, three times more than in cattle, and a substantial increase is projected by 2030³³, the most commonly used antimicrobials for therapeutic purposes in poultry feeding include aminoglycosides, β -lactams, sulfonamides, and tetracyclines.

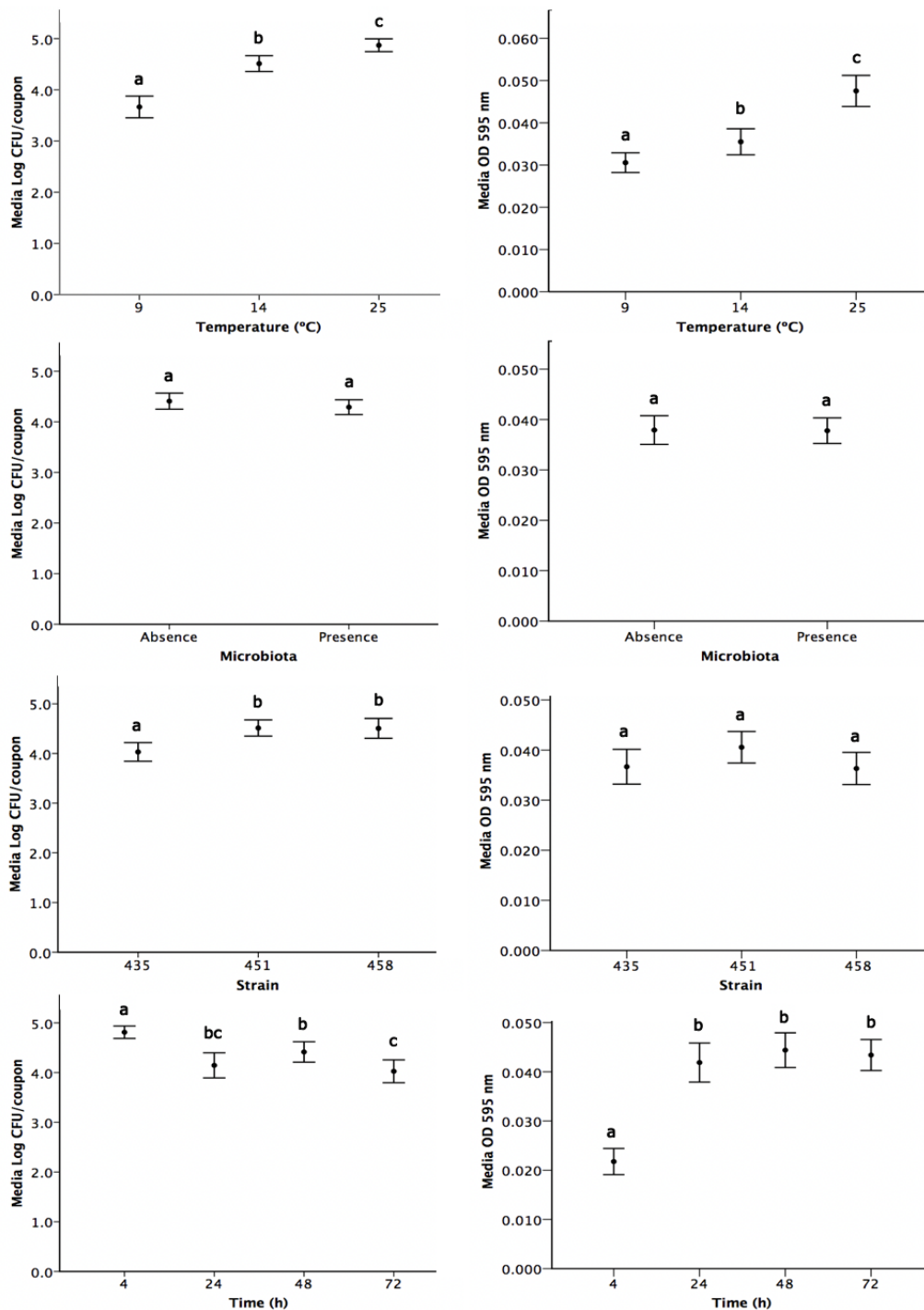
The ACSSuT phenotype (resistance to AM-C-S-SXT-T) was identified in 47 (24 %) isolates. The ACSSuT profile combined with quinolone (CIP and/or NA) resistance was observed in 41 strains (21 %), and with additional cephalosporin (CRO) resistance in 36 strains (18 %). Most of the strains that showed the ACSSuT+Quin+Cef phenotype (n= 28) were recovered from chicken meat from seven different poulterer's shops. ACSSuT strains co-resistant to quinolones and cephalosporins further complicate the treatment of salmonellosis; this phenotype has also been reported in other countries³⁴. In vertically integrated intensive production systems characterized by high cost efficiency, antimicrobials are used to maintain animal health, sustain productivity, and meet global demand for animal protein. Although international health authorities have called for a reduction in the use of antimicrobials in food animals, an increase is

projected in low- and middle-income countries over the next two decades, driven by economic growth, increased meat consumption, and the intensification of animal production systems³⁵.

Biofilm formation

Biofilm formation increased with temperature, with higher production observed at 25 °C ($P < 0.05$), with an average concentration of 4.9 ± 0.8 log CFU/coupon at 72 h of incubation. Average counts at 14 °C (4.5 ± 0.9 log CFU/coupon) were higher ($P < 0.05$) than at 9 °C (3.7 ± 1.3 log CFU/coupon) (Figure 3). Similarly, a higher number of EPS was detected at higher temperatures ($P < 0.05$), with average values of 0.031 ± 0.014 , 0.036 ± 0.018 , and 0.048 ± 0.022 OD₅₉₅ at 9, 14, and 25 °C, respectively. In line with this, previous studies indicate that temperature influences the formation of *Salmonella* biofilms on stainless steel, with greater production of *S. Enteritidis* biofilms at 25 °C than at 9 °C³⁶; by contrast, all *Salmonella* strains ($n = 20$) of different serotypes formed biofilms at 25 °C, but not at 15 °C³⁷, indicating lower biofilm formation at low temperatures, considered stressors. Temperature affects biofilm formation in *Salmonella* by regulating key genes and producing an extracellular matrix; the master regulator *csgD*, which controls curli and cellulose biosynthesis, is activated below 30 °C and repressed above 32 °C³⁸. The expression of *agfA*, which forms curli fimbriae, increases between 20 and 28 °C, favoring the adhesion and development of the biofilm matrix; by contrast, at 37 °C, *Salmonella* represses biofilm genes and activates virulence factors such as SST3, privileging invasion over persistence.

Figure 3. Biofilm formation (log CFU/coupon) and extracellular polymeric substances (OD_{595nm}) of three *Salmonella* strains on stainless steel



Average counts with different letters (a,b,c) are significantly different ($P < 0.05$).

The native microbiota of raw chicken did not affect the ability of the three *Salmonella* strains to form biofilms ($P > 0.05$) (Figure 3); the average counts in the presence and absence of microbiota were 4.4 ± 1.2

and 4.3 ± 1.1 log CFU/coupon, respectively. There were also no differences in the number of EPS produced in the presence (0.038 ± 0.019 OD₅₉₅) and in the absence (0.038 ± 0.021 OD₅₉₅) of microbiota ($P > 0.05$). These findings contrast with other studies showing that raw chicken microbiota can benefit or antagonize the formation of *Salmonella* biofilms³⁹. Although microbial communities exhibit complex interactions, such as competition for nutrients or production of inhibitory compounds, these results did not show a measurable effect of the native microbiota on biofilm formation in the three strains studied. Cooperative behaviors, such as coaggregation or the shared production of a polymer matrix, increase community resilience to adverse environmental factors; nevertheless, these synergistic effects were not visible under the experimental conditions.

Biofilm formation was different between the three strains tested regardless of temperature, as the population of strain 435 was lower (4.0 ± 1.1 log CFU/coupon; $P < 0.05$) compared to strain 451 (4.5 ± 1.0 log CFU/coupon) and strain 458 (4.5 ± 1.2 log CFU/coupon) (Figure 3); however, the number of EPS was not different between the three isolates ($P > 0.05$), with average values of 0.037 ± 0.021 , 0.041 ± 0.019 , and 0.036 ± 0.019 OD₅₉₅ for strains 435, 451, and 458, respectively. These findings coincide with previous reports of considerable variability in biofilm formation between *Salmonella* strains of the same serotype and between different serotypes from clinical, food, and environmental sources^{37,39}. This suggests that biofilm formation is a strain-dependent trait, likely influenced by its genetics and environmental adaptation. These differences can affect the pathogen's persistence in food production environments and its resistance to cleaning and disinfection procedures, thereby affecting food safety. The difference in biofilm production between the strains evaluated could be related to their antimicrobial resistance; strain 435 showed resistance only to ampicillin, whereas strains 458 and 451 were AMR. A relationship between AMR and biofilm formation has been reported in Enterobacteriaceae from poultry feces and in *Salmonella* isolates from foods of animal origin⁴⁰, with AMR strains being likely to form more robust biofilms.

Regarding incubation time, *Salmonella* counts after 4 h (initial adhesion) were 4.8 ± 0.7 log CFU/coupon, decreased at 24 h (4.2 ± 1.3 log CFU/coupon) ($P < 0.05$), and remained unchanged until 72 h ($P > 0.05$) (Figure 3). In contrast, the concentration of EPS increased between 4 h (0.022 ± 0.014 OD₅₉₅) and 24 h (0.042 ± 0.021 OD₅₉₅) ($P < 0.05$) but remained unchanged at 48 h (0.044 ± 0.018 OD₅₉₅) and 72 h (0.043 ± 0.016 OD₅₉₅) ($P > 0.05$). These results suggest that within the first hours of contact with stainless steel, a significant proportion of *Salmonella* cells did not survive, likely due to adverse environmental conditions or limited adhesion capacity. Nonetheless, a resistant subpopulation managed to survive and initiated EPS synthesis, gradually developing structured biofilms. The formation of mature biofilms is of concern in food processing and preparation environments, as they confer greater resistance to cleaning and disinfection procedures, facilitate persistent surface contamination, and increase the risk of food contamination. In a study conducted with three *Salmonella* serotypes, it was observed that time did not affect cell density or EPS production on stainless steel, glass, or plastic at 25 °C for 24, 48, and 72 h ($P > 0.05$)³⁹. The results of the present study also showed no difference in biofilm biomass at 25 °C at 72 h ($P > 0.05$); nevertheless, EPS production did increase between 4 and 24 h ($P < 0.05$). The discrepancies between the two studies may be due to incubation time, as changes in the early stages of biofilm formation may not have been detected in the other study. In addition, variability between strains and different experimental conditions could explain the differences.

Although biofilm formation was evaluated in a limited number of strains in this study, which may represent a methodological limitation, the results reveal relevant trends for the persistence of *Salmonella* on food-contact surfaces. The inclusion of more isolates in future studies will enable the generation of more representative parameters to strengthen the integration of strain heterogeneity into quantitative microbiological risk assessment (QMRA) models and to reduce uncertainty in risk estimation.

Conclusions and implications

Salmonella enterica isolated from chicken meat marketed at retail in Jalisco, Mexico, carries a conserved set of chromosomal virulence genes, and the absence of the *pefA* and *spvC* genes suggests a lower invasive potential in these strains. The high frequency (79 %) of multidrug-resistant isolates, particularly of the Vb virulotype, is noteworthy. Resistance against critically important antimicrobials, such as fluoroquinolones and third-generation cephalosporins, raises concerns about treatment options and the impact on public health. Biofilm formation differed among the three isolates studied and was influenced by temperature, but not by the presence of microbiota, and was higher in AMR strains. These findings provide insight into biofilm development by *Salmonella* strains studied on food-contact surfaces under different environmental conditions and highlight the importance of incorporating strain-specific behavior and early biofilm formation dynamics into QMRA models, in order to improve their persistence and exposure accuracy; however, it is important to conduct more studies with a larger number of strains of diverse origin.

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