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Phenotypic and Genotypic Antibiotic Resistance of Salmonella from Chicken Carcasses Marketed at Ibague, Colombia

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ABSTRACT

Salmonella enterica is responsible for alimentary toxic infections associated with the consumption of contaminated poultry products and the antimicrobial resistant patterns of *Salmonella* circulating in the Tolima region are currently unknown. To address this issue, both the phenotype and genotype antibiotic resistance patterns of 47 *Salmonella* isolated from raw chicken carcasses sold at the Ibague city were analyzed by the disc diffusion, microdilution and PCR assays. All 47 *Salmonella* isolates showed resistance to five or more antimicrobial agents. Resistance to Ampicillin (AMP), Amikacin (AMK), Gentamicin (GEN), Tobramycin (TOB), Cefazoline (CFZ), Cefoxitin (FOX), Nitrofurantoin (NIT), Trimethoprim-Sulfamethoxazole (SXT), Tetracycline (TET), Ciprofloxacin (CIP) and Enrofloxacin (ENR) was observed in 42.35% of *Salmonella* isolates. All tested *S*. Paratyphi B var Java isolates showed resistance to at least 12 antibiotics. *S*. Hvittingfoss showed resistance to 5 antibiotics, whereas *S*. Muenster showed resistance to seven antibiotics. Amplification of a number of antibiotic resistance genes showed that blaTEM (100%) correlated well with resistance to Ampicilin and Cephalosporin, whereas *aad*B (87%) correlated well with resistance to Aminoglycosides. It is concluded that *Salmonella* isolated from raw chicken meat marketed at Ibague showed MDR by both phenotypic and genotypic methods and they may represent an important threat to human health. Additional studies are needed to establish the relationship between antibiotic resistance in *Salmonella* from poultry products and clinical isolates.

INTRODUCTION

Salmonella enterica is a large group of Gram-negative bacteria that cause an infectious disease called salmonellosis. The subspecies *enterica* (I) groups the majority (1547, 60%) of serovars that affect human and domestic animals (Dekker & Frank, 2015; Grimont & Weill, 2007; OIE, 2012), and those serovars are classified as typhoidal and nontyphoidal *Salmonella* (NTS) (Sanderson & Nair, 2013). Contaminated meat and poultry products such as commercial table eggs and raw chicken meat constitute the main sources of *S. enterica* (Ricke & Calo, 2015). The disease in humans is characterized by a self-limiting gastrointestinal infection in immunocompetent patients, who usually develop fever, diarrhea and acute abdominal pain; however, it may progress into a life-threatening disease when the bacteria reach the bloodstream, particularly in young children, elderly and immunocompromised people (Mercado *et al.*, 2012).

NTS, *S*. Enteritidis and *S*. Typhimurium are the most common serovars isolated from clinical cases of human salmonellosis (CDC, 2014; Hendriksen *et al.*, 2011). *S*. Newport, *S*. Javiana, *S*.I4,[5],12:i:-, *S*. Muenchen, *S*. Bareilly, *S*. Monevideo and *S*. Heildelberg, among

others, are also associated in a smaller proportion with human infection (CDC, 2014; Chen *et al.*, 2012). NTS might be responsible for about 80.3 million foodborne illnesses and 115,000 deaths each year in the world (Majowicz *et al.*, 2010), while Typhoid, Paratyphoid and enteric fever cause 25 million infections and 200,000 deaths each year globally (Dekker & Frank, 2015). Antibiotic treatment of salmonellosis is complicated because the microorganism under antibiotic pressure may select for virulence within the host (Diard *et al.*, 2014), acquires tolerance and multiple drug resistance (MDR) phenotypes (fast-, moderate- and low-growing subsets) within host tissues (Claudi *et al.*, 2014), and frequently incorporate new genetic material to resist the antibiotic selective pressure (Brown-Jaque *et al.*, 2015). *Salmonella* isolated from food of animal origin shows higher rates of antimicrobial resistance (Chuanchuen & Padungtod, 2009), which is promoted by the misuse or underuse of antimicrobials incorporated in feed to prevent infectious diseases and to promote bird growth, and those MDR microorganisms may disseminate very quickly with the rapid global food market .

In Colombia, limited information is available on the species of *Salmonella* circulating in poultry products, the serovars responsible for human infections as well as their antibiotic resistance patterns. Serovar Typhimurium variant 5 was isolated from human cases of salmonellosis in Paz del Rio, Boyacá (Díaz Osorio *et al.*, 2014). Recently, our group isolated *Salmonella* Enteritidis and *S.* Shannon from laying-hen farms located in the Tolima region (Rodríguez *et al.*, 2015a), and reported at least 14 different serovars of *Salmonella* from chicken carcasses sold at stores and supermarkets of Ibague (Rodriguez *et al.*, 2015b), pointing out the importance of contaminated eggs and chicken meat as a potential source of human infection. In addition, manipulation, transportation and marketing of poultry products in most of the cases do not meet the standards of good manufacturing practices and instead they may promote contamination with *Salmonella*. In this study the antibiotic resistance patterns of *Salmonella* serovars isolated from chicken meat sold at stores and supermarkets in Ibague city were established.

MATERIALS AND METHODS

Salmonella spp., isolates from chicken meat

A total of 47 strains of *Salmonella* previously isolated from broiler carcasses marketed at Ibague, Colombia were used in this study. The *Salmonella* serovars were collected from a cross-sectional study conducted between February to May 2014 (Rodriguez *et al.*, 2015b). The *Salmonella* isolates were thawed from glycerol stocks and streaked on TSA plates and incubated at 37 °C for 24 hr.

Phenotype of antibiotic resistance

The Kirby-Bauer method (agar-disc diffusion) was used to evaluate the susceptibility of *Salmonella* to Chloramphenicol (CHL, 30 µg), Florfenicol (FFC, 30 µg), Enrofloxacin (ENR, 5 µg), Norfloxacin (NOR, 10 µg) and Fosfomycin (FOF, 50 µg), which are commonly used in veterinary medicine but they are not included in the automatized microdilution Phoenix ™ (Becton Dickinson, Sparks, MD, USA) method. A bacterial suspension in Mueller-Hinton (Oxoid, Germany) agar was calibrated according to 0.5 McFarland scale of turbidity, and bacterial growth inhibition upon culture on plate at 37ºC for 24 hr was evaluated according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2015).

Salmonella isolates were also subjected to an antimicrobial microdilution susceptibility test by using the BD Phoenix™ NMIC/ID-132 panels (Becton Dickinson, Sparks, MD, USA) and the categories established by The Clinical and Laboratory Standards Institute (CLSI, 2015). The antibiotics and concentration included in this assay were Amikacin (AMK, 8-32 µg/mL), Ampicillin (AMP, 4-16 µg/mL), Ampicillin-Sulbactam (SAM, 2/2 µg/mL), Aztreonam (ATM, 2-16 µg/mL), Cefazoline (CFZ, 2-16 µg/mL), Cefepime (FEP, 1-16 µg/mL), Cefoxitin (FOX, 4-16 µg/mL), Ceftriaxone (CRO, 2-32 µg/mL), Ciprofloxacin (CIP, 0.5-2 µg/mL), Ertapenem (ETP, 0.5-8 µg/mL), Gentamicin (GEN, 2-8 µg/mL), Imipenem (IPM, 1-8 µg/mL), Meropenem (MEM, 1-8 µg/mL), Nitrofurantoin (NIT, 16-64 µg/mL), Piperacillin-Tazobactam (TZP, 0.5/16 µg/mL), Tetracycline (TET, 2-8 µg/mL), Ticarcillin-Clavulanate (TIM, 4/32 µg/mL), Tobramycin (TOB, 2-8 µg/mL), and Trimethoprim-Sulfamethoxazole (SXT, 0.05/0.06). In this study, only absolutely but not intermediate resistant isolates of *Salmonella* were considered as resistant strains. Multi-drug resistant (MDR) strains of *Salmonella* were defined as those isolates that showed phenotype resistance to at least three or more classes of antibiotics. *Escherichia coli* ATCC 25922 was used as a reference strain.

Genotype of antibiotic resistance

Salmonella isolates were analyzed by PCR to detect the presence of antibiotic resistance genes that are known to confer resistance to Ampicillin (*bla*TEM) with primer set blaTEM-F-5´-ATCAGTTGGGTGCACGAGTG-3´ and blaTEM-R-5´- ACGCTCACCGGCTCCAGA-3´,

Chloramphenicol (*catB*) with primer set catB-F-5´-CGGATTCAGCCTGACCACC-3´ and catB-R-5´-ATACGCGGTCACCTTCCTG-3´, Tetracycline (*tetB*) with primer set tetB-F-5´- CTGTCGCGGCATCGGTCAT-3´ and tetB-R-5´- CAGGTAAAGCGATCCCACC-3´, Trimethoprim (*dfrA12, drfA1*) with primer sets drfA12-F-5´- TTCGCAGACTCACTGAGGG-3´ and drfA12-R-5´- CGGTTGAGACAAGCTCGAAT-3´, and drfA1-F-5´- CAATGGCTGTTGGTTGGAC-3´and drfA1-R-5´- CCGGCTCGATGTCTATTGT-3´, Spectinomycin (*aadA2*) with primer set aadA2-F-5´-CATTGAGCGCCATCTGGAAT-3´ and aadA2-R-5´-ACATTTCGCTCATCGCCGGC-3´ (Chuanchuen *et al.*, 2008), Gentamicin (*aadB*) with primer set aadB-F-5´-CTAGCTGCGGCAGATGAGC-3´ and aadB-R-5´-CTCAGCCGCCTCTGGGCA-3´, Streptomycin (*strB*) with primer set strB-F-5´- GCGGACACCTTTTCCAGCCT-3´ and strB-R.5´- TCCGCCATCTGTGCAATGCG-3´ and Sulfamethoxazole (*sul2*) with primer set sul2-F-5´-GCGCAGGCGCGTAAGCTGAT-3´, and sul2-R-5´-CGAAGCGCAGCCGCAATTC-3´ (Chuanchuen & Padungtod, 2009). A colony of S*almonella* from each isolate was seeded in tryptone soy agar (TSA), and incubated for 24 hr at 37 °C. Bacterial cells were collected, washed with PBS, pelleted into a 1.5 mL Eppendorf tube and total DNA was extracted using the phenol-chloroform-isoamyl alcohol method (Sambrook & Russell, 2001). Bacterial DNA was diluted in 100 µL 1 × TE buffer and used as template in the PCR mixture to amplify the antibiotic resistance genes.

Polymerase chain reaction, PCR

The PCR was carried out in a total volume of 25 µL containing 1 µL of template DNA, 1µL of forward and 1µL of reverse primers (Invitrogen™, Thermo Fisher Scientific Inc.), 0.5 µL of AccuprimeTaq polymerase, 2.5 µL of 10 × buffer, 2.5 µL of $MgSO_4$, and 16.5 µL of nuclease free water was also added. PCR was performed in a BIO-RAD T100™ thermal cycler after an initial denaturation step of 1 minute at 94°C, 35 cycles of amplification were performed. Each cycle consisted of the following steps: 60s at 94°C (denaturation), 30s at 55°C (primer annealing), and 30s at 68°C (extension), followed by 7 min at 68°C for final extension. *Salmonella* Typhimurium (ATCC 14028) was used as a positive control, whereas the negative control did not contain DNA template. The reaction mixture was mixed with 2,5 µL 10 × gel loading buffer and then resolved by electrophoresis on 2% agarose gel with 100 bp DNA ladder. The reaction products were stained with ethidium bromide and visualized under the UV light by using an ENDURO™ GDS (Labnet International, Inc.), GEL documentation system.

Statistical Analysis

Associations between phenotypic and genotypic antibiotic resistance in *Salmonella* were established by a Spearman correlation test (GraphPad Prism® 5.03 version software).

RESULTS

All 47 *Salmonella* isolates (100%) were resistant to five antibiotics belonging to Aminoglycosides (AMK, GEN, TOB) and Cephalosporin (FOX and CFZ) classes (Table 1). In total 57.4% of *Salmonella* isolates were resistant to Tetracycline (27/47), and 53.19% (25/47) were resistant to Ampicillin. At least 42.35% (20/47) of *Salmonella* isolates were found to be MDR strains that showed resistance to eleven (AMP, AMK, GEN, TOB, CFZ, FOX, NIT, SXT, TET, CIP and ENR) or more antibiotics belonging to seven antibiotic classes (Aminoglycosides, Penicillin, Cephalosporin, Nitrofurans, Sulfonamides/Trimethoprim, Tetracycline and quinolones). *Salmonella* isolates also exhibited resistance to phenicols CHL and FFC at a frequency of 6.38% (3 isolates). All *Salmonella* isolates were susceptible to ATM, FEP, ETP, IPM, MEM, TZP, TIM, NOR and FOF.

All isolates of *S.* Paratyphi B (36.17%), S. Heidelberg, *S*. Typhimurium *S*. Muenster, and *S*. Hvittingfoss were classified as MDR strains with resistance to three or more antibiotics classes. One isolate of *S*. Typhimurium (UT-STm14018), one *S*. Paratyphi B (UT-SPb14010) and one *S*. Hvittingfoss (UT-SHv14023) showed resistance to phenicols and *S.* Heidelberg, *S*. Skansen, *S*. Schwarzengrund, *S*. Budapest and all *S*. Paratyphi B isolates showed resistance to ENR (Table 1).

The genotypic analysis showed the presence of a number of genes associated with antibiotic resistance such as *bla*TEM in 100% of *Salmonella* isolates, *aadB* in 41 out of 47 isolates (87.2%), *strB* in 70.2%, *sul2* in 57.4%, *drfA1* in 51%, *tetB* in 42.5 % and *aadA2* in 38.2%. The *catB* gene that is known to confer resistance to phenicols was present in the same *Salmonella* isolates (100%) that showed phenotypic resistance by the Kirby-Bauer method. Most of the *Salmonell*a isolates exhibited MDR genotype and none of isolates amplified the sequence *drf*A1 2 (Table 2). A good Spearman correlation coefficient between the phenotype and genotype was found for Chloramphenicol (r = 1.00), Gentamicin (r = 0.94), Trimethoprim (r = 0.68) and

Table 1 – Phenotypic and genotypic antibiotic resistance patterns of *Salmonella* serovars isolated from chicken meat sold at Ibague, Colombia (February-May 2014).

Salmonella code	Group	Serovar/(formula)	Phenotype of antibiotic resistance [a]	Genotype of antibiotic resistance
UT-SNp14001	C2	Newport (6,8:e,h:1,2)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, Sul 2, aadB, drfA1
UT-SSk14002	C2	Skansen (6,8:b: 1,2)	AMK, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM , Str B, tet B, Sul 2,aadB, aadA2, drfA1
UT-SKa14003	E1	Kalina (3,10:b: 1,2)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM , aadB, aadA2, Sul 2, drfA1
UT-SSc14004	B	Schwarzengrund (1,4,12,27:d:1,7)	AMK, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadB, drfA1
UT-SPb14005	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM , tet B, Sul 2, aadB, aadA2, Str B, drfA1
UT-SPb14006	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM , tet B, Sul 2,aadB, Str B, aadA2, drfA1
UT-SPb14007	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadA2, aadB, drfA1
UT-SPb14008	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TOB, SXT, ENR.	Bla TEM , Sul 2, aadB, aadA2, Str B, drfA1
UT-SPb14009	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TOB, SXT, ENR.	Bla TEM , Sul 2, aadA2, Str B, aadB, drfA1
UT-SPb14010	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR, FFC.	Bla TEM , tet B, Sul 2, Str B, aadA2, aadB, catB, drfA1
UT-SPb14011	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadA2, aadB, drfA1
UT-SMh14012	C2	Manhattan (6,8:d:1,5)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, Sul 2, aadB, Str B, drfA1
UT-SPb14013	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadA2, aadB, drfA1
UT-SBr14014	C1	Braenderup (6,7,14:e,h:e,n,z,15)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, Sul 2, aadB, Str B
UT-SBr14015	C1	Braenderup (6,7,14:e,h:e,n,z,15)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, tet B, Sul 2, aadB, Str B,
UT-SBm14016	C2	Bovismorbificans (6,8,20:r:1,5)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM Sul 2, aadB,
UT-STm14017	B	Typhimurium (1,4,12:i:-)	AMK, CFZ, FOX, GEN, TET, TOB, CHL, FFC.	Bla TEM, tet B, Sul 2, addB, Str B, catB
UT-STm14018	B	Typhimurium (1,4,12:i:-)	AMK, CFZ, FOX, GEN, TET, TOB,	Bla TEM, Sul 2, aadB,
UT-SOt14019	C1	Othmarschen (6,7:g,m,t:-)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM , Sul 2, aadB
UT-SPb14020	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadB, drfA1
UT-SNp14021	C2	Newport (6,8:e,h:1,2)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, Sul 2, Str B aadB, drfA1
UT-SPb14022	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Sul 2, Str B, aadB, drfA1
UT-SHv14023	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB, FFC.	Bla TEM Sul 2, aadB, catB
UT-SHv14024	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM, Sul 2, aadB,
UT-SHe14025	B	Heidelberg (1,4,12:r:1,2)	AMK, AMP, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM , tet B, Sul 2, Str B, aadB,
UT-SHv14026	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM, aadA2, aadB,
UT-SHe14027	B	Heidelberg (1,4,12:r:1,2)	AMK, AMP, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM. Str B
UT-SHv14028	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM, aadB,
UT-SHv14029	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM, aadB,
UT-SHv14030	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM
UT-SHv14031	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM
UT-SHv14032	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM
UT-SHv14033	I	Hvittingfoss (16:b:e,n,x)	AMK, CFZ, FOX, GEN, TOB,	Bla TEM
UT-SPb14034	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, Str B, drfA1
UT-SMu14035	E1	Muenster (3,10:e,h:1,5)	AMK, AMP, CFZ, FOX, GEN, TET, TOB.	Bla TEM , Str B, addB, aadA2,
UT-SMu14036	E1	Muenster (3,10:e,h:1,5)	AMK, AMP, CFZ, FOX, GEN, TET, TOB.	Bla TEM, Sul 2, Str B, aadB
UT-SMu14037	E1	Muenster (3,10:e,h:1,5)	AMK, AMP, CFZ, FOX, GEN, TET, TOB.	Bla TEM, Str B, aadB
UT-SMu14038	E1	Muenster (3,10:e,h:1,5)	AMK, AMP, CFZ, FOX, GEN, TET, TOB.	Bla TEM, Sul 2, Str B, aadB
UT-SMu14039	E1	Muenster (3,10:e,h:1,5)	AMK, AMP, CFZ, FOX, GEN, TET, TOB.	Bla TEM, Sul 2, Str B, aadB
UT-SKa14040	E1	Kalina (3,10:b: 1,2)	AMK, CFZ, FOX, GEN, TOB.	Bla TEM, tet B, Str B, aadB, drfA1
UT-SPb14041	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Str B, aadB, drfA1
UT-SPb14042	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Str B, aadA2, aadB, drfA1
UT-SPb14043	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Str B, aadA2, aadB, drfA1
UT-SPb14044	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Str B, aadA2, aadB, drfA1
UT-SBu14045	B	Budapest (4,12:g,t:-)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, ENR.	Bla TEM , tet B, Str B, aadA2, aadB, drfA1
UT-SPb14046	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, tet B, Str B, aadA2, aadB, drfA1
UT-SPb14047	B	Parathyphi B (1,4,5,12:b:1,2)	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, SXT, ENR.	Bla TEM, Str B, aadA2, aadB, drfA1

a). Antibiotic resistance patterns of *Salmonella* determined by the disc-diffusion and microdilution assays. AMK, Amikacin; AMP, Ampicillin; SAM, Ampicillin-Sulbactam; CFZ, Cefazoline; FOX, Cefoxitin; CRO, Ceftriaxone; CIP, Ciprofloxacin; GEN, Gentamicin; NIT, Nitrofurantoin; TET, Tetracycline; TOB, Tobramycin; CHL, Chloramphenicol; FFC, Florfenicol; SUL, Sulfamethoxazole; SXT, Trimethoprim-Sulfamethoxazole, ENR, Enrofloxacin.

Tetracycline (r = 0.66) and lower coefficients for Ampicillin (r = 0.57) and Sulfamethoxazole (r = 0.30). The majority of the PCR products were higher in size than those reported in *Salmonella* isolated in Thailand (Chuanchuen & Padungtod, 2009). For example, the expected size of *cat*B was 461bp, however, there were multiple bands with one prominent band of about 800 bp and a similar situation was found with the *aad*B gene (expected band at 300bp) that showed a PCR product of about 800 bp. All other antibiotic resistance genes showed PCR products with similar size to the expected band.

DISCUSSION

The results of this study indicates that *Salmonella* isolated from chicken carcasses sold at Ibague Colombia during February to May 2014 showed a broad spectrum

Table 2 – Frequency of antibiotic resistance genes detected in *Salmonella* spp., isolated from chicken meat sold at Ibague, Colombia (February-May 2014).

Antibiotic	Gene name /	PCR product (bp)	N° of positives (%)	Serovars[(a)] (Number)
Ampicillin	*bla* TEM	(608)	47 (100%)	ParB (17), Hvi (9), Mue (5), Typ (2), New (2), Hei (2), Bra (2), Kal (2), Bov (1), Bud (1), Man (1), Oth (1), Sch (1), Ska (1).
Gentamicin	*aad*B	(300)	41 (87.2%)	ParB (16), Hvi (5), Mue (5), Typ (2), New (2), Hei (1), Bra (2), Kal (2), Bov (1), Bud (1), Man (1), Oth (1), Sch (1), Ska (1).
Streptomycin	*str*B	(621)	33 (70.2%)	ParB (17), Mue (5), Typ (1), New (1), Hei (2), Bra (2), Kal (1), Bud (1), Man (1), Sch (1), Ska (1).
Sulfamethoxazole	*sul*2	(514)	27 (57.4%)	ParB (9), Hvi (2), Mue (3), Typ (2), New (2), Hei (1), Bra (2), Kal (1), Bov (1), Man (1), Oth (1), Sch (1), Ska (1).
Tetracycline	*tet*B	(615)	20 (42.5%)	ParB (13), Typ (1), Hei (1), Bra (1), Kal (1), Bud (1), Sch (1), Ska (1).
Spectinomycin	*aad*A2	(500)	18 (38.2%)	ParB (13), Hvi (1), Mue (1), Kal (1), Bud (1), Ska (1).
Chloramphenicol	*cat* B	(461)	3 (6.38%)	ParB (1), Typ (1), Hvi (1).
Trimethoprim	*drf*A1	(254)	24 (51.0%)	ParB (17), New (2), Kal (2), Man (1), Sch (1), Ska (1).
	*drf*A12	(330)	0 (0%)	

a) ParB, Paratyphi B; Hvi, Hvittingfoss; Mue, Muenster; Typ, Typhimurium; New, Newport; Hei, Heidelberg; Bra, Branderup; Kal, Kalina; Bov, Bovismorbificans; Bud., Budapest; Man, Manhattan; Oth, Othmarschen; Sch, Schwarzengrund; Ska, Skansen.

of antibiotic resistance when compared with isolates from other meat sources (Mąka *et al.*, 2014) or clinical isolates (Vaz *et al.*, 2010). A variety of *Salmonella* serovars resistant to multiple antibiotic classes were present in chicken meat samples and a high percentage (42.35%) of *Salmonella* showed multi-drug resistance (MDR) by the phenotypic method, among them, all *S*. Paratyphi B isolates and *S*. Heildelberg were resistant to 7 different classes of antibiotics, whereas *S*. Muenster and *S*. Typhimurium were resistant to 4 different classes of antibiotics. These results are similar to those reported in *Salmonella* Paratyphi B isolated from broiler farms in Cundinamarca and Santander, where this serovar was found to be resistant to up to 15 antimicrobials (range 9-15) (Donado-Godoy *et al.*, 2012), of which resistance to NIT, SXT, TET, CIP and ENR can be identified as a common resistance pattern that is consistent at least in part with the usage of tetracycline, trimethoprim and quinolones in the regional poultry industry (based on author´s survey). Thus, the choice of fluoroquinolones in treatment of severe infections by *Salmonella* could be seriously impeded, as it has been noted by others (Ricke & Calo, 2015). The percentage of MDR *Salmonella* isolated in this study was lower than that reported in *Salmonella* from different meats (70%) in China, where 252 out of 359 isolates showed MDR phenotypes to at least three classes of antibiotics and the chicken isolates had the higher resistance (80%) rates (Yang *et al.*, 2010).

Salmonella Enteritidis and *S*. Typhimurium are the most widespread serovars and can be isolated from surface water, meat and poultry (Jokinen *et al.*, 2015; Yang *et al.*, 2010), and constitute the principal serovars responsible for human and animal disease (MPS, 2011; Stevens *et al.*, 2009), however, our previous study reported that *Salmonella* Enteritidis was not isolated from chicken meat sold at stores and supermarkets of Ibague, and instead *Salmonella* Paratyphi B var Java was the most prevalent serovar (36.17%) followed by Hvittingfoss (19.15%), and Muenster (10.64%) (Rodriguez *et al.,* 2015b). *S*. Paratyphi B variant Java (76.4%) and *S*. Heildelberg (22.7%) were the most prevalent serovars isolated from broiler farms in two distinct regions (Cundinamarca and Santander) of Colombia (Donado-Godoy *et al.*, 2012), thus, this serovar might be distributed in broiler farms across the country and its potential association with natural outbreaks of paratyphoid disease reported by the National Institute of Health (INS, 2015), is worthy of investigation. *S*. Paratyphi B var Java has been isolated from poultry from Netherlands (Van Pelt *et al.*, 2003) and Germany (Dorn *et al.*, 2001), from chicken viscera at two slaughter plants in the state of Zulia, Venezuela (Boscan *et al.*, 2005), from chicken in Belgium (De Jong *et al.*, 2014), and from breeders and broiler farms in Bangladesh (Barua *et al.*, 2013). In Bangladesh, it was also isolated from blood of patients with clinically diagnosed enteric fever at similar proportions to *S*. Typhi but with higher resistance rates (Afroz *et al.*, 2014), highlighting an increased risk upon its eventual transmission to human. Thus, whether the increased frequency of this serovar relies on changes in the population dynamics (Foley *et al.*, 2011) of *S. enterica* serovars in broiler farms in Colombia is an issue that also needs further investigation.

The MDR resistance rate of *Salmonella* isolates in this study was higher than that reported in *Salmonella* from chicken carcasses (n=123) and chicken meat in Italy, where 30.5% and 36% exhibited multi-drug resistance to AMP, SUL and TET, respectively (Bacci

et al., 2012), however, in that study, authors may have used a limited number of antibiotic agents. Our results are also similar to the MDR *Salmonella* spp., reported in Southeast Asian countries such as Malaysia, Thailand and Vietnam where the resistance rate ranged between 21 to 75%, and resistance to traditional antibiotics such as AMP, SUL and TET was high in *Salmonella* isolated from animals and foods of animal origin in Malaysia (resistance rate 22 - 49%), Thailand (41 - 92%), and Vietnam (17 - 68%) (Van *et al.*, 2012). The results may suggest that misuse or indiscriminate use of antimicrobials in poultry of the Tolima region is contributing to increase the antibiotic resistant strains of *Salmonella*.

S. Typhimurium was found to be resistant to eight antibiotics (AMK, GEN, TOB, TET, CZO, FOX, CHL, FFC), results similar to MDR *S*. Typhimurium isolated from human, chicken and cattle from Malaysia (Benacer *et al.*, 2010) , ducks from Malaysia (Adzitey et al, 2012), and from fresh raw chicken carcasses sold at retail in different markets in central Anatolia, Turkey (Yildirim *et al.*, 2011). MDR *S*. Typhimurium and *S*. Branderup, and *S*. Muenster have been isolated from outbreaks of salmonellosis in Colombia (INS, 2014), suggesting a potential link between poultry and salmonellosis in this region. The MDR phenotype of *S*. Paratyphi B, *S*. Muenster, *S*. Typhimurium and *S*. Heildelberg isolated from chicken carcasses in this study indicate an increased risk and concern in the case of its eventual transmission to humans and suggest the need to search for those serovars in cases of diarrheal disease in the Tolima region.

The phenotypic MDR pattern of *Salmonella* showed partial correlation with the genotypic analysis. The blaTEM gene sequence was present in all *Salmonella* isolates (100%), however it had low correlation (r = 0.577) with the phenotypic AMP resistance phenotype by the Spearman correlation test. In contrast, the presence of *aadB* (87%) gene that confers resistance to Gentamicin had a high correlation with this phenotypic antibiotic resistance (r = 0.94). Although the phenotypic analysis of Streptomycin resistance was not evaluated in this study, we found a high prevalence of the *strB* (70%) gene that has been described to confer such antibiotic resistance (Brenner *et al.*, 2013). Regarding to the phenotypic resistance to Sulfonamides/Trimethoprim (SXT), we found similar frequencies of the *sul2* (57%) and *drfA1* (51%) genes that are associated with Sulfonamides and Trimethoprim resistance, respectively. The *aadA2* (25%) gene involved in Spectinomycin resistance was also frequently detected (Table 1). The differences between the resistance of *Salmonella* to some antibiotics assessed by the phenotypic methods and the resistance pattern obtained by PCR might be due to different antibiotic resistance genes that were not evaluated in this study because of financial constraints. In this regard, a number of antibiotic resistance genes had been described in *Salmonella* (Brenner *et al.*, 2013; Chen *et al.*, 2004). Thus, it is important to increase awareness of the potential impact of antibiotic resistant strains of *Salmonella* present in poultry products in the Tolima region and the need to increase funding to promote this research.

In conclusion, this study found that about 42% of *Salmonella* serovars isolated from chicken meat sold at stores and supermarkets of Ibague city were resistant to multiple classes of antibiotics by both phenotypic and genotypic tests and this data agree with the global health concern imposed by antibiotic resistant strains that may limit the choice of treatment of human infections. The results suggest various needs that include cooperation between the poultry industry, governmental and academic institutions to improve the surveillance of both *Salmonella* and its antibiotic resistance patterns in broiler farms and poultry products, to establish appropriate regulations and funding for antibiotic research, and to promote education and prudent use of antibiotics by poultry farmers.

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