Frequency, territorial distribution and antimicrobial resistance of *Salmonella* spp. on bovine cattle feces from the Altos Sur region of Jalisco State, Mexico

Frecuencia, distribución territorial y resistencia a los antimicrobianos de *Salmonella* spp. aislada de heces de ganado bovino de la región Altos Sur en el estado de Jalisco, México

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ABSTRACT

Multidrug resistance is a global public health problem. In 2017, in Mexico, *Salmonella* caused 192,771 food-borne zoonosis cases. Sonora, Hidalgo, Mexico State and Jalisco have reports of multi-resistant *Salmonella* strains in chicken and beef carcasses in slaughterhouses; however, the prevalence in livestock herds is unknown. From January 2012 to October 2013, 144 diarrheal stool samples were collected from bovine hatchlings from the Altos Sur region of the Jalisco State. As a result, the presence and serotype of *Salmonella* was determined, as well as the antibiotic resistance profile, and the genetic relationship, using PFGE. The *Salmonella* prevalence was 4.16 % (6/144) in feces, identifying the serotypes and the genetic relationship, using PFGE. The *Salmonella* was determined, as well as the antibiotic resistance profile, and some of them, additionally, to amikacin, cefotaxima and/of ceftriaxone. In addition, the persistence and potential spread of two *Salmonella* Anatum strains was discovered in one herd. This is the first study conducted in neonate bovine in the Jalisco State, Mexico, to detect multidrug resistant *Salmonella*. Continuous monitoring of multi-drug resistance in animal biota for human food and ongoing training of veterinary doctors are key elements for efficient prophylaxis and antimicrobial pharmacotherapy.

Key Words: *Salmonella* spp.; Multidrug-resistantance; Bovine cattle.

RESUMEN

La multifarmacorresistencia es un problema mundial de salud pública. En el 2017, en México, *Salmonella* causó 192,771 casos de zoonosis de origen alimentario. Sonora, Hidalgo, Estado de México y Jalisco tienen reportes de *Salmonella* multirresistente en canales de pollo y res en mataderos. Sin embargo, se desconoce la prevalencia en animales en hatos ganaderos. De enero-2012 a octubre-2013 se recolectaron 144 muestras de heces diarreicas de neonatos bovinos de la región Altos Sur del estado de Jalisco. Se determinaron los serotipos de *Salmonella*, el perfil de multifarmacorresistencia y el genotipo mediante PFGE. Se encontró una prevalencia del 4.16% (6/144) de *Salmonella* en heces, recuperándose los serotipos Anatum, Pullorum, Poona, Typhi, Gallinarum y *Salmonella enterica* subsp. arizonae. Todas las cepas mostraron resistencia a ampicilina, cefalotina, trimetoprim-sulfametoxazol y adicionalmente algunas a amikacin, cefotaxima y/o ceftriaxona. Se descubrió una persistencia y propagación de dos cepas de *Salmonella* Anatum en un hato. Este es el primer estudio realizado en bovinos recién nacidos en el estado de Jalisco, México, para la detección de *Salmonella* multirresistente. Es necesario el monitoreo continuo de la multifarmacorresistencia en la biota de animales para alimento humano y una capacitación continua de médicos veterinarios para una eficiente profilaxis y farmacoterapia antimicrobiana.

Palabras claves: *Salmonella* spp.; Multifarmacorresistencia; Ganado bovino.

INTRODUCTION

World beef production grew annually at an average rate of 0.6 percent during the recent decade, reaching a record high of 62.48 million tons of carcass meat in 2018 (United States Department of Agriculture; USDA, 2019). Mexico ranked as the eighth meat producer, with 3.2 percent of the total production (USDA, 2019). In Mexico, the Ministry of Agriculture and Rural Development, through the Agri-Food and Fisheries Information Service, reported a steady growth in beef production over the past decade, at an average annual rate of 1.7 percent, reaching a historical maximum of 1.98 million tons of carcass meat in 2018. Estimates indicate that in 2019 national production increased 2.4 percent, with what could be 2.03 million tons, while projections suggest that in 2020 it will only grow 1.9 percent (USDA, 2019). The...
Salmonella 2019 to the National Center for Epidemiological Surveillance in Infections, with 192,771 salmonellosis cases reported in 2020 (COMECARNE, 2019). Per capita consumption of beef in Mexico increased between 2015 and 2019, from 15.2 to 15.4 kilograms per year. Since 2015, Mexico recorded a surplus balance in the beef trade balance, which historically had been in deficit. In 2019, net exports of 273.5 thousand tons of beef were reported, the highest volume in the last five years, with an annual increase of 36.9 percent (COMECARNE, 2017; SADER, 2019; COMECARNE, 2019). According to USDA Foreign Agricultural Trade of the United States table, the surplus balance in the trade balance could increase 32.6 percent in 2021 (FATUS-USDA, 2020). The most purchased cuts are steak and milanesa, followed by pulp, piece and ground meat (COMECARNE, 2019). Jalisco state is the second largest producer of bovine meat in Mexico, with 13.2 % of the 1,915 million tons of national production in 2019, and this amount represents the mobilization and slaughtering of approximately 280 thousand heads of cattle (SADER, 2019). The Jalisco regional livestock union, in its 2019 annual report noted that the northeast area of the state, which includes the Altos Norte and Altos Sur regions, participates with 46.4% of the state livestock production (UGRJ, 2019). Livestock production in the Altos Sur region maintained an increasing trend during the 2013-2018 period, with 2018 being the year with the highest growth in the value of livestock production in the region, representing 34.4% of the entire state production (IIEG, 2019). Among the livestock products for whose production the Altos del Sur region stands out, the egg is in first place with 47.4% of the total value of the region’s production, followed by pork meat with 19.4 %, bovine milk with 17.2 %, bovine carcass meat with 11.3 % and poultry meat with 4.6 % (IIEG, 2019). In the region, from 2013 to 2019, the number of cattle herds producing beef cattle rose from 106 to 159 (IIEG, 2019). Livestock production in the Altos Sur region in the Jalisco State, Mexico. 

MATERIALS AND METHODS
Design of the investigation
This is a descriptive cross-sectional study to determine the frequency, territorial distribution and antimicrobial resistance of Salmonella spp., on bovine cattle feces from the Altos Sur region in the Jalisco State, Mexico, during the period from January-2012 to October-2013.

Sample collection
A total of 144 diarrheal feces samples were collected from cattle from fourteen different herds, located in eleven of the twelve municipalities in the Altos Sur region of Jalisco State, Mexico, during a 21-month period (January-2012 to October-2013), on the condition that the cattle did not exceed four months of age. Samples were collected from at least one herd of cattle in each of the municipalities of the region; unfortunately, it was not possible to collect a sample from San Ignacio Cerro Gordo. Five grams of cattle diarrheal feces were collected in sterile bags (Speci-Sponge, Nasco Whirl-Pak, Modesto, CA) with 20 mL of buffered peptone water (BD, Franklin Lakes, NJ), thus fulfilling the procedure described by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS, 1996). 

Salmonella isolation
Each sample was added to 40 mL of buffered peptone water for a total volume of 60 mL, homogenized for 2 min with a bag mixer (Stomacher® 80 Biomaster), and incubated at 35 °C for 18 to 22 h. After incubation, 1.0 and 0.1 mL aliquots were inoculated into 9 and 9.9 mL of tetraphionate broth (BD) and Rapaport-Vassiliadis broth (BD), respectively. 

The emergence of multidrug-resistant (MDR) Salmonella isolates among animals and humans has been documented, and represents a public health concern (Arthur et al., 2008). Some reports have indicated that the use of antimicrobials in animal production for disease therapy, prophylaxis, and growth enhancement promotes the selection of resistant bacteria, although the impact of these uses on human health is not clearly understood yet (Mathew et al., 2007; WHO, 2008). Resistance of pathogenic bacteria to antimicrobials used in human therapy may result in lower efficacy of these drugs against infections and may subsequently threaten public health (Geornaras et al., 2012). 

Guidelines for prudent use of antimicrobial agents may help to slow down the selection for resistance, and should be based on knowledge regarding the normal susceptibility patterns of the causative agents and consider the potential human health problems (Aarestrup, 2005). According to the World Health Organization (WHO, 2008), surveillance programs are needed to monitor the antimicrobial prevalence and resistance of Salmonella isolates from animals, humans, and food (Aidara-Kane et al., 2018). 

In Mexico, there are no surveillance reports of Salmonella strains with antimicrobial resistance in newborn cattle. It is necessary to generate monitoring programs for MDR Salmonella presence in the main livestock areas. These programs can be useful to develop public health policies for the regulation of drugs used in food-producing animals and to design control measures to prevent the spread of MDR bacteria (Whichard et al., 2010). The purpose of this study was to determine the frequency, territorial distribution and antimicrobial resistance of Salmonella on cattle feces from the Altos Sur region of Jalisco State, Mexico.

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Both selective enrichment broths were incubated at 42 °C for 16 h. After incubation, 1.0 mL aliquots of each enrichment broth were individually transferred to tubes containing 10 mL of M broth (BD) and incubated at 35 °C for 6 to 8 h. After incubation, 0.5 mL aliquots of M broth culture from each tube were combined for an enzyme-linked immunosorbent assay (Salmonella VIA, TECRA International) (Hughes et al., 2003) according to the manufacturer’s instructions. Aliquots from tetrathionate and Rapapport-Vassiliadis cultures from samples that were positive for Salmonella with the enzyme-linked immunosorbent assay were individually streaked onto brilliant green sulfua agar (BD), bismuth sulfite agar (Bioxon), and xylose lysine Tergitol 4 agar (BD). All plates were incubated at 35 °C for 24 to 48 h. From each selective agar type, we selected and streaked at least three colonies with characteristics typical for Salmonella, onto triple sugar iron agar (BD) and lysine iron agar (BD) and incubated at 35 °C for 24 h. Isolates with typical biochemical reactions were then streaked onto Tryptic Soy agar (TSA; BD), incubated at 35 °C for 24 h, and tested for slide agglutination using polyvalent serum A-Vi (BD). Isolates that produced nontypical triple sugar iron agar and lysine iron agar reactions, and/or negative serological reactions, were tested for additional biochemical analysis in Methyl Red–Voges Proskauer medium, Simmons Citrate agar, Urease Rustigian and Stuart broth, motility medium, and Phenol Red Salicin and Dulcitol Fermentation broths (Bioxon) (USDA-FSIS, 2008). One isolate from each positive sample was randomly chosen for serotyping and antimicrobial susceptibility testing.

Serotyping

Salmonella cultures were reactivated in TSB at 35 °C for 24 h and then individually streaked on Brilliant Green Sulfua agar (BD) plates. From each culture, one colony with typical Salmonella characteristics was individually inoculated on TSA slants, incubated at 35 °C for 24 h, reconfirmed by biochemical and serological testing as previously described, and then shipped to the Institute of Epidemiological Diagnosis and Reference "Dr. Manuel Martínez Báez" (InDRE, Mexico City, Mexico) for serotype identification, according to the Kauffman-White scheme (1974).

Pulsed-field gel electrophoresis (PFGE) for subtyping of Salmonella serotypes

Individual bacterial colonies of Salmonella strains, grown over 24 h at 37 °C on Trypticase Soy Agar (BD) plates were directly suspended using cotton swabs in 3 mL of TE buffer (100 mM Tris and 100 mM EDTA, pH 8.0). Cell suspensions were adjusted with TE buffer to 0.700 absorbance using a Varian Cary 50 Scan UV-visible spectrophotometer. Cell suspension aliquots (400 µL) were transferred to 1.5 mL microcentrifuge tubes. Lysozyme (10 mg/mL stock solution) and proteinase K (20 mg/mL stock solution) were added at a final concentration of 1 mg/mL each, and mixed several times by pipetting up and down. The bacterial suspensions were incubated at 37 °C for 15 min. UltraPure™ Agarose gel (Invitrogen) was prepared in 0.5X TBE to a final concentration of 1 % and maintained at 55 °C in a water bath. Following the lysozyme-proteinase K incubation, 7 µL of 20 % sodium dodecyl sulfate and 140 µL of 1 % UltraPure™ Agarose gel (Invitrogen) were mixed with each bacterial suspension with the help of a pipette. This bacterium-agarose mixture was immediately added to plug molds (Bio-Rad Laboratories). The plugs were allowed to solidify for 10 min at 4 °C, then transferred to 2 mL round-bottom tubes containing 1.5 mL of TESP buffer (50 mM Tris, 50 mM EDTA, pH 9.0; 1 % sodium lauryl sarcosine; 1 mg of proteinase K per mL), and incubated with gentle mixing in a shaker water bath at 55 °C for 2 h. After the completion of proteolysis, the plugs were transferred to 1.5 mL microcentrifuge tubes containing 200 µL of sterile, preheated (50 °C) distilled water and incubated for 10 min at 50 °C with gentle mixing in a shaker water bath. Subsequently, four 50 °C washes were done in a shaker water bath for 15 min each with 400 µL of preheated (50 °C) TE buffer (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0), and plugs cooled to room temperature in TE buffer. For restriction endonuclease digestion, two 1 mm thick slices of each plug were incubated at 37 °C for 3 h with 3 µL of XbaI (Invitrogen), in 100 µL of the restriction enzyme buffer (containing 4 µL of restriction enzyme buffer (10x), 0.1 % BSA and 29 µL of sterile DNase/ RNase-Free Distilled Water) as recommended by the manufacturer.

The plug slices of the samples were loaded and electrophoresed in 1 % UltraPure™ agarose (Invitrogen) with 2 liters of standard 0.5X TBE running buffer. Electrophoresis of the prepared samples was performed on the CHEF-DR III system (Bio-Rad). The electrophoretic conditions used were as follows: initial switch time, 2.16 s; final switch time, 55 s; run time, 22 h; angle, 120 °; gradient, 6.0 V/cm; temperature, 14 °C; ramping factor, linear. After electrophoresis, the gels were stained for 30 min in 1 liter of sterile distilled water containing 50 mL of ethidium bromide (10 mg/mL) and destained with three 1-liter distilled water washes (30 min each); then, photographed under UV illumination with Kodak film (Edas 290) in 8-bit negative format in gray scale. Additional analysis and construction of dendrograms and trees were done with GelCompar II software (version 2.0; Applied Math, Sint-Martens-Latem, Belgium). The molecular size marker (XbaI-digested DNA from S. enterica serotype Braenderup H9812) was included in all runs as a control. The consistency of the control DNA patterns confirmed the reproducibility of the procedure.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined according to the disk diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI, 2009; CLSI, 2010). Antimicrobial susceptibility test disks (BBL, BD, Sparks, MD) were used for the following antimicrobials of veterinary and human health importance: ampicillin (AMP, 10 µg), gentamicin (GEN, 10 µg), amikacin (AN, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25 and 23.75 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), polymyxin
B (PB, 300 units) and cephalothin (CEP, 30 µg). *Escherichia coli* ATCC 25922 was used as a quality control. Inhibition zones were measured as MIC breakpoints according to the M100-S20, (CLSI, 2010). Multidrug resistance was reported when resistance to three or more antimicrobials was observed (Miranda et al., 2009). It is worth mentioning that the antibiotics selection was made according to the therapeutic recommendation against suspected gastrointestinal diseases caused by resistant and non-resistant *Salmonella* (CDC, 2009).

**Complementary study**

Additionally, in October 2013 and January 2017, a survey was conducted to evaluate the sale frequency of the different groups of antibiotics in veterinary pharmacies of the region, with the aim of explaining the possible resistance of isolated *Salmonella* strains. Thirty-six veterinary pharmacies were included in this study. The number of sold vials of each antibiotics group in the last two months was recorded, as an indicator of the pharmacological preferences of the region's veterinary doctors or livestock farmers.

**Data analysis**

The differences significance (p≤ 0.05) in *Salmonella* isolation frequency by cattle farm were evaluated with the Kruskal-Wallis test and the Fisher’s LSD test and the preference for the best-selling antimicrobials in the region’s veterinary pharmacies were evaluated with the chi-square test in the Statistical Package for Social Science, version 11.5 for Windows (SPSS, Chicago, IL).

**RESULTS**

**Table 1.** Frequency of *Salmonella* serotypes in cattle feces from the Altos Sur region in Jalisco State, Mexico (Jan-2012 to Oct-2013).

| Municipalities | Cattle herd | No. of stool samples of cattle feces per herd | No. of *Salmonella* spp. positive samples (Percentage with respect to the total samples analyzed per herd,%)²/ (No. of strains isolated) | *Salmonella* serotype isolate and another subsp. (No. of strains isolated) |
|----------------|-------------|-----------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Mexticacán     | A           | 13                                            | 2 (15.38)²/2                                                                                                                     | Pullorum(1) *Salmonella enterica* subsp. arizonae(1)                      |
|                | B           | 9                                             | 0²                                                                       |                                                                          |
|                | C           | 3                                             | 0²                                                                       |                                                                          |
|                | D           | 4                                             | 0²                                                                       |                                                                          |
|                | E           | 13                                            | 0²                                                                       |                                                                          |
|                | F           | 6                                             | 0²                                                                       |                                                                          |
|                | G           | 7                                             | 0²                                                                       |                                                                          |
|                | H           | 16                                            | 0²                                                                       |                                                                          |
|                | I           | 24                                            |                                                                           |                                                                          |
|                | J           | 10                                            | 1 (10.00)²/1                                                            Gallinarum(1)                                                               |
|                | K           | 14                                            | 2 (14.29)²/3                                                            Anatum(2) Typhi(1)                                                          |
|                | L           | 18                                            | 0²                                                                       |                                                                          |
|                | M           | 6                                             | 0²                                                                       |                                                                          |
|                | N           | 1                                             | 1 (100)²/1                                                              Poona(1)                                                                    |
| Total          |             | 144                                           | 6 (4.16)¹/7                                                                                                                                     |

¹Within these columns, values with different letters are significantly different (p≤0.05) by Kruskal-Wallis test and Fisher’s LSD test.

**Frequency and territorial distribution of *Salmonella* in cattle**

*Salmonella* spp. was isolated from 6 (4.16 %) of 144 diarrheal feces samples of bovine neonates of exploitation, collected from eleven municipalities from the Altos Sur region in Jalisco State, Mexico (Table 1). The isolated *Salmonella enterica* subsp. enterica serotypes were Anatum, Pullorum, Poona, Typhi and Gallinarum, and a sample contained *Salmonella enterica* subsp. arizonae. *Salmonella* was isolated in bovine diarrheal stool samples from four of the eleven municipalities that were included in the study (Figure 1).

**Antimicrobial susceptibility and PFGE patterns of *Salmonella* serotypes**

Seven *Salmonella* strains were characterized to determine their antimicrobial susceptibility and PFGE patterns (Figure 2). The antimicrobial resistance criterion is represented by gray scale boxes. Five antibiotypes were identified. All *Salmonella* strains have resistance to AMP, CEP and STX. The *Salmonella* Anatum, Typhi and Gallinarum serotype strains additionally showed resistance to AN, CTX and/or CRO. The *Salmonella* Anatum strains identified as 15A44 (obtained in July 2012) and 21A52 (January 2013) showed a similar susceptibility pattern, and these strains were identified by PFGE as clones.

**Sale frequency of the different antibiotic groups in veterinary pharmacies of the region**

Table 2 shows the trends for the antibiotics demand in veterinary pharmacies of the region in 2013, and the changes in preferences after an equal evaluation four years later.
Based on observations, the most requested chemical groups in veterinary pharmacies are aminopenicillins, sulfonamides (with and without dihydrofolate reductase inhibitors), penicillins, cephalosporins and first, third and fourth-generation fluoroquinolones. The 2014 results were reported to the Commission for the Protection against Sanitary Risks of the State of Jalisco (COPRISJAL), which in 2015, with the support of the College of Veterinary Doctors of the State of Jalisco (Civil Association), started an awareness and training campaign on the public health impact of multi-drug resistant pathogens, the use of antibiotics in livestock feed and antimicrobial therapeutic alternatives for farm cattle. In early 2017, the veterinary pharmacies survey in the Altos Sur region of Jalisco was repeated to observe changes in the trend of the veterinary doctors’ preferences for the different antimicrobial drugs. The results of the survey indicate that the demand for aminopenicillins and sulfonamides (with and without dihydrofolate reductase inhibitors) has increased. These are the antimicrobial drugs recommended by the FDA National Antimicrobial Resistance Monitoring System (NARMS, 2010).
for veterinary use in wounds, mastitis, gastrointestinal and respiratory infection treatments. In contrast, there was a reduction in the demand of first and third-generation cephalosporins, while the consumption of cefquinome raised, which is a fourth-generation cephalosporin recommended exclusively for veterinary use.

**DISCUSSION**

As an infectious, contagious pathogen, *Salmonella* is probably rivaled just by bovine viral diarrhea virus in its ability to cause clinical diseases, such as enteritis, septicemia, pneumonia, and reproductive losses (Holschbach and Peek, 2018). The increasing prevalence of *Salmonella* presents new challenges to meat producers and veterinarians. No current discussion on bovine salmonellosis is complete without acknowledging the increasing public health concern. Increasing antimicrobial resistance among enteric pathogens brings the use of antimicrobials by veterinarians and producers under an increasingly strict scrutiny. There are no studies in Mexico on this pathogen prevalence in newborn cattle. However, the presence of the pathogen can be assumed as a commensal biota in their intestine, which will accompany them during their life to the slaughterhouse. Hence, it can be also assumed that the contamination of the carcass can be configured, if hygienic practices are not maintained in the gutted during the slaughter. In this respect, there are studies on the prevalence of this pathogen in slaughterhouse meat that report the contamination of the carcass.

In Mexico, Narvaez *et al.*, (2013) and Pérez-Montano *et al.*, (2012) reported information related to the presence of *Salmonella* on beef carcasses in abattoirs and cattle feedlots, indicating a *Salmonella* presence of 5.2 % and 55.56 %, respectively. They also reported that all abattoirs included in her study had failed to comply with good manufacturing practices (GMPs) and sanitation standard operating procedures (SSOPs), and none had implemented a food safety system. Fecal contamination on beef carcasses was visible and cross-contamination was common during operations.

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**Table 2.** Changes in the antimicrobials dispensing preference in veterinary pharmacies at the Altos Sur region of Jalisco State, Mexico.

| Class of drugs                        | Antibiotics                        | Number of veterinary pharmacies\(^1\) (Relative frequency)\(^2\) | Change in acquisition preference\(^3\) |
|---------------------------------------|------------------------------------|---------------------------------------------------------------|---------------------------------------|
|                                       | September 2013 October 2017        | 36 veterinary outlets                                        | 36 veterinary outlets                 |
| Aminoglycoside                        | Amikacin \(^b\)                    | 2(5.6 %)                                                      | 2(5.6 %)                              |
| Aminoglycoside                        | Gentamicin \(^b\)                  | 3(8.3 %)                                                      | 3(8.3 %)                              |
| Aminopenicillins                      | Ampicillin \(^*\)                  | 15(41.7 %)                                                   | 18(50.0 %)                            |
| Beta-lactam                           | Penicillin G \(^*\)                | 8(22.2 %)                                                     | 7(19.4 %)                             |
| Beta-lactam                           | Oxacillin \(^*\)                   | 1(2.8 %)                                                      | 0(0 %)                                |
| Beta-lactam/beta-lactamase inhibitors | Amoxicillin with clavulanic acid \(^*\) | 5(13.9 %)                                                   | 8(22.2 %)                             |
| Cephalosporin (First-generation)      | Cefalothin \(^ab,d\)              | 8(22.2 %)                                                     | 5(13.9 %)                             |
| Cephalosporin (Third-generation)      | Ceftriaxone \(^ab,d\)             | 7(19.4 %)                                                     | 5(13.9 %)                             |
| Cephalosporin (Third-generation)      | Cefotaxime \(^ab,d\)              | 4(11.1 %)                                                     | 1(2.8 %)                              |
| Cephalosporin (Fourth-generation aminothiazolyl) | Cefquinome \(^ab,g\)               | 2(5.6 %)                                                      | 3(8.3 %)                              |
| Cefepime \(^ab,d\)                    |                                    | 9(25.0 %)                                                     | 4(11.1 %)                             |
| Cyclic non-ribosomal polypeptide      | Polymyxin B \(^b\)                | 2(5.6 %)                                                      | 1(2.8 %)                              |
| Fluoroquinolone                       | Ciprofloxacain \(^*\)             | 1(2.8 %)                                                      | 1(2.8 %)                              |
| Fluoroquinolone                       | Danofloxacain \(^*\)              | 7(19.4 %)                                                     | 9(25.0 %)                             |
| Sulfonamide                           | Sulfadiazine \(^*\)               | 14(38.9 %)                                                    | 19(52.8 %)                            |
| Sulfonamide/inhibitor of dihydrofolate reductase | Trimethoprim-Sulfamethoxazole \(^abf\) | 17(47.2 %)                                                   | 18(50.0 %)                            |
| Tetracycline                          | Oxytetacycline \(^c\)             | 1(2.8 %)                                                      | 1(2.8 %)                              |
| Tetracycline                          | Minocycline \(^b\)                | 2(5.6 %)                                                      | 0(0 %)                                |
| Tetracycline                          | Doxycycline \(^b\)                | 0(0 %)                                                        | 3(8.3 %)                              |

\(^1\) Number of veterinary pharmacies that reported it as one of the three best-selling antibiotics of 36 veterinary outlets surveyed. \(^2\) (No. of veterinary pharmacies that reported it as one of the three best-selling antibiotics/36 veterinary outlets surveyed) x 100. \(^3\) Changes with statistical significance (p≤ 0.05).

\(^a\)For wounds treatment

\(^b\)In livestock feed to prevent diseases and infections in cattle

\(^*\)Is contraindicated in neonates cattle

\(^c\)For respiratory disease treatment

\(^d\)For mastitis treatment in dairy cattle

\(^e\)It is only used in veterinary applications
at all abattoirs. Without excluding GMPs and SSOPs during rearing, dressing and slaughter, it is important to evaluate and control the presence of Salmonella and other pathogens in farm cattle at the different stages of their life (neonate, reproductive age and prior to slaughter). In addition to this, Barkocy-Gallagher et al. (2003) reported that Salmonella prevalence on pre-eviscerated beef carcasses was higher during summer and fall (19.7 to 24.9 %) than in winter and spring (3.0 to 4.1 %). During the wet season, cattle hides are more likely to be soiled with mud and feces, increasing the possibility of carcass contamination during hide removal and evisceration (Rivera-Betancourt et al., 2004). It is worth mentioning that the mere presence of the pathogen in the intestine of the animal of origin and, unfortunately, in its carcass, does not necessarily have an impact on the consumer’s disease, cross contamination with other raw or cooked foods must occur and/or an inadequate thermal elimination of the pathogen in the food of meat origin. However, all the Salmonella serotypes and Salmonella enterica subsp. arizonae that were isolated in this study have been previously related, as ethological agents, to disease outbreaks due to poorly cooked meats intake (Geimba et al., 2004; Gould et al., 2004; Duggan et al., 2012; Evangelopoulos et al., 2014).

Regarding geographical distribution, the municipalities that present cattle carrying Salmonella make up the western area of the region, connected by federal highways 71 and 207. Here, six of the fourteen studied cattle ranches are located, and is the only land route by which feed and supplies for the livestock feeding are distributed, as well as the products of its exploitation. Therefore, it will be necessary to evaluate if there is any source or mechanism of Salmonella contamination in the bovine feeding sources in this area of the region and in the particular practices of antimicrobial treatment and prophylaxis of veterinary doctors who take care of livestock in the area.

All of the above results because of the process of animal food production, which involves large amounts of antimicrobial agents either for therapy, metaphylactic, prophylaxis of bacterial infections or, in feed, to promote growth (Van Boeckel et al., 2015). Globally, intensive livestock farming has increased food production at a low cost per unit, but perhaps at the unrecognized price of increased antimicrobial resistance (FDA, 2010a).

Linking antimicrobial consumption in animals to drug-resistant infections in humans is inherently complex, due to the ecological nature of selection pressure for drug-resistant pathogens, as well as to the existence of indirect routes of transmission through the environment (Roca et al., 2015).

In the United States, the use of antimicrobial for animal feeding is estimated to account for 80% of the nation’s annual antimicrobial consumption (FDA, 2010b). A significant fraction of this, involves antimicrobials that are important for the treatment of common human infections, and for performing medical procedures such as major surgeries, organ transplantation, and chemotherapy (Laxminarayan et al., 2013). Modern animal production practices are associated with the regular use of antimicrobials, potentially increasing selection pressure on bacteria to become resistant (Van Boeckel et al., 2015).

Van Boeckel et al. (2015) report that in 2010, the five countries with the largest shares of global antimicrobial use for animal food production were China (23 %), the United States (13 %), Brazil (9 %), India (3 %), and Germany (3 %). However, by 2030, this ranking is projected to be China (30 %), the United States (10 %), Brazil (8 %), India (4 %), and Mexico (2 %). With this trend and without particular actions for its containment, the problem of drug multiresistance will increase. In Mexico, particularly in the Sonora, Hidalgo, Jalisco and State of Mexico, studies have shown the presence of multidrug-resistant Salmonella in chicken and beef carcasses and cooked foods (Miranda et al., 2009; Pérez-Montaño et al., 2009; Camacho et al., 2010; Gordillo-Benavente, 2019). It is worth mentioning that all the Salmonella strains isolated from neonatal cattle from the Altos Sur region of the state of Jalisco show multiresistance to drugs, according to the criteria of Miranda et al. (2009). Particularly, the strains show resistance to drugs (AMP, CEP, STX, AN, CTX and CRO) that belong to the chemical groups of greater use in human and veterinary antibiotherapy. In addition, it is striking those two samples of diarrheal feces from the same cattle herd belonged to the same Salmonella Anatum clone. The two samples were recovered from different calves, on different sampling dates (July 2012 and January 2013), which indicates an operating source of contamination in the cattle herd. Likewise, between strain 15A44 (from July 2012) and strain 21A52 (from January 2013), an increase of resistance to CTX (transition from and intermediate category to a resistant one) was observed, even though the use of CTX is contraindicated in newborn cattle.

Finally, the high demand for aminopenicillins, sulfonamides (with and without dihydrofolate reductase inhibitors), penicillins, first, third and fourth-generation cephalosporins and fluoroquinolones might relate to the multidrug resistance shown by Salmonella strains in diarrheal feces of newborn cattle. Although the awareness campaign offered to veterinary doctors was useful to modify the consumption preferences of veterinary drugs in favor of those recommended by the FDA (FDA, 2010b), we also recommend to raise awareness among farm livestock producers, to limit the spread of multi-resistant strains and the consumption of feed prepared with antibiotics for prophylactic or growth improvement purposes. We recommend reducing the prophylactic use of antimicrobials, eliminating the use of antimicrobial agents as development promoters and making the choice of antimicrobials more efficient, selecting alternatives for exclusive veterinary use.

CONCLUSIONS

In conclusion, this study is, as far as we know, the first study conducted in neonate bovine in the Jalisco State, Mexico, for detecting multidrug resistant Salmonella. Our findings call for initiatives to preserve antibiotic effectiveness while
ensuring food security in low and middle-income countries, as ours. Information on the occurrence of resistance is crucial at local, regional and international levels to guide policy and detect changes that require a response strategy. In order to fulfill this requirement, we need systems for the continuous monitoring on the changes in the occurrence of resistance.

CONFLICT OF INTEREST:
Authors declare that they have no conflict of interest.

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