



## Characterization of Antimicrobial Resistant (AMR) *Salmonella Enterica* Isolates Associated With Cattle at Harvest in Mexico

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**Abstract:** Despite being the target of control efforts for many decades, *Salmonella enterica* continues to be linked with a large amount of foodborne illnesses and outbreaks worldwide. Over the years, *Salmonella* isolated from meat products have exhibited a high level of antibiotic resistance. In this study, a total of 351 *Salmonella* isolates, recovered from cattle fecal samples ( $n = 31$ ), hides ( $n = 105$ ), and beef carcasses ( $n = 215$ ) from 3 abattoirs in Mexico were analyzed for antimicrobial susceptibility. Resistance to at least one antimicrobial drug was found in 205 (58.4%) isolates and 20 different resistance phenotypes were observed among this *Salmonella* isolates set. Resistance to tetracycline (40.2%) and nalidixic acid (21.1%) was most commonly observed. Additionally, the most common multidrug-resistant (MDR) phenotypes shared resistance to chloramphenicol, streptomycin, tetracycline, and trimethopim/sulfamethoxazole (11.3%), resistance to ampicillin, tetracycline, and trimethopim/sulfamethoxazole (3.4%), and resistance to ampicillin, streptomycin, and tetracycline (2.5%). When it came to antimicrobial resistance phenotypes in each abattoir, we determined there was no statistical difference in the frequency of resistant vs. susceptible *Salmonella* isolates among the three abattoirs ( $P > 0.05$ ). These data indicate that *Salmonella* isolates recovered from beef cattle in Mexico are commonly resistant to antimicrobials and often multiple antimicrobials. In Mexico, antimicrobial resistance, and in particular, multidrug-resistance, maybe of particular concern due to the much higher prevalence of *Salmonella* in retail beef. This may lead to the spread of resistance and to the reduction of antibiotic efficacy for the control of animal and human infections. Promoting control measures and inspection standards on imported animals and food products should be applied to avoid the spread of antibiotic resistance in various populations and among countries.

**Keywords:** antimicrobial resistance, beef, cattle, Mexico, *Salmonella*

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## Introduction

Antimicrobial resistance (AMR) is defined by the CDC as the resistance of bacteria to an antibiotic that was originally intended to treat the infections caused by bacteria (Center for Disease Control, 2015). Multidrug-resistance (MDR) is defined as the resistance of a microorganism to several antimicrobial classes (Center for Disease Control, 2013). There

have been some reports indicating that the use of antimicrobials in animal production, for either treatment or prophylactic purposes, can lead to antimicrobial resistant bacteria. However, the effect of the relationship between antimicrobial resistance in food animals and human health is still not clearly understood (Mathew et al., 2007; Maron et al., 2013; WHO, 2014).

In the Mexican Animal Health Federal Law of 2007, the use of antimicrobial growth promoters (AGPs) in animal feed is very limited, with many commonly used antimicrobial drugs requiring a veterinary prescription (Maron et al., 2013). After the elimination of most AGPs, were provided exception and some of these include: avoparcin, spiramycin, salinomycin,

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vancomycin, avilamycin, bambermycin, bacitracin, tylosin, virginiamycin, and monensin. Present intends to eliminate the use of these antimicrobial drugs as growth promoters in Mexican animal feeds do not exists. The ministry of agriculture stated that the 25 million head of cattle on the other hand, are still given substantial doses of antimicrobials, however these require a veterinary prescription (Maron et al., 2013).

In public health, one of the greatest challenges in predicting outbreaks caused by MDR pathogens, such as *Salmonella*, is to have qualified monitoring agencies. Based on the specific recommendations by the World Health Organization (WHO), there are very few countries (Holland, France, Norway, Sweden, Denmark, Finland, and the United States) that have implemented monitoring agencies (Salas, 2015). Despite the fact that Mexico does not have a monitoring agency equivalent to the National Antimicrobial Resistance Monitoring System (NARMS) in the U.S., the Mexican government has built up measures, for example, NOM-064-ZOO-2000, NOM-051-ZOO-1995 and NOM-012-ZOO-1993 among others, to oversee veterinary antimicrobials sales, animal transport, and specifications for the improvement of drugs intended for food animal use. The biggest challenge for the Mexican government, however, lies in guaranteeing that all producers consent to all these rules and carry out robust antimicrobial stewardship programs (Salas, 2015).

*Salmonella* is a foodborne pathogen that is commonly found in beef cattle (Kunze et al., 2008; Laufer et al., 2015). The emergence of antimicrobial drug resistance in *Salmonella* has become a major public health concern in recent years (Lynne et al., 2008; Hur et al., 2012). *Salmonella* is a common food contaminant that can develop resistance to antimicrobial drugs used to treat human 78 or animal illnesses (Hur et al., 2012; Louden et al., 2012). In Mexico, the majority of gastrointestinal infections are caused by *Salmonella*, and in 2010 alone, more than 100,000 salmonellosis cases were reported to the National Center for Epidemiological Surveillance and Control of Diseases (Secretaria de Salud, 2010). The fact that *Salmonella* can develop resistance to antimicrobial drugs used to treat human infections may hinder the efficacy of these drugs against *Salmonella* infections resulting in a public health threat (Hur et al., 2012; Perez-Montano et al., 2012; Center for Disease Control, 2014).

Recently, increased reports of *Salmonella* prevalence and antimicrobial resistance have surfaced (Hur et al., 2012; Michael and Schwarz, 2016). An overall increase in the percentage of *Salmonella* antimicrobial resistance was reported by Su et al. (2004). Antimicrobial resistance in *Salmonella* ranged from 20 to 30% in the 90s, to an increased 70% in some countries in the 2000s.

Though there is variation in the resistance rate, it is dependent on the serovars and the antimicrobials (Su et al., 2004). MDR to several antimicrobial drugs, including third-generation cephalosporins, has been observed in certain commonly reported *Salmonella* serovars (i.e., Typhimurium, Montevideo, Kentucky, and Newport). Third-generation cephalosporins are among the last line of antibiotics used to treat severe human infections. Significant evidence in recent years show that the same plasmids that are encoding for resistance in these serovars, also encoding additional virulence characteristics, which can induce more severe human illnesses (Foley and Lynne, 2008; Fricke et al., 2009; Chuanchuen et al., 2010). Albeit most illnesses caused by nontyphoidal *Salmonella* serovars are typically self-constraining, effective antimicrobial treatment is imperative if the infection spreads past the digestive tract (Secretaria de Salud, 2010). The lack of regulation when it comes to antimicrobial drug use in many developing countries, has led to the misuse and overuse of antimicrobials. Actions must be taken to reverse new trends showing increased multi-drug resistance in *Salmonella*, as demonstrated in several studies from developing countries (Weisner et al., 2009; Perez-Montano et al., 2012; WHO, 2014).

Antibiotic resistance is encoded by several genes, many of which are readily transferred among different bacteria. Selective pressure is not the only factor that plays a critical role in *Salmonella*'s drug resistance. Evidence of clonal dissemination of drug resistance genes also play a critical role for both human and animal *Salmonella* infections (Butaye et al., 2006; Lucarelli et al., 2010; Hauser et al., 2012). Additionally, there is also evidence that *Salmonella* resistance genes are located on mobile genetic elements such as plasmids, transposons, gene cassettes and genomic islands, which to some extent, can then be transferred to other bacteria, thus, posing a public health threat (Alcaine et al., 2007; Lindsey et al., 2009; Brichta-Harhay et al., 2011; Frye and Jackson, 2013). The objective of this study was to determine the antimicrobial resistance profiles of 351 *Salmonella* enterica isolates randomly selected from previous studies (Gragg et al., 2013; Narvaez-Bravo et al., 2013; Pond et al., 2016), recovered from cattle feces, hides, and carcasses in three Mexican abattoirs.

## Materials and Methods

### Selection of *Salmonella* isolates

From a collection of *Salmonella* isolates obtained in previous studies, 351 *Salmonella* isolates were random-

ly selected for characterization in this study. *Salmonella* isolates were obtained from fecal grab samples ( $n = 31$ ), beefhides ( $n = 104$ ), and beef carcasses ( $n = 215$ ) samples. Isolates included in this study were obtained from three abattoirs in Southeastern Mexico (Veracruz, Merida, and Cancun). The *Salmonella* isolates from Veracruz included in this study were obtained during five separate sampling periods: July and December 2009, and April, August, and December 2010. The Merida isolates were recovered during 4 time periods: March, September, and December 2012, and March 2013. Finally, the Cancun isolates were recovered from the Cancun abattoir during three time periods in October and December 2012, and March 2013 (Table 1).

### Antimicrobial resistance characterization

All 351 *Salmonella* presumptive positive isolates were streaked onto tryptic soy agar (Beckton Dickinson, Sparks, MD) containing 5% defibrinated sheep blood and incubated at 37°C for 18 to 20 h. Antibiotic susceptibility was evaluated using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) following the manufacturer's instructions. The following quality control organisms were used: *E. coli* 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. Additionally, as defined by NARMS, a single isolate exhibiting resistance to three or more antimicrobial classes was classified as multidrug-resistant (MDR; FDA, 2015).

Fourteen antibiotics were used for testing: amoxicillin/clavulanic acid (2:1 ratio), ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. The Minimum Inhibitory Concentration (MIC) breakpoints for 12 of the antimicrobials tested were interpreted using the National Committee for Clinical Laboratory Standards for microdilution broth methods, and the MIC breakpoints for streptomycin and erythromycin were interpreted with the National Antimicrobial Resistance Monitoring System (NARMS) breakpoints (Center for Disease Control, 2011; Clinical and Laboratory Standards Institute, 2015; FDA, 2015).

### Statistical analysis

Statistical analyses were performed using R (R, Version 3.2.2, The R Foundation for Statistical

**Table 1.** Total *Salmonella* isolates from cattle and beef in Mexico tested for antimicrobial resistance

Total no. isolates	City	Sample type
31	Veracruz	Fecal
85	Veracruz	Carcass
45	Veracruz	Hides
83	Merida	Carcass
32	Merida	Hides
48	Cancun	Carcass
25	Cancun	Hides

Computing, Vienna, Austria). Fisher's Exact Test of independence with a 95% confidence limit (CL) was used for each comparison with a  $2 \times 2$  factorial design. Additionally, Fisher's Exact Test with a 95% confidence limit (CL) with Bonferroni's multiple comparison post-test was used for comparisons with a  $2 \times 3$  factorial design. The variation in the probability of recovery of resistant isolates versus susceptible isolates within each abattoir from a specific area (Veracruz vs. Yucatan Peninsula abattoirs), and within each animal sampling location (fecal, hides, and carcass) was evaluated. Additionally, the variation in the probability of recovery of multi-drug resistant isolates versus single resistant isolates within each abattoir from a specific area, and within each animal sampling location was evaluated. *P*-values less than 0.05 were considered significant.

## Results and Discussion

### Prevalence of *Salmonella* and MDR *Salmonella* on cattle fecal, hide, and carcass samples

We analyzed a total 351 *Salmonella* isolates for antimicrobial sensitivity screening. Our findings revealed that 205 (58.4%) *Salmonella enterica* isolates exhibited resistance to at least one or more antimicrobial drug. Resistance to tetracycline was the most common profile, and it was exhibited by 40.2% of the isolates (82 isolates), followed by resistance to nalidixic acid in 21.1% (43 isolates). No isolates presented resistance to gentamicin, azithromycin, and sulfisoxazole.

In addition, 26.3% (54 of 205) of these *Salmonella* isolates had a multidrug-resistance (MDR) phenotype. The most common MDR phenotypes exhibited by these isolates shared resistance to chloramphenicol, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole (11.3%), followed by resistance to ampicillin, tetracycline, and trimethoprim/sulfamethoxazole (3.4%),

**Table 2.** Antimicrobial resistance phenotypes of *Salmonella enterica* isolates recovered from the feces, carcass, and hides of beef cattle at harvest in three Mexican abattoirs ( $n = 351$ )

Total no.	Resistance-types <sup>1</sup>	Veracruz	Merida	Cancun	Total %
1	AMC, AMP, FOX, TIF, CRO, CHL, NAL, STR, TET, SXT	1	0	0	0.28%
2	STR, TET	14	0	2	4.56%
3	STR, TET, SXT	1	0	0	0.28%
4	TET	67	12	4	23.65%
5	CHL, STR, TET, SXT	19	0	4	6.55%
6	AMP, TET, SXT	0	4	3	1.99%
7	AMP, STR, TET, SXT	0	4	1	1.42%
8	NAL	0	32	12	12.54%
9	CHL, STR, TET	0	1	1	0.57%
10	AMP, SXT	0	1	0	0.28%
11	AMP, NAL, STR, TET, SXT	0	1	0	0.28%
12	AMP, CHL, STR, TET, SXT	0	1	2	0.85%
13	NAL, TET	0	3	4	1.99%
14	AMP, NAL, SXT	0	1	0	0.28%
15	CHL, NAL, STR, TET, SXT	0	1	3	1.14%
16	AMP, TIF, CRO, CHL, CIP, STR, TET, SXT	0	0	1	0.28%
17	CHL, NAL, STR, TET	0	1	0	0.28%
18	NAL, STR, TET, SXT	0	0	1	0.28%
19	TET, SXT	0	0	1	0.28%
20	AMP STR SXT	0	2	0	0.57%
21	*PANSUSCEPTIBLE	60	56	30	41.60%
	TOTAL	162	120	69	100.00%

<sup>1</sup>AMC, Amoxicillin-clauvulonic Acid; AMP, Ampicillin; FOX, Cefoxitin; TIF, Ceftiofur; CRO, Ceftriaxone; CHL, Chloramphenicol; NAL, Nalidixic Acid; STR, Streptomycin; TET, Tetracycline; SXT, Trimethoprim/Sulfomethoxazole.

and resistance to ampicillin, streptomycin, and tetracycline (2.5%; Table 2). Notably, when it came to antimicrobial resistance phenotypes in each abattoir, we determined there was no statistical difference in the frequency of resistant vs. susceptible *Salmonella* isolates among the three abattoirs (Table 3).

In this study, there was no observed statistical difference in the frequency of resistant vs. susceptible *Salmonella* isolates across the 3 abattoirs or animal sampling locations (fecal, hides and carcasses) within each abattoir. However, we were able to determine that there was an effect on the recovery of tetracycline and nalidixic acid among the abattoirs. These findings are consistent with a study by Perez-Montano et al. (2012), where they reported that resistance to tetracycline (46.2%) was the most common profile among the *Salmonella* isolates recovered from beef carcasses ( $n = 78$ ), followed by resistance to nalidixic acid (17.9%) in the state of Jalisco, Mexico. Authors suggested that based on their data and data in other studies that antimicrobial resistance to tetracycline, streptomycin, and chloramphenicol is very common among *Salmonella* isolates due to a longer and frequent use of these antimicrobials in animal production (Perez-Montano et al., 2012).

*Salmonella* isolates obtained from slaughter processing plants (carcass swabs and ground product) report Tetracycline resistance as the most common antimicrobial resistance profile in the U.S. In the latest NARMS Annual Animal Report (Center for Disease Control, 2011), of the 340 *Salmonella* isolates tested from cattle at slaughter, tetracycline resistance was the most common with 30.6%, this is consistent with our findings and those by Perez-Montano (Perez-Montano et al., 2012). Additionally, tetracycline resistance is also among the common antimicrobial resistance profiles found in retail meats in the U.S. based on the latest NARMS retail meat report (FDA, 2015). However, when it came to nalidixic acid, only 1.8% of the isolates from retail meat in the U.S. were resistant (Center for Disease Control, 2011).

In developing countries, Tetracycline is commonly used for treatment in human infections, and as a prophylactic agent in veterinary medicine. Tetracycline is classified as a broad-spectrum agent, meaning it can affect a wide range of gram-positive and gram-negative bacteria (Pezzella et al., 2004; Stevenson et al., 2007). Our findings are similar to those of previous studies, in that resistance to tetracycline is very common among beef cattle bacterial isolates (Thaker et al., 2010; Center for Disease Control, 2011; Perez-

**Table 3.** Prevalence of antimicrobial susceptibility profiles of *Salmonella enterica* isolates by abattoir type ( $n = 351$ )<sup>1</sup>

Abattoir type	N	% Susceptible isolates	% Resistant isolates
Veracruz	164	37.20	62.80
Peninsula Abattoirs	187	45.45	54.55

<sup>1</sup>Values were not significantly different based on an odds ratio of 1.128925 (95% CI: 0.7878931 to 1.6178969, p-value of 0.538).

Montano et al., 2012). However, we are not inferring that this resistance is necessarily attributed to the selective pressure of antibiotics use in the cattle, as this information was not collected.

Additional findings in our study include high resistance to fluoroquinolones and resistance to extended-spectrum cephalosporins. The high levels of resistance to nalidixic acid observed in this study may be a concern due to the fact that nalidixic acid resistance has been associated with reduced susceptibility to fluoroquinolones in bacteria belonging to the *Enterobacteriaceae* family (Veldman et al., 2011). Fluoroquinolones are the last line of treatment for severe *Salmonella* infections, and continued antimicrobial resistance is of concern due to the potential threat to global public health (Acheson and Hohmann, 2001).

Additionally, 7.84% (16 of 205) of the *Salmonella* isolates showed resistance to a combination of tetracycline and streptomycin. Streptomycin is an antimicrobial that has limited current usage in human medicine, but plays a critical role in veterinary medicine, where it is used for the treatment of bacterial infections in cattle, sheep and pigs. Streptomycin is also critical for bacterial disease control in plants (Pezzella et al., 2004). Our findings are consistent with the latest NARMS Annual Animal Report (2011), where the percentage of streptomycin resistance in *Salmonella* isolates from slaughtered cattle has steadily increased over the years from 1997 to 2011 (Center for Disease Control, 2011).

Based on the definition for multidrug-resistance, a total of 26.3% (54 of 205) of the *Salmonella* isolates were found to be MDR. Traditional first-line antimicrobial drugs, such as chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, tend to have higher reports of widespread resistance, which is consistent with our findings (Sjölund-Karlsson et al., 2011). Treatment of invasive and severe *Salmonella* infections include the use of fluoroquinolones (e.g., ciprofloxacin) or extended-spectrum cephalosporins (e.g., ceftriaxone), due to the widespread resistance of first-line antimicrobial drugs (Su et al., 2004; Miranda et al., 2009).

Comparing our findings to those reported by NARMS in the U.S., we can see that the percentages of

MDR phenotypes observed for the Mexican *Salmonella* isolates in our study vary in comparison to what is reported in the U.S. (Center for Disease Control, 2011). In the U.S., resistance to at least ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole was 1.5% ( $n = 340$ ) in *Salmonella* isolates from cattle in 2011. Additionally, resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline was 12.6% ( $n = 340$ ) in all *Salmonella* isolates tested (Center for Disease Control, 2011).

Interestingly, our findings of MDR phenotypes in the isolates from the Veracruz abattoir are an indication that we can have antimicrobial resistance in the bacterial isolates, despite the implementation of food safety measures in an abattoir. Evidence of this also happens in the U.S., as NARMS reports show there are resistant pathogens in U.S. beef abattoirs (Kunze et al., 2008; Center for Disease Control, 2011). A major limitation of our study was the lack of serotype information. This information is certainly warranted to determine if the serovars of these *Salmonella* isolates are serovars commonly associated with human illnesses. In serovars such as *Salmonella* Typhimurium, Newport, and Montevideo, antimicrobial resistance is more significant than in other serovars due to the fact that these serovars tend to have the phenotypic trait of MDR; hence serotype information can help us understand the epidemiology of drug-resistant *Salmonella*.

## Conclusions

In conclusion, data presented in this study clearly illustrates the presence of *Salmonella* drug-resistant isolates in beef cattle and the variability of antimicrobial susceptible profiles present in *Salmonella* isolates from cattle in Mexico. Antimicrobial resistant *Salmonella* in cattle feces, and on hides and carcasses have the potential to reach consumers by means of cross-contamination of the carcass during the slaughter and dressing procedure.

Tetracyclines, penicillins, and sulfonamides are among the most common antimicrobials used in animal production in developing countries, although they are no longer used to treat human infections. Therefore, the presence of drug-resistant isolates in animal production could potentially lead to the development and spread of resistance in humans.

One of the best risk management practices to prevent widespread antimicrobial drug resistance in *Salmonella*, and other foodborne pathogens present in food-producing animals such as beef cattle, is to monitor and report antimicrobial drugs use. Additionally,

mitigation of antimicrobial resistance in *Salmonella*, can also be achieved by educating veterinary and human medicine specialists on the proper usage of antimicrobial drugs. Finally, additional research is necessary to characterize the genetic mechanisms responsible for the resistance profiles encountered among these *Salmonella* isolates.

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