Genomic surveillance of antimicrobial resistance shows cattle are a moderate source of multi-drug resistant non-typhoidal *Salmonella* in Mexico

Multi-drug resistant *Salmonella* in cattle

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Abstract

Multi-drug resistant (MDR) non-typhoidal *Salmonella* (NTS) is a major public health concern globally. This study reports antibiotic susceptibility testing and genotypic antimicrobial resistance (AMR) profiles of NTS isolates from bovine lymph nodes (n=48)

and ground beef (n=29). Furthermore, we compared genotypic AMR data of our isolates with those of publicly available NTS genomes from Mexico (n=1637). The probability of finding MDR isolates was higher in ground beef as compared to lymph nodes: $\chi^2=12.0$, $P=0.0005$. The most common resistant phenotypes involved tetracycline (40.3%), carbenicillin (26.0%), amoxicillin-clavulanic acid (20.8%), chloramphenicol (19.5%) and trimethoprim-sulfamethoxazole (16.9%), while over 55% of the isolates showed decreased susceptibility to ciprofloxacin and 26% were MDR. Occurrence of MDR isolates was strongly associated with NTS serovar ($\chi^2=24.5$, $P<0.0001$), with Typhimurium accounting for 40% of MDR strains. Most of these (9/10), carried *Salmonella* genomic island 1, which harbors multiple AMR genes (*aadA2*, *blaCARB-2*, *floR*, *sul1*, *tetG*) that confer a penta-resistant phenotype. Moreover, 100% of MDR isolates had mutations in the *ramR* gene. Among public NTS isolates from foods and clinical cases in Mexico to date, those from cattle had the highest proportion of MDR genotypes. Our results suggest attaining significant improvements in AMR meat safety may require the identification and removal (or treatment) of product harboring MDR NTS, instead of screening for *Salmonella* spp. or for isolates showing resistance to individual antibiotics. In that sense, massive integration of whole genome sequencing (WGS) technologies in AMR surveillance provides the shortest path to accomplish these goals.

Introduction

For decades, experimental data has supported concerns that antimicrobials use (AMU) in animal production is a key factor contributing to the ever-growing crisis of bacterial

antimicrobial resistance (AMR). However, there is limited evidence linking food consumption with AMR emergence in humans [1, 2]. Moreover, an increasing number of studies have found that AMU in food animals has a limited impact on the AMR profile of foodborne pathogens [3-6]. While this may be true in developed countries, the situation is likely different in low and middle-income countries (LMIC) where controls of AMU in veterinary practice and human health are less rigorous. Therefore, in the context of an intense trade of foods between countries differing in AMR food safety, it is vital to identify relevant sources of AMR pathogens along the food chain. This would help to prevent their dissemination, as well as human exposure to multi-drug resistant (MDR) bacteria, which is a global public health issue.

In Mexico, source attribution of foodborne illnesses is in an early stage. Nonetheless, official epidemiological surveillance reports high incidence rates for infections commonly transmitted through foods. That of non-typhoidal salmonellosis has been above 60 cases per 100 thousand inhabitants in the last 5 years [7]. In addition, recent studies conducted in Mexico show non-typhoidal *Salmonella* (NTS) contamination is unusually high (15 to nearly 100% positive samples) in bovine lymph nodes, beef carcasses and ground beef, with isolates exhibiting rates of resistance that vary from 30 to nearly 98%, across several important antimicrobial classes (i. e. penicillins, aminoglycosides, cephalosporins, quinolones) [8, 9]. This evidence suggests beef is likely to play a role as a relevant reservoir of foodborne MDR salmonellosis in Mexico. Especially, if considering it is often involved in salmonellosis outbreaks in countries with lower NTS contamination rates [10, 11].

The increasing availability of whole genome sequencing (WGS) technologies have helped to improve genomic surveillance as it provides a high-resolution method for the

characterization of an organism features. Regarding AMR in NTS, however, most research from LMIC deal with phenotypes [12-16]. Although these studies raise concerns, they do not provide insights into the genetic basis of AMR, its evolution or dissemination within bacterial populations. Such information is crucial to devise new strategies to contain the dissemination of AMR pathogens. However, it can best be obtained by addressing phenotypic and genomic profiling of AMR simultaneously, an area with a limited number of studies in Mexico and other countries [17].

In the present investigation, we conducted antibiotic susceptibility testing and WGS of 77 NTS isolates collected in the course of a previous research project involving bovine lymph nodes (n=800) and ground beef (n=745) across a two-year sampling period [18]. Assembled genomes were used to predict the AMR genomic profiling of NTS isolates, and this was further compared to their corresponding AMR phenotypes. We also conducted comparative genomics of AMR genotypes of publicly available NTS isolates collected in Mexico from bovines, vegetables, seafood, aquatic environments, human stools and clinical cases. Consequently, we managed to thoroughly characterize the role of cattle as a reservoir of NTS harboring AMR genes of human clinical significance. Moreover, we identified the dominant genetic determinants that sustain resistance against specific antibiotic classes, as well as MDR phenotypes.

Materials and Methods

Animal Care and Use Committee approval was not obtained for this study since live animals were not directly involved in the experiment.

Salmonella isolates

We used 77 strains from a previous study conducted by our research group [18]. These were isolated from bovine lymph nodes (LN, n= 800) and ground beef (GB, n=745) collected between April 2017 and December 2018. Each isolate was obtained from a different sample across the two-year sampling period.

Antibiotic Susceptibility Testing (AST)

The phenotypic AMR profile of NTS isolates was determined with a panel of 13 antibiotics included in the WHO list of critically important and highly important antimicrobials [19]. We used the disk diffusion method [20], with the Bencton Dickinson AST test disks and concentrations reported in Table 1.

Table 1. Antimicrobial agents tested, their concentrations and zone diameter clinical break points used in the AST

Antimicrobials	C, μg^2	Zone diameter breakpoint ¹ , mm	
		R	I
Amikacin (AMK)	30	≤ 14	15-16
Gentamicin (GEN)	10	≤ 12	13-14
Carbenicillin (CB)	100	≤ 19	20-22
Amoxicillin/clavulanic acid (AMC)	20/10	≤ 13	14-17
Cefotaxime (CTX)	30	≤ 22	23-25
Ceftriaxone (CRO)	30	≤ 19	20-22
Cefepime (FEP)	30	≤ 18	19-24

Imipenem (IPM)	10	≤19	20-22
Ertapenem (ETP)	10	≤18	19-21
Meropenem (MEM)	15	≤19	20-22
Chloramphenicol (CHL)	30	≤12	13-17
Trimethoprim-sulfamethoxazole (SXT)	1.25/23.75	≤10	11-15
Ciprofloxacin (CIP)	5	≤20	21-30
Tetracycline (TET)	30	≤11	12-14

¹Criteria to consider isolates as clinically resistant (R) or intermediate (I) [21]. For meropenem, we also considered epidemiological cutoff values (ECOFF) set by EUCAST to classify strains as wild-type (>27 mm) or non-wild-type (≤27 mm) [22].

²Antimicrobial's disk concentration.

Isolates were classified as susceptible, intermediate or resistant, according to the Clinical Laboratory Standards Institute (CLSI) guidelines [21]. Strains of *Escherichia coli* ATCC 8739, *Enterococcus fecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 9027 were used as quality control organisms. Isolates showing intermediate and resistance phenotypes were considered as non-susceptible in this study. Likewise, isolates showing resistance to ≥3 antimicrobial classes were classified as MDR [23]. The detailed AST protocol is available at protocols.io (doi: dx.doi.org/10.17504/protocols.io.bpympvvn).

Whole-genome sequencing and genome assembly

Genomic DNA (gDNA) was extracted from fresh colonies grown overnight at 37° C in tryptic soy broth. For that purpose, we used the Roche PCR High Purity Template

Preparation Kit (Roche México, Mexico City, Mexico), following manufacturer's instructions. Subsequently, gDNA was quantified using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific México, Mexico City, Mexico). Afterwards, sequencing libraries were prepared from 1 ng gDNA using the Nextera XT Library Preparation Kit v.3 (Illumina) and sequenced on the Illumina NextSeq system (paired end 2 x 150 bp insert size). Raw sequences are publicly available at the National Center for Biotechnology Information (NCBI). The accession numbers and metadata are listed in supplementary S1 Table.

The quality of raw reads was first assessed with FastQC [24] and we used Trimmomatic [25] to filter poor-quality reads and Illumina adaptors. Trimmed sequences were analyzed again with FastQC to ensure only high-quality reads (i. e. Q $\geq$ 30) were used for *de novo* genome assembly. Finally, genome assembly was conducted in the PATRIC web server [26] using the SPAdes algorithm [27].

Genotypic AMR profiling and comparative genomics

AMR genes and point mutations associated with AMR were predicted with the aid of AMRFinderPlus program version 3.8 using assembled genomes [28]. The study also aimed to compare the genetic AMR profile of our isolates in the context of NTS population circulating in the country. For that purpose, we identified all *Salmonella* isolates from Mexico that were publicly available at NCBI as of September 21, 2020 (n=2400). Among these, we selected groups of isolates of known isolation source: bovines (n=114, including the 77 from this study), vegetables (n=1,064), seafood (n=129), aquatic environment (n=385), and human stools/clinical cases (n=22). The human isolates group will be referred to as "human clinical cases" from now on. The full

list of accessions and metadata of these isolates is provided in supplementary S2 Table. AMR genotypes of these isolates were collected from the NCBI Pathogen Detection web site (<https://www.ncbi.nlm.nih.gov/pathogens>), which are generated by the AMRFinderPlus database and program.

Most serovar Typhimurium isolates (8/10) exhibited a penta-resistant phenotype similar to that reported for the Typhimurium DT104 strain, which is sustained by *Salmonella* genomic island 1 (SGI1) [29]. Hence, we conducted a BLAST atlas analysis to determine if the MDR profile of these isolates was associated with this genomic feature. For that purpose, we used assembled genomes at the GView web server [30], configured as follows: expect e-value cutoff=0.001, genetic code=bacterial and plant plastid, alignment length cutoff=50, percent identity cutoff=70 and tblastx as the BLAST program. The SGI1 reference sequence (AF261825.2) was collected from the Pathogenicity Island Database [31]. Furthermore, considering the epidemiological importance of this serovar, we also analyzed the whole set of Typhimurium isolates from Mexico deposited at NCBI (n=38, refer to S3 Table for accession numbers and AMR genotypes of this group of isolates). This analysis was performed in order to estimate how common MDR profiles are in strains of this serovar circulating in Mexico.

Plasmid profiling

Plasmids are known as strong contributors to AMR dissemination. Hence, we used PlasmidFinder 2.1 [32] to predict the isolates plasmid profile using assembled genomes and a threshold identity of 95%. When plasmid replicons were detected, we collected each plasmid's reference sequence from NCBI and mapped it against assembled genomes. If most plasmid sequences (>70%) were represented in a genome and the

genomic context of genes matched that of the plasmid, these isolates were proposed to carry the predicted plasmid.

Data analysis

We used Chi-square tests and odds ratio calculations to determine if there was an association between AMR profiles (both phenotypic and genotypic) and NTS serovars and/or isolation sources. These analyses were performed both with our experimental isolates, as well as with the additional public NTS genomes from Mexico included in the study.

We also conducted a correlation analysis between phenotypic and genotypic AMR results. Moreover, the genomic AMR profile of public NTS from Mexico was analyzed with the aid of a heatmap. For that purpose, we first determine the number of isolates from each source harboring specific AMR genes. Using these figures, we calculated the proportion of isolates from each source having the feature and used these data to generate a heatmap with the Heatmapper software [33].

Results

Phenotypic and genotypic antimicrobial resistance profiles

Approximately three quarters of the isolates were non-susceptible to at least one antibiotic class, while 26% were susceptible to all the tested antibiotics (Fig 1). The most common resistant phenotypes included tetracycline (40.3%), carbenicillin (26.0%), amoxicillin-clavulanic acid (20.8%), chloramphenicol (19.5%) and trimethoprim-sulfamethoxazole (16.9%). Resistance to cephalosporins and aminoglycosides was less

frequent (1.3-7.8% across these antibiotic classes), while for carbapenems only intermediate resistance was observed at a frequency of 1.3 and 9.1% for ertapenem and imipenem, respectively. On the other hand, although only one isolate resisted ciprofloxacin, 54.5% of the strains showed intermediate resistance to this drug.

Fig 1. AMR profile of 77 *Salmonella* isolates from lymph nodes (LN) and ground beef (GB). Cells are color coded according to antibiotic class: orange, aminoglycosides; blue, beta-lactams; purple, phenicols; green, sulfonamides; red, fluoroquinolones; black, tetracyclines. Resistant strains are indicated with “R” and intermediate with “I”. For genotypes, cells with the antibiotic class color indicate presence of the gene. Genes in gray are not associated with any antibiotic class included in the AST panel. Antibiotic abbreviations are as follows: amikacin (AMK), gentamycin (GEN), carbenicillin (CB), amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP), meropenem (MEM), ertapenem (ETP), imipenem (IMP), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), tetracycline (TET).

The rate of MDR strains was 26%, with the most common MDR profiles involving penicillins, chloramphenicol, trimethoprim-sulfamethoxazole, ciprofloxacin and tetracycline. Strikingly, there was one serovar Typhimurium isolate that resisted all antibiotic classes but carbapenems. In fact, the probability of isolates having MDR profiles was significantly higher in strains of serovar Typhimurium as compared to other serovars (OR=45.8, 95CI 5.3-399.2, P<0.0001). Likewise, there was a higher probability of finding MDR strains in ground beef as compared to lymph nodes (OR=6.5 95CI 2.1-20.1, P=0.0005).

The WGS-based *in-silico* prediction of non-susceptible phenotypes was generally good, as shown by the strong correlation between AMR genotypes and phenotypes (Fig 2).

Fig 2. Overall correlation of phenotypic resistance with the presence of antimicrobial resistance genes for the 77 *Salmonella* isolates ($r=0.979$, $P<0.0001$). Antibiotic abbreviations: ciprofloxacin (CIP), tetracycline (TET), carbenicillin (CB), amoxicillin-clavulanic acid (AMC), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), imipenem (IMP), ceftriaxone (CRO), cefotaxime (CTX), gentamycin (GEN), amikacin (AMK), cefepime (FEP), meropenem (MEM), ertapenem (ETP).

Genomic AMR profiling also identified the presence of several additional resistance genes that are not associated with any specific antimicrobial included in the AST panel. Particularly, those encoding resistance to fosfomycin (*fosA7.7*), quaternary ammonium compounds (*qacEDelta1*), as well as components of the multidrug and metal RND-type efflux complex (*mdsAB*).

Assembled genomes were also analyzed for point mutations associated with resistance. However, the occurrence of mutations was mostly inconsistent with the observed phenotypes (Table 2).

Table 2. Relative frequency of chromosomal point mutations detected in the studied *Salmonella* isolates (n=77) and their associated resistance phenotypes¹

Gene	Detected point mutations	Relative frequency, %	Resistance phenotype
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<i>gyrA</i>	A67P, D72G, V73I, G81C, G81D, G81H, G81S, D82G, D82N, S83A, S83F, S83Y, D87G, D87K, D87N, D87Y, S97P, L98V, A119E, A119S, A139A	100.0	Quinolones
<i>gyrB</i>	Y421C, R438L, S464T, S464Y, E466D	100.0	Quinolones
<i>parC</i>	T66I, G78D, S80I, S80R, E84G, E84K, W106G	1.3	Quinolones
<i>parE</i>	M438I, E454G, S458P, V461G, H462Y, A499T, V514G, V521F	100.0	Quinolones
<i>pmrA</i>	G15R, G53E, G53R, R81C, R81H	98.7	Colistin
<i>pmrB</i>	L14F, L22P, S29R, T92A, P94Q, E121A, S124P, N130Y, T147P, R155P, T156M, T156P, V161G, V161L, V161M, E166K, M186I, G206R, G206W, S305R	98.7	Colistin
<i>acrB</i>	R717L, R717Q	88.3	Azithromycin

<i>soxR</i>	R20H, G121D	100.0	Penicillins, phenicols, quinolones, rifampin, tetracyclines
<i>soxS</i>	E52K	100.0	Penicillins, phenicols, quinolones, rifampin, tetracyclines
<i>ramR</i>	T18P, G25A, R46P, T50P, Y59H, M84I, E160D	68.8	Penicillins, phenicols, quinolones, rifampin, tetracyclines

¹As reported by *in silico* analysis of assembled genomes with AMRFinderPlus 3.8[28]

For instance, all isolates carried multiple mutations in the quinolone resistance-determining region (QRDR), *gyrAB* and *parE* genes, regardless of being susceptible or not to ciprofloxacin. Likewise, 100% isolates had mutations in *soxRS* genes, which are known to confer MDR profiles [34]. Still, only 26% of isolates were classified as MDR. Conversely, mutations in *ramR* were strongly associated with the occurrence of MDR strains ($\chi^2=17.7$, $P<0.0001$).

Genomic analysis also revealed additional widespread mutations. Those of *pmrAB* genes, which are associated with colistin resistance, were present in all isolates.

Likewise, mutations in the *acrB* gene, which confer resistance to azithromycin, were detected in 88.3% of the strains. These two drugs, however, were not included in the

AST panel. Below, a detailed description of the relationship between AMR phenotypes

and genotypes will be presented for each of the tested antibiotic classes, as well as for MDR strains.

Aminoglycoside resistance

Aminoglycoside resistance was supported by the presence of genes encoding enzymatic inactivation mechanisms, such as phosphorylation (several *aph* alleles) and adenylation (*aadA2* gene). Comparative analysis with public *Salmonella* genomes revealed a strong association between aminoglycoside resistant and the source of the isolates ($\chi^2=89.2$, $P<0.0001$). Clinical strains had the highest proportion of aminoglycoside resistance genotypes (Fig 3) and were more likely to carry AMR genes against this antibiotic class as compared to those from food related sources (OR=7.7, 95CI 3.2-18.3). Among non-clinical samples, bovines were the most significant contributors of aminoglycoside resistant NTS (OR=5.0, 95CI 3.1-7.9). In general, however, the resistance profile was similar across sources, with a predominance of *aadA* and *aph* genes over the *acc* alleles.

Fig 3. Comparative genomic AMR profile of public NTS isolated from human clinical cases, foods, and food related sources in Mexico. Refer to S2 Table for accessions and metadata of isolates included in this analysis.

Beta-lactam resistance

Experimental isolates harbored only the Ambler's class A beta-lactamase-encoding genes *blaCARB-2* and *blaTEM-1*. These enzymes are known to confer resistance to all penicillins, as well as first, second and third generation cephalosporins [35]. The

276 *blaCARB-2* gene was the most abundant among isolates showing resistance to
 277 penicillins, especially in those of serovar Typhimurium (9/10). One isolate of serovar
 278 Anatum and one of monophasic (14,[5],12:i:-) Typhimurium also carried *blaTEM-1*,
 279 which encode another extended-spectrum beta-lactamase (ESBL) that hydrolyzes
 280 penicillins and first-generation cephalosporins [35]. There were 11 non-susceptible
 281 isolates that did not carry any of the known ESBL-encoding genes. Strikingly, however,
 282 10 out of these isolates had *ramR* mutations, which are associated with resistance
 283 towards several antibiotic classes, including penicillins.

284 Despite eight isolates were non-susceptible to third generation cephalosporins (3GC)
 285 and one to both 3GC and fourth generation cephalosporins (4GC), none carried ESBL-
 286 encoding genes associated with these phenotypes. As observed with penicillins,
 287 mutations in *ramR* appeared to be associated with non-susceptibility to cephalosporins
 288 since 100% of non-susceptible isolates to 3GC/4GC had these mutations.

289 Non-susceptibility to carbapenems was the least frequent among the tested antibiotic
 290 classes. No isolate resisted meropenem, while only one showed intermediate resistance
 291 to ertapenem and seven to imipenem. Again, no carbapenemase-encoding genes were
 292 found in the genome of any of the non-susceptible isolates. Interestingly, however,
 293 when using the epidemiological cutoff value of the inhibition zone (≤ 27 mm) set for
 294 meropenem in the European Union [22], 17 isolates (22%) were classified as non-wild
 295 type, which indicates some mechanism of carbapenem resistance could be emerging in
 296 the studied population.

297 In relation to other public *Salmonella* genomes from Mexico, clinical cases were also the
 298 main source of beta-lactam-resistant *Salmonella*: $\chi^2=72.5$, $P<0.0001$, $OR=12.8$, 95CI

4.5-36.5 (Fig 3). Again, bovines were the most important reservoir, among food related samples, of these AMR genotypes: $\chi^2=39.3$, $P<0.0001$, OR=6.1, 95CI 3.0-12.7. Class-A extended spectrum beta-lactamase (ESBL)-encoding genes (i. e. *blaCARB*, *blaTEM*) were the most abundant among isolates from all sources. Genes encoding class-C ESBLs (i. e. *blaCMY*) were also present in a small proportion of isolates from clinical cases (4.6%), vegetables (0.3%), and aquatic environments (1.6%), while the *blaOXA* gene, which encodes a class-D ESBL, was only detected in 9% of clinical strains. Fortunately, class-B ESBL-encoding genes were not detected in the genome of the studied public isolates.

Fluoroquinolone resistance

The main resistance mechanism we detected was that encoded by plasmid-mediated quinolone resistance (PMQR) genes, such as *qnrB19* and *qnrA1*, involved in quinolone target protection (DNA gyrase). This genomic feature, which is known to confer low-level quinolone resistance, was strongly associated with the intermediate resistance against ciprofloxacin observed in our study ($\chi^2=36.8$, $P<0.0001$). As mentioned before, we did not find statistical association between point-mutations in the QRDR and the observed phenotypes.

Among public *Salmonella* genomes, those from cattle origin were the major source of PMQR genes (Fig 3), with 32.5% of these isolates carrying *qnr* alleles ($\chi^2=196.9$, $P<0.0001$, OR=13.8, 95CI 8.5-22.2). Conversely, the proportion of PMQR-positive isolates was 18.2% in clinical strains, while it ranged from 1.6 to 6.0% across isolates from vegetables, seafood, and aquatic environments. Public genomes encoded additional quinolone resistance factors, such as efflux mechanisms (*oqxAB* genes).

However, these genes were not as abundant as *qnr* alleles and were restricted mostly to clinical strains (13.6%) and a very small proportion of vegetable isolates (0.4%).

Chloramphenicol resistance

Resistance to chloramphenicol in experimental isolates was mainly associated with efflux mechanisms (*floR* gene). This gene was present in most isolates of serovar Typhimurium (8/10), accounting for over 50% of chloramphenicol non-susceptible phenotypes. We detected a second resistance mechanism (antibiotic inactivation), encoded by a chloramphenicol acetyltransferase gene (*catA2*). However, this gene was present just in one isolate of serovar Give. Moreover, there were six non-susceptible isolates that were predicted as genotypically susceptible. Again, *ramR* mutations were strongly associated with chloramphenicol resistance ($\chi^2=8.1$, $P=0.0045$), OR=21.1 95CI 1.2-368.4.

As observed for previous antibiotic classes, there was a strong association between source and the proportion of phenicol-resistant *Salmonella* genotypes ($\chi^2=187.7$, $P<0.0001$) among public NTS isolates. Clinical strains were more likely to carry phenicols resistance genes than isolates from any other source: OR=11.7, 95CI 4.6-29.7. Likewise, cattle were the dominant reservoir, among food related samples, of these AMR genotypes (OR=15.6, 95CI 8.9-27.1). Still, the genomic AMR profile was similar across sources (Fig 3), with a predominance of efflux factors (i. e. *floR*) over enzymatic inactivation mechanisms (i. e. *catAB*).

Folate pathway inhibitors resistance

Regarding folate pathway inhibitors, the most abundant resistance mechanism among experimental isolates was that encoded by *sul* alleles (i. e. *sul1* and *sul2*), which were present in 11 isolates. Conversely, just one isolate carried the *dfrA12* gene along with the *sul1* gene. Overall, there was a discrete proportion of experimental isolates that resisted trimethoprim-sulfamethoxazole (16.9%).

The resistance profile of public genomes again revealed isolates of bovine origin are more likely to carry trimethoprim-sulfonamide resistance genes as compared to those from vegetables, seafood and aquatic environments ($\chi^2=142.0$, $P<0.0001$), OR=11.7 95CI 7.1-19.2 (Fig 3). In all these sources, *sul* and *dfrA* alleles were the most common. In general, however, clinical isolates represent a more significant source of trimethoprim-sulfonamide resistant *Salmonella* as compared to those from food related isolates ($\chi^2=175.9$, $P<0.0001$), OR=11.5 95CI 4.7-28.2.

Tetracycline resistance

Among experimental isolates, resistance to tetracycline was mainly associated with the presence of genes encoding efflux mechanisms (i. e. *tetABCG*). There were seven non-susceptible isolates that did not carry any of the known tetracycline resistance genes. However, all these isolates carried *ramR* mutations, which are associated with MDR profiles involving tetracyclines and other antibiotic classes [36].

Comparison with public *Salmonella* genomes revealed the highest proportion of tetracycline-resistance genotypes corresponded to bovine sources ($\chi^2=189.1$, $P<0.0001$), OR=10.9 95CI 7.1-16.9, as compared to vegetables, seafood, aquatic environments and even clinical cases (Fig 3). Regarding the genomic AMR profile,

isolates from all sources carried some variants of the same efflux-mediated resistance determinants (*tet* alleles).

MDR profiles

Among experimental isolates, MDR profiles were associated with the presence of a resistance island and, to a lesser extent, the occurrence of mutations in their genomes. For instance, most serovar Typhimurium isolates (9/10) carried SGI1. This genomic island harbors a class-1 integron containing multiple gene cassettes (i. e. *aadA2*, *blaCARB-2*, *floR*, *sul1*, *tetG*), which explains the observed MDR phenotypes of these isolates (Fig 4). This feature appears to be common in *S. enterica* ser. Typhimurium circulating in Mexico. The analysis of the whole set of Typhimurium isolates from Mexico deposited at NCBI (n=38) showed 55.3% of them have MDR genotypes. Among these, nearly 70% harbor multiple AMR genes associated with the ACSSuT phenotype. Refer to supplementary S3 Table for accession numbers and AMR genotypes of this group of isolates.

Fig 4. BLAST atlas analysis of SGI1 of 10 serovar Typhimurium isolates and one monophasic Typhimurium. The black slot corresponds to the backbone and the brown inner ring to the reference sequence. The most inner ring shows gene annotation, with AMR genes highlighted in bold text. The outer rings correspond to the serovars and sample names indicated. Regions with shared synteny between genomes and reference sequence are filled with intense color while blank spaces indicate lack of synteny. Refer to S1 Table for accession numbers and metadata of isolates.

As mentioned before, mutations in the *ramR* gene (Table 2) were strongly associated with MDR phenotypes as well. In fact, the probability of finding MDR strains was 50.5 times higher (95CI 2.9-881.3) in isolates carrying *ramR* mutations as compared to those lacking them.

Interestingly, plasmids had a rather discrete contribution to AMR phenotypes in general (Table 3). Only one third of the studied isolates (n=26) were predicted to carry seven different plasmids. Of these, three were small plasmids harboring few replication-related genes. The most abundant plasmid was the *Salmonella* virulence plasmid (pSLT), which was detected in all serovar Typhimurium isolates. The pSLT is a hybrid plasmid that carries a class-1 integron with several AMR gene cassettes against beta-lactams (*blaTEM*), chloramphenicol (*catA*), aminoglycosides (*aadA1*, *strAB*), and folate pathway inhibitors (*sul1*, *sul2*, *dfrA1*). However, the integron carried by our serovar Typhimurium isolates had a genomic context matching that of SGI1 instead of pSLT (see Fig 4 and supplementary S1 Figure).

406 Table 3. General features of the plasmids predicted per *Salmonella* serovar

Plasmid (NCBI accession)	Size, bp	Incompatibility group	Plasmid AMR genes ¹	Serovar (n) ²
pSLT (FN432031)	117,047	IncFIB(S)	aadA1 , <i>blaTEM</i> , <i>catA1</i> , <i>dfrA1</i> , <i>strAB</i> , sul1 , <i>sul2</i>	Typhimurium (10)
pK245 (DQ449578)	98,264	IncR	<i>aacC2</i> , <i>blaSHV2A</i> , catA2 , <i>dfrA14</i> , <i>strAB</i> , <i>sul2</i> , <i>tetD</i> , <i>tetR</i>	Give (1)
R64 (AP005147)	120,826	IncI1	<i>tetACD</i> , <i>strAB</i>	Fresno (4) Anatum (1)
pOLA52	51,602	IncX1	blaTEM , <i>oqxAB</i>	1,4,[5],12:i:- (1)
RSF1010	8,684	IncQ1	---	1,4,[5],12:i:- (1)
pBS512_2	2,089	--	--	Anatum (2) London (4) Reading (1)
pVCM01	1981	---	---	Anatum (2)

407 ¹Only AMR genes in bold were present in both the plasmid and the genomes.

408 ²Number of isolates of the same serovar carrying replicons of the plasmid.

Among the plasmids detected, the pK245 has the strongest resistance profile, carrying a class-1 integron with a sole resistance cassette (*dfrA14*), as well as another seven AMR genes (*tetAR*, *sul2*, *strAB*, *catA2*, *blaSHV-2*) distributed across the plasmid sequence. However, it is unlikely that pK245 plasmid was actually present in that isolate. Only 30% of this plasmid aligned to the genome of the single isolate (a serovar Give strain) carrying its replicons. Moreover, this isolate carried a chromosomal class-1 integron with seven resistance cassettes in a genomic context different from that of pK245 (see supplementary S2 Figure).

Replicons of the resistance plasmid R64 were also found in the genomes of serovar Fresno isolates (n=4), as well as one serovar Anatum isolate. This plasmid carries AMR genes against tetracyclines (*tetADR*) and aminoglycosides (*strAB*). Although over 70% of R64 aligned to the assembled genomes, none of its AMR genes were found in the genomes of isolates carrying its replicons, except *tetA*, which was detected in the serovar Anatum isolate (see supplementary S3 Figure). Finally, the monophasic Typhimurium isolate carried replicons of the pOLA52 plasmid. This is also a hybrid plasmid that harbors genes encoding a type IV secretion system, as well as AMR genes against quinolones (*oqxAB*) and beta-lactams (*blaTEM*). Although over 70% of this plasmid aligned to the referred genome, only the *blaTEM* gene was also present in the isolate, while *oqxAB* genes were missing (see supplementary S4 Figure).

Finally, the analysis of public genomes showed bovines and clinical cases are the most important sources, among those studied, of MDR NTS genotypes (Fig 3). In the other matrices, the proportion of MDR genotypes ranged from 2 to nearly 6%.

Discussion

Our results confirmed previous observations of widespread resistance to older antibiotics (i. e. tetracyclines, penicillins) among NTS from different sources. For instance, in Mexico, previous studies from over 10 years ago, as well as recent research, report these phenotypes at very high frequencies (up to 90% or higher) in beef isolates [12, 15, 17, 37-39]. Likewise, these AMR phenotypes are also common in NTS isolated in developed countries, a phenomenon that is thought to be driven by the use of these antimicrobials in food-producing animals [40].

Regarding tetracyclines, selective pressure has likely resulted in the extensive acquisition of efflux mechanisms (*tetABCG* alleles), which are usually carried either in plasmids or in the chromosome of NTS [41]. Our results also support certain point mutations could sustain this phenotype in isolates lacking *tet* alleles, providing evidence of convergent evolution. As observed here, 100% of isolates that did not carry *tet* alleles had *ramR* mutations. These mutations are known to induce over expression of the AcrAB-RND efflux system [42], which confer resistance against multiple drugs, including tetracyclines.

Strikingly, results showed cattle seemed more relevant than any other source, including clinical cases, as a source of tetracycline resistance NTS. Although the number of clinical strains that were available for comparative analysis was low (n=22), these findings are relevant from a public health perspective. Tetracyclines are highly important antimicrobials [19] since they are among the few alternatives to treat human infections caused by *Brucella* spp., a pathogen that is associated with cattle.

The rate of resistance to penicillins observed here was not as high as that reported recently in NTS isolated from beef in Mexico [14, 15, 17, 43]. However, this variation is likely associated with the region where isolates originated. Hence, the relevance of cattle as a relevant source of penicillin resistant NTS could not be discarded, as shown by the genomic comparison with other public isolates from Mexico conducted here. In beta-lactam resistance, class-C and class-B ESBLs are the leading concerns. Class-C ESBLs confer resistance to most beta-lactams (except carbapenems) and are not inhibited by clavulanic acid, while class-B metallo-beta-lactamases confer the strongest resistance phenotype, involving all known beta-lactams and clavulanic acid [35]. Fortunately, resistance to 3GC, 4GC and carbapenems was rare among experimental isolates, which is in line with recent studies [9]. Apparently, food isolates in Mexico, as well as in the US and Europe [40], do not seem to be a significant source of NTS resistant to 3GC/4GC and carbapenems. Still, we observed some strains (10-15%) showed non-susceptibility to 3GC, 4GC and carbapenems, despite lacking ESBL-encoding genes. This could be the result of selective pressure and may lead to the emergence of resistance against these critically important antimicrobials, as shown by the moderate proportion of isolates (22%) exceeding the EUCAST's ECOFF value for meropenem. In Mexico, 3GC used in human medicine (i. e. cefotaxime, ceftriaxone) are also approved, besides ceftiofur, to treat several bovine diseases [44]. Ceftiofur is associated with the emergence of ceftriaxone resistance among livestock and poultry NTS in the United States [40]. Hence, it is important to revise approval of both ceftiofur and other 3GC for veterinary use in Mexico. It has been reported that restrictions (voluntary or law-enforced) in 3GC use in the United States and Canada have helped reduce 3GC resistance among NTS associated with animals [40]. In any case, given the

limited scientific evidence available in Mexico, continuous surveillance of NTS from foods is of upmost importance to support future risk management decisions. We also observed decreased susceptibility to ciprofloxacin among experimental isolates, a phenomenon that has been associated with the presence of PMQR genes. Particularly, the *qnrB19* allele, which was present in over 50% of our experimental isolates, has also been reported as predominant among NTS strains from the United States [40]. Recent studies from Mexico have also documented high rates (36-44%) of decreased susceptibility to ciprofloxacin in beef NTS isolates [12], although these authors did not determine AMR genotypes. Moreover, a previous study by our research group documented *qnr* and *oqx* alleles were widely distributed among NTS isolated from cattle feces, carcasses and ground beef [17]. Furthermore, the genomic AMR profiling of public NTS isolates from Mexico conducted here, showed *qnr* alleles are widely disseminated among bovine isolates but not in those from vegetables, seafood, aquatic environments or even clinical strains.

In the context of Mexico's cattle production, acquisition and conservation of PMQR genes in NTS is likely the result of selective pressure associated with the use of quinolones (i. e. enrofloxacin), which are approved to treat bovine respiratory, digestive, urinary and skin diseases, among others [44]. Since PMQR genes are carried in plasmids, this increases the risk of their dissemination under positive selective pressure. Many studies report quinolone resistance is rare in NTS isolated from cattle [3, 45]. Hence, it has been suggested that the use of these drugs in cattle could not be linked to the emergence of quinolone resistance among cattle NTS isolates [1]. However, PMQR genes are known to confer low-level quinolone resistance, which usually goes undetected when using CLSI breakpoints [46]. Therefore, we believe the role of PMQR

genes as a contributing factor to quinolone resistance in NTS from cattle should not be minimized. Especially, considering PMQR genes have been associated with increasing resistance to quinolones in NTS isolated from foods [47, 48]. In summary, cattle appear to be a relevant source of low-level quinolone resistant NTS. However, to which extent this could result in the emergence of clinical fluoroquinolone resistance among cattle NTS isolates is yet to be determined.

Regarding aminoglycoside resistance, although the number of non-susceptible isolates in the experiment was low, the CLSI guidelines emphasize these antimicrobials may appear active *in vitro* but are not effective clinically against *Salmonella* and, thus, susceptible isolates should not be reported as such [21]. This may explain why isolates carrying *aadA* and *aph* alleles exhibited susceptible phenotypes, a phenomenon that has been observed in previous experiments [17]. Hence, it appears that WGS is a better tool to monitor aminoglycoside resistance as compared to AST. In that sense, our comparative genomics of public NTS from Mexico showed aminoglycoside resistant is less frequent in isolates from food related sources as compared to clinical cases.

Resistance to chloramphenicol and trimethoprim-sulfamethoxazole was also frequently observed among experimental isolates (approximately 17 and 20%, respectively). These phenotypes were sustained by acquired AMR genes (i. e. *cat*, *flo*, *sul*, and *dfrA* alleles). In the case of chloramphenicol, *ramR* mutations, which are known to confer phenicol resistance [36], seemed to play a role as well.

These findings could be explained by the selective pressure exerted by the use of these drugs in food-producing animals. For instance, trimethoprim-sulfamethoxazole is approved as a wide spectrum antibiotic to treat all sorts of bacterial infections in livestock and poultry in Mexico [44]. Likewise, although chloramphenicol is no longer

approved for these purposes, there are other phenicols (i. e. florfenicol) registered. This is likely the reason why phenicol resistance is consistently reported in NTS from animal foods in Mexico, in proportions that vary from moderate (16-23%) [12, 15, 39] to very high (>90%) [14, 43]. Our comparative genomics further supported this analysis since *floR*, which confer resistance to both phenicols [49], was the dominant resistance factor harbored by NTS from food related sources and clinical cases in Mexico.

Regarding MDR profiles, it is interesting to note the higher frequency of MDR isolates in ground beef as compared to lymph nodes. Perhaps this was influenced by the high number of MDR serovar Typhimurium isolates that were exclusively present in ground beef. However, some other factors could further explain these findings. For instance, research on ground beef consistently report high rates of MDR profiles in NTS, regardless of the relative serovar representation [12, 13, 15]. Conversely, studies involving NTS isolated from bovine lymph nodes usually report low levels of MDR phenotypes [8, 50]. To date, researchers have not been able to decipher the route of entry of NTS circulating in bovine lymph nodes. It has been suggested that fly bites could directly introduce the pathogen into the host's blood [51]. Since this is reasonably likely to occur in feedlots, and NTS survives within the host cells [52], which are not affected by antibiotic treatments, these isolates may not be exposed to the same antimicrobial selective pressure as those circulating in the feces. Hence, they exhibit a weaker AMR profile. This study confirmed pan-susceptible phenotypes are more common among NTS isolates from bovine lymph nodes, while those from ground beef tend to have stronger AMR profiles, as well as higher rates of MDR phenotypes.

Among the genetic determinants associated with MDR phenotypes, SGI1 was the most frequently observed. It was present in all MDR serovar Typhimurium isolates (8/10),

which accounted for 40% of the total number of MDR strains. The SGI1 contains a class-1 integron and multiple AMR gene cassettes (*aadA2*, *floR*, *tetG*, *bla-CARB-2*, *sul1*) conferring resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline [53]. This penta-resistance profile is typical of MDR *S. enterica* ser. Typhimurium DT104 carrying SGI1 [29] and was very similar to that observed here. Although SGI1 is not self-mobilizable, it can be transferred to other hosts by donor cells harboring conjugative plasmids [54]. In fact, SGI1 has been recognized as a key factor for the rapid dissemination of strains [55]. Unfortunately, although serovar Typhimurium is known to be dominant in NTS circulating in foods and clinical cases in Mexico, it has been poorly characterized in terms of its genomic AMR profile [9]. According to this review, the SGI1 associated ACSSuT phenotype has been reported in a modest proportion of MDR isolates (9.5%) collected from clinical cases, chicken, pork, and beef between 2006 and 2013. Analysis of public serovar Typhimurium isolates from Mexico deposited at NCBI (n=38) showed nearly 37% of them carry AMR genes supporting DT104-like or even stronger phenotypes. Nonetheless, further research is needed to establish if the high rate of DT104-like isolates observed here is a local dissemination phenomenon. Especially, considering half of our MDR Typhimurium isolates also showed decreased susceptibility to ciprofloxacin, which further amplifies the risks posed by these strains to human health. Moreover, it is important to better assess the contribution of point mutations and plasmids to AMR in NTS from foods, which appeared to be modest in the studied sample.

In summary, this research showed beef is a moderate source of MDR NTS harboring several AMR genes of human clinical significance. Among the sequenced public NTS isolates from foods and clinical cases in Mexico to date, those from cattle have the

highest proportion of MDR genotypes. However, this should be interpreted with caution since the number of sequenced isolates from other species (i. e. chicken, pigs) and clinical cases is very limited in public databases.

Taken together, our results show it is vital to improve NTS control in apparently healthy animals to prevent its dissemination along the food chain and, consequently, human exposure to MDR strains. Particularly, we believe attaining significant improvements in AMR meat safety may require the identification and removal (or treatment) of product harboring MDR NTS, instead of screening for isolates showing resistance to individual antimicrobial classes. Such measures do not seem realistic, in the context of the meat industry of many countries, where testing practices are limited to *Salmonella* spp. isolation and confirmation. However, most nations have embraced the WHO global action plan on AMR. Thus, they should eventually set up existing technologies, such as WGS, which provide the shortest path to accomplish these goals.

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References

1. Schmidt JW. SMART Antimicrobial Resistance Goals to Drive Meat Safety Improvement. *Meat and Muscle Biology*. 2020;4(2). doi: 10.22175/mmb.11218.
2. Wall BA, Mateus A, Marshall L, Pfeiffer DU, Lubroth J, Ormel HJ, et al. Drivers, dynamics and epidemiology of antimicrobial resistance in animal production. Rome, Italy: Food and Agriculture Organization of the United Nations; 2016. Available from: www.fao.org/publications
3. Vikram A, Rovira P, Agga GE, Arthur TM, Bosilevac JM, Wheeler TL, et al. Impact of "Raised without Antibiotics" Beef Cattle Production Practices on Occurrences of Antimicrobial Resistance. *Appl Environ Microbiol*. 2017;83(22). doi: 10.1128/AEM.01682-17. PMID: 28887421
4. Levent G, Schlottermeier SE, Ives KN, Norman SD, Lawhon GH, Loneragan GH, et al. Population dynamics of *Salmonella enterica* within beef cattle cohorts followed from single-dose metaphylactic antibiotic treatment until slaughter. *Appl Environ Microbiol*. 2019;85(23): e01386-19. doi: <https://doi.org/10.1128/AEM.01386-19>. PMID: 31519659
5. Ohta N, Norby B, Loneragan GH, Vinasco J, den Bakker HC, Lawhon SD, et al. Quantitative dynamics of *Salmonella* and *E. coli* in feces of feedlot cattle treated with ceftiofur and chlortetracycline. *PloS One*. 2019;14(12): e0225697. doi: 10.1371/journal.pone.0225697. PMID: 31791047
6. Rovira P, McAllister T, Lakin SM, Cook SR, Doster E, Noyes NR, et al. Characterization of the Microbial Resistome in Conventional and "Raised Without

Antibiotics" Beef and Dairy Production Systems. *Front Microbiol.* 2019;10: 1980.

doi: 10.3389/fmicb.2019.01980. PMID: 31555225

7. SSA. Anuarios de Morbilidad 1984-2019: Secretaría de Salud, México; 2019.

Available from: <https://www.gob.mx/salud/acciones-y-programas/anuarios-de-morbilidad-1984-2019>

8. Gragg SE, Loneragan GH, Nightingale KK, Brichta-Harhay DM, Ruiz H, Elder JR, et al. Substantial within-animal diversity of *Salmonella* isolates from lymph nodes, feces, and hides of cattle at slaughter. *Appl Environ Microbiol.* 2013;79(15): 4744-50. doi: 10.1128/AEM.01020-13. PMID: 23793628

9. Godinez-Oviedo A, Tamplin ML, Bowman JP, Hernandez-Iturriaga M. *Salmonella enterica* in Mexico 2000-2017: Epidemiology, Antimicrobial Resistance, and Prevalence in Food. *Foodborne Pathog Dis.* 2020;17(2): 98-118. doi: 10.1089/fpd.2019.2627. PMID: 31647328

10. Laufer AS, Grass J, Holt K, Whichard JM, Griffin PM, Gould LH. Outbreaks of *Salmonella* infections attributed to beef -United States, 1973-2011. *Epidemiol Infect.* 2015;143(9): 2003-13. doi: 10.1017/S0950268814003112. PMID: 25427978

11. CDC. Outbreak of *Salmonella* Infections Linked to Ground Beef: Centers for Disease Control and Prevention (CDC) 2018. Available from: <https://www.cdc.gov/salmonella/newport-10-18/index.html>

12. Realpe-Quintero M, Barba-Leon J, Perez-Montano JA, Pacheco-Gallardo C, Gonzalez-Aguilar D, Dominguez-Arias RM, et al. Genetic diversity and antimicrobial resistance of *Salmonella* serotypes recovered throughout the beef production chain and from patients with salmonellosis. *PeerJ.* 2018;6: e5482. doi: 10.7717/peerj.5482. PMID: 30155367

13. Cabrera-Diaz E, Barbosa-Cardenas CM, Perez-Montano JA, Gonzalez-Aguilar D, Pacheco-Gallardo C, Barba J. Occurrence, serotype diversity, and antimicrobial resistance of salmonella in ground beef at retail stores in Jalisco state, Mexico. J Food Prot. 2013;76(12): 2004-10. doi: 10.4315/0362-028X.JFP-13-109. PMID: 24290673
14. Aguilar-Montes de Oca S, Talavera-Rojas M, Soriano-Vargas E, Barba-Leon J, Vazquez-Navarrete J, Acosta-Dibarrat J, et al. Phenotypic and genotypic profile of clinical and animal multidrug-resistant Salmonella enterica isolates from Mexico. J Appl Microbiol. 2018;124(1): 67-74. doi: 10.1111/jam.13615. PMID: 29044980
15. Ballesteros-Nova N, Rubio-Lozano MS, Delgado-Suárez EJ, Méndez-Medina RD, Braña-Varela D, Rodas Suárez O. Perfil de resistencia a antibióticos de serotipos Salmonella spp. aislados de carne de res molida en la Ciudad de México. Salud Publica Mex. 2016;58(3): 1-7. doi: <https://doi.org/10.21149/spm.v58i3.7897>. PMID: 27598935
16. Quesada A, Reginatto GA, Ruiz Español A, Colantonio LD, Burrone MS. Resistencia antimicrobiana de Salmonella spp aislada de alimentos de origen animal para consumo humano. Rev Peru Med Exp Salud Publica. 2016;33(1): 32. doi: 10.17843/rpmesp.2016.331.1899.
17. Delgado Suárez EJ, Ortiz López R, Gebreyes WA, Allard MW, Barona-Gomez F, Rubio MS. Genomic surveillance links livestock production with the emergence and spread of multi-drug resistant non-typhoidal Salmonella in Mexico. J Microbiol. 2019;57(4). doi: DOI 10.1007/s12275-019-8421-3. PMID: 30721457
18. Palós Gutiérrez T, Rubio Lozano MS, Delgado Suárez EJ, Rosi Guzmán N, Soberanis Ramos O, Hernández Pérez CF, et al. Lymph nodes and ground beef

- 666 as public health importance reservoirs of Salmonella spp. Rev Mex Cienc Pecu.
667 2020;11(3):795-810. doi: 10.22319/rmcp.v11i3.5516.
- 668 19. WHO. Critically important antimicrobials for human medicine. 6th Revision 2018:
669 World Health Organization; 2019. Available from:
670 <https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/>
- 671 20. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a
672 standardized single disk method. Am J Clin Pathol. 1966;45(4): 493-6. PMID:
673 5325707
- 674 21. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed.
675 CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute;
676 2020. Available from: <http://em100.edaptivedocs.net/dashboard.aspx>
- 677 22. EUCAST. MIC and zone diameter distributions and ECOFFs: European
678 Committee on Antimicrobial Susceptibility Testing; 2020. Available from:
679 https://www.eucast.org/mic_distributions_and_ecoffs
- 680 23. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al.
681 Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an
682 international expert proposal for interim standard definitions for acquired
683 resistance. Clin Microbiol Infect. 2012;18(3): 268-81. doi: 10.1111/j.1469-
684 0691.2011.03570.x. PMID: 21793988
- 685 24. Andrews S. FastQC: a quality control tool for high throughput sequence data;
686 2010. Available from: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
- 687 25. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina
688 Sequence Data. Bioinformatics. 2014;btu170. doi: 10.1093/bioinformatics/btu170.
689 PMID: 24695404

26. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, et al. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res.* 2014;42(D1): D581-D91. doi: 10.1093/nar/gkt1099. PMID: 24695404
27. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 2012;19(5): 455-77. doi: 10.1089/cmb.2012.0021. PMID: 22506599
28. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, et al. Validating the AMRFinder Tool and Resistance Gene Database by Using Antimicrobial Resistance Genotype-Phenotype Correlations in a Collection of Isolates. *Antimicrob Agents Chemother.* 2019;63(11). doi: 10.1128/AAC.00483-19. PMID: 31427293
29. Leekitcharoenphon P, Hendriksen RS, Le Hello S, Weill FX, Baggesen DL, Jun SR, et al. Global Genomic Epidemiology of Salmonella enterica Serovar Typhimurium DT104. *Appl Environ Microbiol.* 2016;82(8): 2516-26. doi: 10.1128/AEM.03821-15. PMID: 26944846
30. Petkau A, Stuart-Edwards M, Stothard P, Van Domselaar G. Interactive microbial genome visualization with GView. *Bioinformatics.* 2010;26(24):3125-6. doi: 10.1093/bioinformatics/btq588. PMID: 20956244
31. Yoon SH, Park YK, Kim JF. PAIDB v2.0: exploration and analysis of pathogenicity and resistance islands. *Nucleic Acids Res.* 2015;43(Database issue): D624-30. doi: 10.1093/nar/gku985. PMID: 25336619

32. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, et al. In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. *Antimicrob Agents Chemother.* 2014;58(7): 3895-903. doi: 10.1128/AAC.02412-14. PMID: 25336619
33. Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, Maciejewski A, et al. Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Res.* 2016;44(W1): W147-53. doi: 10.1093/nar/gkw419. PMID: 27190236
34. Koutsolioutsou A, Martins E, White DG, Levy SB, Demple B. A soxRS-Constitutive Mutation Contributing to Antibiotic Resistance in a Clinical Isolate of *Salmonella enterica* (Seroovar Typhimurium). *Antimicrob Agents Chemother.* 2001;45(1): 38-43. doi: 10.1128/AAC.45.1.38-43.2001. PMID: 11120941
35. Lee JH, Takahashi M, Jeon JH, Kang LW, Seki M, Park KS, et al. Dual activity of PNGM-1 pinpoints the evolutionary origin of subclass B3 metallo-beta-lactamases: a molecular and evolutionary study. *Emerg Microbes Infect.* 2019;8(1): 1688-700. doi: 10.1080/22221751.2019.1692638. PMID: 31749408
36. Abouzeed YM, Baucheron S, Cloeckeaert A. ramR mutations involved in efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrob Agents Chemother.* 2008;52(7): 2428-34. doi: 10.1128/AAC.00084-08. PMID: 18443112
37. Zaidi MB, McDermott P, Fedorka-Cray P, Leon V, Canche C, Hubert S, et al. Nontyphoidal *Salmonella* from human clinical cases, asymptomatic children and raw retail meats in Yucatan, Mexico. *Clin Infect Dis.* 2006;42: 21-8. doi: 10.1086/498508. PMID: 16323087

38. Miranda JM, Mondragón AC, Martínez B, Guarddon M, Rodríguez JA. Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. *J Food Protect.* 2009;72(5): 966-71. doi: <https://doi.org/10.4315/0362-028X-72.5.966>. PMID: 19517722
39. Perez-Montaña JA, González-Aguilar D, Barba J, Pacheco-Gallardo C, Campos-Bravo CA, García S, et al. Frequency and Antimicrobial Resistance of *Salmonella* Serotypes on Beef Carcasses at Small Abattoirs in Jalisco State, Mexico. *J Food Prot.* 2012;75(5): 867-73. doi: 10.4315/0362-028X.JFP-11-423. PMID: 22564935
40. McDermott PF, Zhao S, Tate H. Antimicrobial Resistance in Nontyphoidal *Salmonella*. *Microbiol Spectr.* 2018;6(4). doi: 10.1128/microbiolspec.ARBA-0014-2017. PMID: 30027887
41. Nguyen F, Starosta AL, Arenz S, Sohmen D, Donhofer A, Wilson DN. Tetracycline antibiotics and resistance mechanisms. *Biol Chem.* 2014;395(5): 559-75. doi: 10.1515/hsz-2013-0292. PMID: 24497223
42. Liu YY, Chen CC. Computational Analysis of the Molecular Mechanism of RamR Mutations Contributing to Antimicrobial Resistance in *Salmonella enterica*. *Sci Rep.* 2017;7(1): 13418. doi: 10.1038/s41598-017-14008-5. PMID: 29042652
43. Villalpando-Guzman S, Vázquez-Quñones CR, Natividad-Bonifacio I, Curiel-Quesada E, Quiñones-Ramírez EI, Vázquez-Salinas C. Frecuencia, susceptibilidad antimicrobiana y patrón de adherencia de *Salmonella enterica* aislada de carne de pollo, res y cerdo de la Ciudad de México. *Rev Chilena Infectol.* 2017;34(5): 458-66. doi: 10.4067/S0716-10182017000500458. PMID: 29488588

- 760 44. SADER. Productos químico-farmacéuticos vigentes: Secretaría de Agricultura y
761 Desarrollo Rural, Gobierno de México; 2020. Available from:
762 [https://www.gob.mx/cms/uploads/attachment/file/512374/PRODUCTOS_VIGENT](https://www.gob.mx/cms/uploads/attachment/file/512374/PRODUCTOS_VIGENTES_QF_2019.pdf)
763 [ES_QF_2019.pdf](https://www.gob.mx/cms/uploads/attachment/file/512374/PRODUCTOS_VIGENTES_QF_2019.pdf)
- 764 45. Smith AB, Renter DG, Cernicchiaro N, Shi X, Nagaraja TG. Prevalence and
765 Quinolone Susceptibilities of Salmonella Isolated from the Feces of Preharvest
766 Cattle Within Feedlots that Used a Fluoroquinolone to Treat Bovine Respiratory
767 Disease. Foodborne Pathog Dis. 2016;13(6): 303-8. doi: 10.1089/fpd.2015.2081.
768 PMID: 26990393
- 769 46. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone
770 resistance: a multifaceted threat. Clin Microbiol Rev. 2009;22(4): 664-89. doi:
771 10.1128/CMR.00016-09. PMID: 19822894
- 772 47. Pribul BR, Festivo ML, Rodrigues MS, Costa RG, Rodrigues EC, de Souza MM, et
773 al. Characteristics of Quinolone Resistance in Salmonella spp. Isolates from the
774 Food Chain in Brazil. Front Microbiol. 2017;8: 299. doi: 10.3389/fmicb.2017.00299.
775 PMID: 28352250
- 776 48. Karp BE, Campbell D, Chen JC, Folster JP, Friedman CR. Plasmid-mediated
777 quinolone resistance in human non-typhoidal Salmonella infections: An emerging
778 public health problem in the United States. Zoonoses Public Health. 2018;65(7):
779 838-49. doi: 10.1111/zph.12507. PMID: 30027554
- 780 49. Arcangioli MA, Leroy-Sétrin S, Martel JL, Chaslus-Dancla E. A new
781 chloramphenicol and florfenicol resistance gene flanked by two integron structures
782 in Salmonella typhimurium DT104. FEMS Microbiol Lett. 1999;74: 327-32. doi:
783 10.1111/j.1574-6968.1999.tb13586.x. PMID: 10339826

- 784 50. Gragg SE, Loneragan GH, Brashears MM, Arthur TM, Bosilevac JM,
785 Kalchayanand N, et al. Cross-sectional study examining *Salmonella enterica*
786 carriage in subiliac lymph nodes of cull and feedlot cattle at harvest. *Foodborne*
787 *Pathog Dis.* 2013;10(4): 368-74. doi: 10.1089/fpd.2012.1275. PMID: 23566273
- 788 51. Olafson PU, Brown TR, Lohmeyer KH, Harvey RB, Nisbet DJ, Loneragan GH, et
789 al. Assessing Transmission of *Salmonella* to Bovine Peripheral Lymph Nodes
790 upon Horn Fly Feeding. *J Food Prot.* 2016;79(7): 1135-42. doi: 10.4315/0362-
791 028X.JFP-15-414. PMID: 27357032
- 792 52. Ohi ME, Miller SI. *SALMONELLA: A Model for Bacterial Pathogenesis.* *Annu Rev*
793 *Med.* 2001;52: 259-74. doi: 10.1146/annurev.med.52.1.259. PMID: 11160778
- 794 53. Mulvey MR, Boyd DA, Olson AB, Doublet B, Cloeckert A. The genetics of
795 *Salmonella* genomic island 1. *Microbes Infect.* 2006;8(7): 1915-22. doi:
796 10.1016/j.micinf.2005.12.028. PMID: 16713724
- 797 54. Hall R. *Salmonella* genomic islands and antibiotic resistance in *Salmonella*
798 *enterica*. *Future Microbiol.* 2010;5(10): 1525-38. doi: 10.2217/fmb.10.122. PMID:
799 21073312
- 800 55. Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP. The genomic
801 island SGI1, containing the multiple antibiotic resistance region of *Salmonella*
802 *enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other
803 *S. enterica* serovars. *J Bacteriol.* 2005;187(13): 4401-9. doi:
804 10.1128/JB.187.13.4401-4409.2005. PMID: 15968049

Supporting information

S1 Table. NCBI accessions and metadata of our fully sequenced *Salmonella* isolates collected from bovine lymph nodes and ground beef (XLSX).

S2 Table. NCBI accessions, metadata and antimicrobial resistance genotypes of fully sequenced public *Salmonella* isolates from Mexico included in this study

S3 Table. NCBI accesions, metada and antimicrobial resistance genotypes of fully sequenced public *Salmonella enterica* ser. Typhimurium isolates from Mexico included in this study

S1 Figure. BLAST atlas of *Salmonella* virulence plasmid pSLT of ten experimental serovar Typhimurium isolates. The black slot corresponds to the backbone and the inner ring to the reference plasmid sequence and genes. Map generated with GView, version 1.7 through the tblastx program, with e-value=0.001, alignment cutoff=50, identity cutoff=70, and no filtering of low complexity sequences. Refer to S1 Table for isolates accessions and metadata.

S2 Figure. BLAST atlas of plasmid pK245 of one experimental serovar Give isolate. The black slot corresponds to the backbone and the inner ring to the reference plasmid sequence and genes. Map generated with GView, version 1.7 through the tblastx program, with e-value=0.001, alignment cutoff=50, identity cutoff=70, and no filtering of low complexity sequences. Refer to Table S1 for isolate accession and metadata.

828

829 S3 Figure. BLAST atlas of plasmid R64 of experimental serovar Fresno and Anatum

830 isolates. The black slot corresponds to the backbone and the inner ring to the reference

831 plasmid sequence and genes. Map generated with GView, version 1.7 through the

832 tblastx program, with e-value=0.001, alignment cutoff=50, identity cutoff=70, and no

833 filtering of low complexity sequences. Refer to S1 Table for isolate accessions and

834 metadata.

835

836 S4 Figure. BLAST atlas of plasmid pOLA52 of one experimental serovar monophasic

837 Typhimurium isolate. The black slot corresponds to the backbone and the inner ring to

838 the reference plasmid sequence and genes. Map generated with GView, version 1.7

839 through the tblastx program, with e-value=0.001, alignment cutoff=50, identity cutoff=70,

840 and no filtering of low complexity sequences. Refer to Table S1 for isolate accession and

841 metadata.

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ID_serovar	Source	Collection date	AMK	GEN	GB	AMC	CTX	CRO	FEP	MEM	ETP	IPM	CH	ST	CP	TEI	aadA2	aph(3)-Ia	aph(3'')-Ib	aph(6)-Id	blaTEM-1	blaCARB-2	catA2	floR	su1	su2	dfrA12	qnrA1	qnrB19	tetA	tetB	tetC	tetG	fosA7.7	qacEDelta1	mdsAB
AK69_Anatum	LN	4/3/17																																		
AK70_Anatum	LN	4/24/17				R								I																						
AK71_Anatum	LN	4/24/17																																		
AK72_Anatum	GB	4/24/17																																		
AK73_Anatum	GB	4/24/17																																		
AK74_Anatum	LN	4/24/17														I																				
AK75_Anatum	LN	5/1/17																																		
AK76_Anatum	LN	5/1/17																																		
AK78_Anatum	LN	5/3/17																																		
AK79_Anatum	LN	5/8/17																																		
AK81_Anatum	LN	5/10/17																																		
AK85_Anatum	GB	5/22/17														I	R																			
AM39_Anatum	LN	5/24/17												R	I	R																				
AM40_Anatum	GB	5/24/17																																		
AM44_Anatum	LN	6/5/17																																		
AM46_Anatum	LN	6/12/17																																		
AM58_Anatum	LN	8/9/17														I																				
AM68_Anatum	LN	9/25/17																																		
AM79_Anatum	LN	10/16/17																																		
AM89_Anatum	LN	4/23/18																																		
AM90_Anatum	GB	4/23/18																																		
AM95_Anatum	LN	4/23/18																																		
AM98_Anatum	LN	4/30/18											I																							
AK80_Fresno	GB	5/8/17											I				R																			
AM78_Fresno	LN	10/16/17			I												R																			
AM86_Fresno	LN	4/9/18															R																			
AM87_Fresno	GB	4/9/18					I										R																			
AS56_Gve	LN	9/24/18	R	R	R	R							R		I	R																				
AN13_I4,[5],12;:-	LN	5/7/18			R	R											R																			

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ID_serovar	Source	Collection date	AMK	GEN	GB	AMC	CTX	CRO	FEP	MEM	ETP	IPM	CH	ST	CP	TEI	aadA2	aph(3)-Ia	aph(3'')-Ib	aph(6)-Id	blaTEM-1	blaCARB-2	catA2	floR	su1	su2	dfrA12	qnrA1	qnrB19	tetA	tetB	tetC	tetG	fosA7.7	qacEDelta1	mdsAB
AM81_Kentucky	LN	1/29/18										I																								
AN03_Kentucky	LN	5/7/18														I																				
AN22_Kentucky	GB	5/28/18			I		R	I	I			I				I																				
AN23_Kentucky	LN	5/28/18			R			I					R	R	I	R																				
AN24_Kentucky	LN	6/4/18			R	I							R	R	I	R																				
AN27_Kentucky	LN	6/4/18			R	I							R	R	I	R				</

Fig2

Percentage of isolates having the feature

Fig3

Fig4