Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2021 / v.23 / n.4 / 001-008

http://dx.doi.org/10.1590/1806-9061-2021-1460

Original Article

Plasmid-Mediated Antibiotic Resistance and Class 1 Integron in Salmonella Heidelberg Isolated from Poultry Farms in Santander - Colombia

■Author(s)

Castro-Vargas RE[I,II]
https://orcid.org/0000-0003-1949-7515
Herrera-Sánchez MP[I,II]
https://orcid.org/0000-0002-1138-1625
Rondón-Barragán IS[I,II]
https://orcid.org/0000-0001-6980-892X

[I] Research Group in Immunobiology and Pathogenesis, Laboratory of Immunology and Molecular Biology, Faculty of Veterinary Medicine and Zootechnics, University of Tolima, Santa Helena Highs, postal code 730006299, Ibagué, Colombia.
[II] Poultry Research Group, Laboratory of Immunology and Molecular Biology, Faculty of Veterinary Medicine and Zootechnics, University of Tolima, Santa Helena Highs, postal code 730006299, Ibagué, Colombia.

■Mail Address

Corresponding author e-mail address
Iang Schroniltgen Rondón-Barragán
Faculty of Veterinary Medicine and Zootechnics, University of Tolima, Santa Helena Highs, postal code 730006299, Ibagué, Tolima, Colombia.
Phone: +57-316-8320642
Email: isrondon@ut.edu.co

■Keywords

Antibiotic resistance, integrons, Salmonella, poultry.

Submitted: 11/February/2021
Approved: 27/August/2021

ABSTRACT

Salmonella enterica is a zoonotic pathogen transmitted to humans by the consumption of contaminated poultry products. *Salmonella* causes around 93 million cases of gastroenteritis and 155,000 deaths worldwide. A high number of multidrug-resistant *Salmonella* isolates has been found from different segments of poultry production, and it is often associated with horizontal antibiotic resistance gene transfer through mobile elements such as plasmids, integrons, and transposons. The emergence of antibiotic-resistant *Salmonella* has been associated with the misuse of antibiotics in animal production and it is classified as a highly important pathogen from public health due to its zoonotic character and its high dissemination ability. This study aimed to determine the antibiotic resistance associated with plasmids, and class 1 and 2 integrons from *Salmonella* Heidelberg isolates from healthy chickens in poultry farms from Santander, Colombia. 15 *Salmonella* Heidelberg isolates obtained from cloacal samples, were evaluated through endpoint PCR to determine the presence of plasmid-encoded resistance genes, and class 1 and 2 integrons. The bla_{CMY2}, *strA* and *strB*, *sul1* and *sul2,* and *tetB* genes were found in all 15 *Salmonella* Heidelberg. The class 1 integron was present in 11 out of 15 isolates, harbored the gene cassette *dfra7.*The results indicate the presence of a high number of antibiotic resistance genes associated with plasmids and class 1 integrons in *Salmonella* Heidelberg strains isolated from poultry farms, resulting in a public health concern, both in humans and poultry production in Colombia.

INTRODUCTION

Salmonella enterica is a zoonotic pathogen, considered as one of the main causes of foodborne disease, associated with the consumption of poultry products (Donado-Godoy *et al.*, 2012; McMillan *et al.*, 2019). In humans, the infectious dose is generally 10^6 to 10^8 organisms and the disease manifestations include acute abdominal pain, diarrhea, and fever (Cosby *et al.*, 2015; Antunes *et al.*, 2016). The indiscriminate use of antibiotics in animal production as prophylactic or as growth promoters has participated in the selection of multidrug-resistant strains, hindering treatment in humans (Barreto *et al.*, 2016; Zhu *et al.*, 2017).

The antibiotic resistance can be shared between bacteria of different or same genus by horizontal gene transfer via mobile genetic elements such as integrons associated to transposons and plasmids (Zhou *et al.*, 2018). Plasmids are extra-chromosomal DNA containing an origin of replication and genes that can be transferred by conjugation, transformation, and transduction into a new bacterial host (Smillie *et al.*, 2010). In *Salmonella*, plasmids that carry quinolone and beta-

lactam resistance genes have been reported (Chen *et al.*, 2010). Integrons are composed by an integrase gene (*intI*), the attachment site (*attI*), and the promoter (*Pant*) are classified based on the variation in the amino acid sequence of integrase, some of the most common classes in the clinical context are *intI1* that is classified as class 1 integron, followed by *intI2* as class 2 integron, and *intI3* as class 3 integron (Carattoli, 2001; Severino & Magalhães, 2004; Gillings, 2014).

The present study aims to characterize the genotypic resistance associated to plasmids in *Salmonella* Heidelberg strains, and characterize class 1 and 2 integrons from the strains isolated from broiler farms of Santander, Colombia.

MATERIALS AND METHODS

Sample collection

15 isolates were obtained from 8 poultry farms with a capacity of 70,000 broilers, located in the region of Santander, Colombia (Table 1). Samples were taken by cloacal swabbing from healthy Ross 308 broiler chickens on the 35th day of production. All strains were processed according to the international guidelines ISO 6579-1 (ISO, 2017) and identified as *Salmonella* Heidelberg by microbiological, serological, and molecular methods.

Table 1 – Distribution of 15 *S.* Heidelberg isolates from healthy broiler chickens in 8 farms in Santander, Colombia.

Farm number	Number of isolates
Farm 1	1
Farm 2	1
Farm 3	2
Farm 4	5
Farm 5	3
Farm 6	3
Farm 7	None
Farm 8	None

Resistance genes in plasmids

The DNA was extracted from fresh colonies using the Easy-DNA kit (Invitrogen, USA) and stored at -20°C until its use. Bacterial DNA was used as a template in a PCR reaction to determine the presence of resistance genes for antibiotics, using specific primer sets (Table 2). A total volume of 25 µL was prepared for each sample containing 1 µL of the DNA template, 1 µL of each primer (forward and reverse) (Invitrogen, USA), 1 µL of Taq DNA polymerase (Invitrogen, USA), 2.5 µL of dNTPs (Invitrogen, USA) and buffer, 2 µL of $MgCl_2$ and 14 µL of nuclease-free water for PCR. The PCR was run in a T100 thermal cycler (BIO-RAD, USA) with an initial denaturation step of 3 minutes at 95°C, followed by 35 cycles as follows: 30 seconds at 95°C for denaturation, 30 seconds at 55°C for annealing, 30 seconds at 72°C for extension and a final extension step of 7 minutes at 72°C. Amplification products were revealed by horizontal electrophoresis on 2% agarose gel stained with GelGreen® (Biotium, Russia) using the PowerPac HC (BIO-RAD, USA). The gel was visualized and documented using the gel documentation system ENDURO GDS (Labnet International, USA).

Class 1 and 2 integrons

Genomic DNA (gDNA) was extracted from bacterial colonies using the Invisorb® Spin Universal Kit (Stratec, Berlin, Germany). PCR was performed for amplification of the gene cassettes inserted in the variable regions of class 1 using primers Forward (5'-GGCATCCAAGCAGCAAG-3') and Reverse (5'-AAGCAGACTTGACCTGA-3') (Levesque *et al.*, 1995) and class 2 integrons using primers Forward (5'-CGGGATCCCGGACGGCATGCACGATTTGTA-3') and Reverse (5'-GATGCCATCGCAAGTACGAG-3') (White *et al.*, 2001). PCR conditions were the same as described above and the annealing at 55°C.The PCR products were sequenced by the Sanger method (Macrogen Inc., Korea). The DNA sequences were assembled using the software Geneious version 8 (Kearse *et al.*, 2012) and analyzed using the online software BLASTN of the National Center for Biotechnology Information.

RESULTS

Antibiotic resistance genes in plasmids

Resistance genes such as bla_{CMY2} (ceftriaxone) (n=15/15; 100%), *strA* and *strB* (streptomycin) (n=15/15; 100%), *sul1* and *sul2* (sulfamethoxazole) (n=15/15; 100%), *tetB* (tetracycline) (n=15/15; 100%) were detected in *S.* Heidelberg isolates (Table 3). Low prevalence was determined for bla_{PSE-1} (n=6/15; 40%) related with ampicillin resistance. In contrast, none of the isolates were positive to *qnrA,* bla_{TEM}, bla_{CTX-M}, *catA, catB*, *cmlA, aadA1, aadA2, aadB, oqxA, sul3, tetA, dfrA1, dfrA10,* and *dfrA12* genes.

Class 1 and 2 integrons

11 out of 15 strains showed the class 1 integron with a cassette structure size of 741 bp (Figure 1). None of the strains showed class 2 integron. Resistance gene mapping showed 100% of identity in all 11 sequences with the *dfrA7* gene of *Escherichia coli* (accession number MF465028.1) and 99.8% identity with the

Table 2 – Primer sequences used to detect resistance genes in *S.* Heidelberg isolates*.

Antibiotic	Gene	Primer sequence	Annealing temperature (°C)	Amplicon size (bp)
Ampicillin	bla_{PSE-1}	F-GCAAGTAGGGCAGGCAATCA R-GAGCTAGATAGATGCTCACAA	55	422
	bla_{TEM}	F-ATCAGTTGGGTGCACGAGTG R-ACGCTCACCGGCTCCAGA	55	608
Chloramphenicol	*catA*	F-CCAGACCGTTCAGCTGGATA R-CATCAGCACCTTGTCGCCT	55	454
	catB	F-CGGATTCAGCCTGACCACC R-ATACGCGGTCACCTTCCTG	55	461
	cmlA	F-TGGACCGCTATCGGACCG R-CGCAAGACACTTGGGCTGC	55	641
Gentamicin	*aadB*	F-CTAGCTGCGGCAGATGAGC R-CTCAGCCGCCTCTGGGCA	55	300
Spectinomycin	*aadA1*	F-CTCCGCAGTGGATGGCGG R-GATCTGCGCGCGAGGCCA	55	631
	aadA2	F-CATTGAGCGCCATCTGGAAT R-ACATTTCGCTCATCGCCGGC	55	500
Tetracycline	*tetA*	F-GCTGTCGGATCGTTTCGG R-CATTCCGAGCATGAGTGCC	55	658
	tetB	F-CTGTCGCGGCATCGGTCAT R-CAGGTAAAGCGATCCCACC	55	615
Piperacillin/tazobactam	*dfrA1*	F-CAATGGCTGTTGGTTGGAC R-CCGGCTCGATGTCTATTGT	55	254
	dfrA10	F-TCAAGGCAAATTACCTTGGC R-ATCTATTGGATCACCTACCC	55	432
	dfrA12	F-TTCGCAGACTCACTGAGGG R-CGGTTGAGACAAGCTCGAAT	55	330
Streptomycin	*strA*	F-TGGCAGGAGGAACAGGAGG R-AGGTCGATCAGACCCGTGC	55	405
	strB	F-GCGGACACTTTTCCAGCCT R-TCCGCCATCTGTGCAATGCG	55	621
Ceftriaxone	bla_{CMY2}	F-AAATCGTTATGCTGCGCTCT R-CCGATCCTAGCTCAAACAGC	55	224
	bla_{CTX-M}	F-TTCGCTAAATACCGCCATTC R-TATCGTTGGTTGTGCCGTAA	55	236
Trimethoprim/sulfamethoxazole	*sul1*	F-CGGACGCGAGGCCTGTATC R-GGGTGCGGACGTAGTCAGC	55	591
	sul2	F-GCGCAGGCGCGTAAGCTGAT R-CGAAGCGCAGCCGCAATTC	55	514
	sul3	F-GGGAGCCGCTTCCAGTAAT R-TCCGTGACACTGCAATCATTA	55	500
Quinolones and fluoroquinolones	*oqxA*	F-GGTGAAGTCGATCAGTCAGT R-ATCTATCGTGAACAGCACCT	55	154
Nalidixic acid	*qnrA*	F-CCGCTTTTATCAGTGTGACT R-ACTCTATGCCAAAGCAGTTG	55	188

*Based in Chuanchuen & Padungtod (2009).

cassette containing the *dfrA7* gene of *Salmonella* Typhi (accession number AY245101.1) (Figure 2). The *dfrA7* gene codes for a Dihydrofolate reductase that confers resistance to trimethoprim.

DISCUSSION

In recent years, the role of antimicrobial resistance in livestock has been suggested as a potential source of bacterial infections and antibiotic resistance, representing a risk at the clinical level with great challenges for clinical treatment (Berg *et al.*, 2017; Ewers *et al.*, 2012).

The antibiotic resistance genotypes as bla_{CMY2} have been found in poultry farms in Brazil (Biffi *et al.*, 2014) and in Colombia, where 168 isolates from different poultry production levels (farms, slaughter, and retail) were positive to bla_{CMY2} gene (Castellanos *et al.*, 2018). The presence of Extended Spectrum β-lactamase (ESBL) codified by bla_{CMY2} and other genes from this group reduce the effectiveness of antibiotics such as ceftriaxone, ceftazidime, and cefotaxime by the hydrolyzing of the β-lactam ring preventing the union with the penicillin-binding protein (PBP) (De Souza *et al.*, 2019; Kong *et al.*, 2010).

Table 3 – Genotypic profiles of resistance in *S.* Heidelberg isolates associated with plasmids and class I and II integrons.

Isolate code	Antimicrobial resistance profile in plasmid DNA	Class 1 integron*	Class 2 integron
1	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	-	-
2	bla_{CMY2}, bla_{PSE-1}, *strA, strB, sul1, sul2, tetB*	+	-
3	bla_{CMY2}, bla_{PSE-1}, *strA, strB, sul1, sul2, tetB*	+	-
4	bla_{CMY2}, bla_{PSE-1}, *strA, strB, sul1, sul2, tetB*	+	-
5	bla_{CMY2}, bla_{PSE-1}, *strA, strB, sul1, sul2, tetB*	-	-
6	bla_{CMY2}, bla_{PSE-1}, *strA, strB, sul1, sul2, tetB*	+	-
7	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	-	-
8	bla_{CMY2}, bla_{PSE-1},*strA, strB, sul1, sul2, tetB*	+	-
9	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
10	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
11	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
12	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
13	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
14	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	+	-
15	bla_{CMY2}, *strA, strB, sul1, sul2, tetB*	-	-

*+: presence of the mobile element.

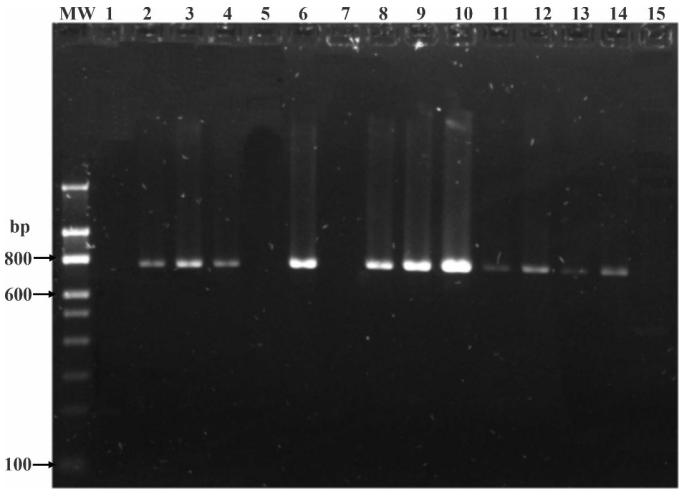

Figure1 – PCR product of the variable region of class 1 integron from *Salmonella* Heidelberg strains. MW: 100 bp DNA ladder (Corpogen, Colombia).

The *strA* and *strB* genes confer resistance to streptomycin by encoding a phosphotransferase that catalyzes the ATP-dependent phosphorylation of hydroxyl groups in streptomycin (Ashenafi *et al.*, 2014). The presence of these genes was also found in isolates of *S.* Typhimurium from poultry farms from Pakistan (20.5% and 41.1%) (Wajid *et al.*, 2019) and poultry carcasses in Iran, with values of 37.6% and 22.4%, respectively (Doosti *et al.*, 2016). In the case of *sul1,* it is normally found in the conserved region of class 1 integrons, while *sul2* is located on small plasmids such as IncQ family (RSF1010) and pBP1 and both encode a variant of sulfonamide resistant dihydropteroate synthase protein that has no affinity with the antibiotic (Van Treeck *et al.*, 1981; Sköld, 2000; Chen *et al.*, 2015; Deng *et al.*, 2015). These genes were found in poultry farms from Egypt with values of 97.3% and 5.3%, respectively (Abdel-Maksoud *et al.*, 2015) and *sul2* gene in isolates from chickens sold at retail in Colombia (57.4%) (Cortés *et al.*, 2017). The *tetB* gene was found in broiler chickens from Portugal (45.4%) (De Souza *et al.*, 2019) and in chicken carcasses retailed in Colombia (42.5%) (Cortés *et al.*, 2017). This gene codes for a tetracycline resistance protein, which promotes the elimination of tetracycline from the inside of cells by an energy-dependent efflux pump (Grossman, 2016).

Castro *et al.* (2019) reported the phenotypic resistance of these strains, and all 15 isolates showed resistance to nalidixic acid, ciprofloxacin, levofloxacin, cefotaxime, ceftazidime, cefazolin and ceftriaxone, ampicillin, ampicillin/sulbactam, streptomycin, and tetracyclines as well as high resistance to trimethoprim/sulfamethoxazole (n=14/15; 93%), aztreonam and cefepime (n=7/15; 46%). In our study, phenotypic resistance to trimethoprim/sulfamethoxazole may be mediated mainly by the *sul1* and *sul2* genes and by the *dfrA7* gene found in class 1 integron. In addition, the presence of non-endemic serotypes in Colombia, such as *S.* Heidelberg has been supported by a high number of resistance genes as well as class 1 integrons in the first part of the poultry production segment causing a major public health impact.

Class 1 integrons are adapted to capture, integrate, and express around 130 different gene cassettes that encoding proteins with resistance to cationic surfactants and main antibiotic families such as quaternary ammonium-compound family, erythromycin, aminoglycosides, sulfonamides, quinolones, chloramphenicol, fosfomycin, trimethoprim, and β-lactamases (Stokes & Hall, 1989; Partridge *et al.*, 2009; Cambray *et al.*, 2010; Kung *et al.*, 2010). This

allows the appearance of multi-drug resistance (MDR) strains in different bacteria species (Firoozeh *et al.*, 2012). In addition, other pathogenic bacteria such as nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) 42.5% (n=76/179), *Escherichia coli* 31% (n=340/1098), *Aeromonas* spp. 39.02% (n=16/41), *Stenotrophomonas maltophilia* 22% (n=20/93), *Salmonella* spp. 42% (n=77/183), and *Shigella* spp. 3.85% (n=1/26) have reported class 1 integrons (Ahmed *et al.*, 2006; Chang *et al.*, 2004; Odetoyin *et al.*, 2017; Pérez-Valdespino *et al.*, 2009; S. Rao *et al.*, 2020; Xu *et al.*, 2011).

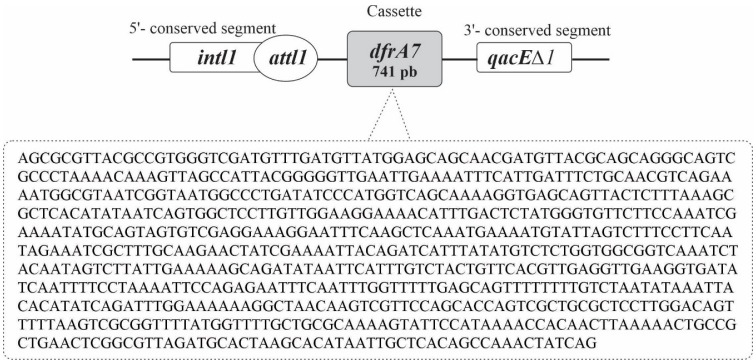

Figure 2 – Graphical representation of the class 1 integron found in *Salmonella* Heidelberg strains isolated from broiler farms located in Santander. *intI1*: integrase class 1; *attI*: recombination and integration site; *dfrA7*: Dihydrofolate reductase gene; *qacEΔ1*: quaternary ammonium resistance gene.

In the case of *Salmonella*, class 1 integron has been reported from different sources in the food chain. In animal farms from China class 1 integron was identified in chicken, ducks, and pigs that carried resistance genes cassettes against β-lactams and aminoglycosides (Zhao *et al.*, 2017). Other sources have presented *Salmonella* as in broiler samples (n=20/26; 76.9%), poultry (n=4/5; 80%), human (n=1/2; 50%) and calves (n=5/5; 100%) in Egypt (Abdel Aziz *et al.*, 2018; Elkenany *et al.*, 2018). In addition, *Salmonella* strains were isolated from dead-in-shell chicken embryos (n=24/86; 27.9%), raw chicken meat (n=1/7; 14.2%), beef meat (n=1/8; 12.5%), and seafood (n=3/40; 7.5%) (Deekshit *et al.*, 2012; Moawad *et al.*, 2017; Zhao *et al.*, 2021).

In Colombia, few data on molecular resistance to antibiotics in different segments of the poultry chain is available and to date, this is the first report of class 1 integrons characterized in poultry production in Colombia. In this study, 73.33% (n=11/15) of *S.* Heidelberg isolates contained class 1 integron which is higher than reported in *S.* Typhi from human isolates 30% (n=24/80) (Afzal *et al.*, 2013), in *Salmonella* spp. isolates from lettuce samples 40% (n=40/100) in Burkina Faso (Somda *et al.*, 2018), and isolates from humans, food products, and pork 13% (n=154/1183) (Antunes *et al.*, 2006). In addition, in Brazilian strains isolated from broiler chicken and pork, 45% (n=9/20) had the class 1 integron, lower than the values obtained in this study (Ribeiro *et al.*, 2011). In our study, only class 1 integron was found, which is a common finding in *Salmonella* and other members of *Enterobacteriaceae*, contributing to the dissemination of gene cassettes (White *et al.*, 2001; Rao *et al.*, 2006).

The class 1 integron from the present study contains a cassette with the *dfrA7* gene that confers resistance to trimethoprim by the expression of a modified dihydrofolate reductase enzyme that catalyzes the reduction of dihydrofolic acid to tetrahydrofolate, which is necessary for the synthesis of purines and pyrimidines (Brolund *et al.*, 2010; Joyner *et al.*, 1984). The importance of this finding is because antibiotic treatment is based on the use of trimethoprim/sulfamethoxazole, fluoroquinolones, and oxyimino-cephalosporins, even in children, elderly, and immunocompromised people (Guerrant *et al.*, 2001; Mthembu *et al.*, 2019). In agreement with authors such Argüello *et al.* (2018), *dfrA7* gene is one of the classic genes carried by class 1 integrons and this was first reported by Wain *et al.* (2003) in isolates from Vietnam in 1994. Additionally, the gene has been found in isolates from livestock production in South Africa (Mthembu *et al.*, 2019), isolates of *Salmonella* Heidelberg from poultry in Colombia (Donado-Godoy *et al.*, 2015), and in class 1 intregrons from *S.* Typhi isolates from clinical patients in Pakistan (Afzal *et al.*, 2013).

In conclusion, this study found a high presence of resistance genes associated with commonly used antibiotics in both human and animal medicine, as well as class 1 integron with the gene cassette *dfrA7* in *Salmonella* Heidelberg strains isolated from farms in Santander that belong to the first part of the Colombian poultry segment.

COMPETING OF INTERESTS

The authors declare no competing of interest.

ACKNOWLEDGMENTS

Financial support was provided by the Laboratory of Immunology and Molecular Biology – LIBM of the University of Tolima and its head professor Iang S. Rondón.

REFERENCES

Abdel-Maksoud M, Abdel-Khalek R, El-Gendy A, Gamal RF, Abdelhady HM, House BL. Genetic characterisation of multidrug-resistant *Salmonella enterica* serotypes isolated from poultry in Cairo, Egypt. African Journal of Laboratory Medicine 2015; 4(1):1–7.

Abdel Aziz SA, Abdel-Latef GK, Shany SAS, Rouby SR. Molecular detection of integron and antimicrobial resistance genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-Suef governorate, Egypt. Beni-Suef University Journal of Basic and Applied Sciences 2018; 7(4):535–542.

Afzal A, Sarwar Y, Ali A, Maqbool A, Salman M, Habeeb MA, Haque A. Molecular evaluation of drug resistance in clinical isolates of *salmonella enterica* serovar Typhi from Pakistan. Journal of Infection in Developing Countries 2013; 7(12):929–940.

Ahmed AM, Furuta K, Shimomura K, Kasama Y, Shimamoto T. Genetic characterization of multidrug resistance in *Shigella* spp. from Japan. Journal of Medical Microbiology 2006; 55(12): 1685–1691.

Antunes P, Mourão J, Campos J, Peixe L. Salmonellosis: the role of poultry meat. Clinical Microbiology and Infection 2016; 22(2):110–121.

Antunes P, Machado J, Peixe L. Characterization of antimicrobial resistance and class 1 and 2 integrons in *Salmonella enterica* isolates from different sources in Portugal. Journal of Antimicrobial Chemotherapy 2006; 58(2):297–304.

Argüello H, Guerra B, Rodríguez I, Rubio P, Carvajal A. Characterization of antimicrobial resistance determinants and class 1 and class 2 integrons in *salmonella enterica* spp, multidrug-resistant isolates from pigs. Genes 2018; 9(5);1–13.

Ashenafi M, Ammosova T, Nekhai S, Byrnes M. Purification and characterization of aminoglycoside phosphotransferase APH (6)-Id, a streptomycin inactivating enzyme. Molecular Cell Biochemistry 2014; 387(1):207–216.

Barreto M, Castillo-Ruiz M, Retamal P. *Salmonella enterica*: una revisión de la trilogía agente, hospedero y ambiente, y su trascendencia en Chile. Revista Chilena de Infectología 2016;33(5):547–557.

Berg ES, Wester AL, Ahrenfeldt J, Mo SS, Slettemeås JS, Steinbakk M, et al. Norwegian patients and retail chicken meat share cephalosporin-resistant *Escherichia coli* and IncK/bla_{CMY-2} resistance plasmids. Clinical Microbiology and Infection 2017; 23(6):407e9-407.e15.

Biffi CP, Stefani LM, Miletti LC, Matiello CA, Backes RG, Almeida JM, et al. Phenotypic and genotypic resistance profile of *Salmonella* Typhimurium to antimicrobials commonly used in poultry. Revista Brasileira de Ciencia Avicola 2014; 16(2):93–96.

Brolund A, Sundqvist M, Kahlmeter G, Grape M. Molecular characterisation of trimethoprim resistance in *Escherichia coli* and *Klebsiella pneumoniae* during a two year intervention on trimethoprim use. PLoS ONE 2010; 5(2):1–5.

Cambray G, Guerout A, Mazel D. Integrons. Annual Reviews 2010; 44(1):141–166.

Carattoli A. Importance of integrons in the diffusion of resistance. Veterinary Research 2001; 32(3–4):243–259.

Castellanos LR, Graaf-van Bloois L, Donado-Godoy P, León M, Clavijo V, Arévalo A, et al. Genomic Characterization of Extended-Spectrum Cephalosporin-Resistant *Salmonella enterica* in the Colombian Poultry Chain. Frontiers in Microbiology 2018; 9(10):1–11.

Chang LL, Chen HF, Chang CY, Lee TM, Wu WJ. Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*. Journal of Antimicrobial Chemotherapy 2004; 53(3):518–521.

Chen B, Liang X, Nie X, Huang X, Zou S, Li X. The role of class I integrons in the dissemination of sulfonamide resistance genes in the Pearl River and Pearl River Estuary, South China. Journal of Hazardous Materials 2015; 282:61–67.

Chen CY, Lindsey RL, Strobaugh TP, Frye JG, Meinersmann RJ. Prevalence of ColE1-like plasmids and kanamycin resistance genes in *salmonella enterica* serovars. Applied and Environmental Microbiology 2010; 76(20):6707–6714.

Chuanchuen R, Padungtod P. Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. The Journal of Veterinary Medical Science / the Japanese Society of Veterinary Science 2009; 71:1349–1355.

Cortés D, Rodríguez V, Verjan N. *Salmonella* from Chicken Carcasses Marketed at. Revista Brasileira de Ciência Avícola 2017; 19:347–354.

Cosby DE, Cox NA, Harrison MA, Wilson JL, Jeff Buhr R, Fedorka-Cray, PJ. *Salmonella* and antimicrobial resistance in broilers: A review. Journal of Applied Poultry Research 2015; 24(3):408–426.

De Souza M, Brito DAP, Menck-Costa MF, Oba A, Kobayashi RKT, Justino L, Baptista, AAS. Multidrug resistant and ESBL-producing *Salmonella* spp. isolated from poultry. Semina: Ciencias Agrarias 2019; 40(6):3045–3056.

Deekshit VK, Kumar BK, Rai P, Srikumar S, Karunasagar I, Karunasagar I. Detection of class 1 integrons in *Salmonella* Weltevreden and silent antibiotic resistance genes in some seafood-associated nontyphoidal isolates of *Salmonella* in south-west coast of India. Journal of Applied Microbiology 2012; 112(6):1113–1122.

Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, et al. Resistance integrons: Class 1, 2 and 3 integrons. Annals of Clinical Microbiology and Antimicrobials 2015; 14(1):45

Donado-Godoy P, Gardner I, Byrne BA, Leon M, Perez-Gutierrez E, Ovalle MV, et al. Prevalence, risk factors, and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. Journal of Food Protection 2012; 75(5):874–883.

Donado-Godoy P, Byrne BA, Hume M, León M, Pérez-Gutiérrez E, Vives Flores MJ, et al. Molecular characterization of *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg from poultry and retail chicken meat in Colombia by pulsed-field gel electrophoresis. Journal of Food Protection 2015; 78(4):802–807.

Doosti A, Mahmoudi E, Jami MS, Mokhtari-Farsani A. Prevalence of *aadA1*, *aadA2*, *aadB*, *strA* and *strB* genes and their associations with multidrug resistance phenotype in *Salmonella* Typhimurium isolated from poultry carcasses. Thai Journal of Veterinary Medicine 2016; 46(4):691–697.

Elkenany RM, Eladl AH, El-Shafei RA. Genetic characterisation of class 1 integrons among multidrug-resistant *Salmonella* serotypes in broiler chicken farms. Journal of Global Antimicrobial Resistance 2018; 14(9):202-208.

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: A global perspective. Clinical Microbiology and Infection 2012; 18(7):646–655.

Firoozeh F, Zahraei-Salehi T, Shahcheraghi F, Karimi V, Aslani MM. Characterization of class I integrons among *Salmonella enterica* serovar Enteritidis isolated from humans and poultry. FEMS Immunology and Medical Microbiology 2012; 64(2):237–243.

Gillings MR. Integrons : Past , Present , and Future Structure of Integrons. Microbiology and Molecular Biology Reviews 2014; 78(2):257–277.

Grossman TH. Tetracycline antibiotics and resistance. Cold Spring Harbor Perspectives in Medicine 2016: 6(4):1–24.

Guerrant RL, Van Gilder T, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, et al. Practice guidelines for the management of infectious diarrhea. Clinical Infectious Diseases 2001; 32(3):331–351.

ISO - International Organization for Standardization. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp [ISO 6579-1]; 2017. Available from: https://www.abntcatalogo.com.br/norma.aspx?ID=367735

Joyner SS, Fling ME, Stone D, Baccanari DP. Characterization of an R-plasmid dihydrofolate reductase with a monomeric structure. The Journal of Biological Chemistry 1984; 259(9):5851–5856.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012; 28(12):1647–1649.

Kong KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. Apmis 2010; 118(1):1–36.

Kung VL, Ozer EA, Hauser AR. The Accessory Genome of *Pseudomonasae ruginosa*. Microbiology and Molecular Biology Reviews 2010; 74(4):621–641.

Levesque C, Piche L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrobial Agents and Chemotherapy 1995; 39(1):185–191.

McMillan EA, Gupta SK, Williams LE, Jové T, Hiott LM, Woodley TA, et al. Antimicrobial resistance genes, cassettes, and plasmids present in *Salmonella enterica* associated with United States food animals. Frontiers in Microbiology 2019; 10(APR):1–18.

Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafez HM, et al. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. Gut Pathogens 2017;9(1):1–13.

Mthembu TP, Zishiri OT, El Zowalaty ME. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. Infection and Drug Resistance 2019; 12:3537–3548.

Odetoyin BW, Labar AS, Lamikanra A, Aboderin AO, Okeke IN. Classes 1 and 2 integrons in faecal *Escherichia coli* strains isolated from mother-child pairs in Nigeria. PLoS ONE 2017; 12(8):e0183383.

Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons: Review article. FEMS Microbiology Reviews 2009; 33(4):757–784.

Pérez-Valdespino A, Fernández-Rendón E, Curiel-Qesada E. Detection and characterization of class 1 integrons in *Aeromonas* spp. isolated from human diarrheic stool in Mexico. Journal of Basic Microbiology 2009;49(6):572–578.

Rao AN, Barlow M, Clark LA, Boring JR, Tenover FC, McGowan JE. Class 1 integrons in resistant *Escherichia coli* and *Klebsiella* spp, US hospitals. Emerging Infectious Diseases 2006; 12(6):1011–1014.

Rao S, Linke L, Doster E, Hyatt D, Burgess BA, Magnuson R, et al. Genomic diversity of class I integrons from antimicrobial resistant strains of *Salmonella* Typhimurium isolated from livestock, poultry and humans. PLoS ONE, 2020; 15(12):e0243477.

Ribeiro VB, Lincopan N, Landgraf M, Franco BDGM, Destro MT. Characterization of class 1 integrons and antibiotic resistance genes in multidrug resistant *Salmonella enterica* isolates from foodstuff and related sources. Brazilian Journal of Microbiology 2011; 42(2):685–692.

Severino P, Magalhães VD. Integrons as tools for epidemiological studies. Clinical Microbiology and Infection 2004; 10(2):156–162.

Sköld O. Sulfonamide resistance: Mechanisms and trends. Drug Resistance Updates 2000; 3(3):155–160.

Smillie C, Garcillan-Barcia MP, Francia MV, Rocha EPC, Cruz F. Mobility of plasmids. Microbiology and Molecular Biology Reviews 2010; 74(3):434–452.

Somda SN, Savadogo A, Bonkoungou OJI, Traore O, Sambe-Ba B, Wane A-A, et al. Molecular detection of virulence and resistance genes in. 2018. p.1–21. Available from: doi: https://doi.org/10.1101/436501

Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Molecular Microbiology 1989; 3(12):1669–1683.

Van Treeck U, Schmidt F, Wiedemann B. Molecular nature of a streptomycin and sulfonamide resistance plasmid (pBP1) prevalent in clinical *Escherichia coli* strains and integration of an ampicillin resistance transposon (TnA). Antimicrobial Agents and Chemotherapy 1981; 19(3):371–380.

Wain J, Nga LTD, Kidgell C, James K, Fortune S, Parry C, et al. Molecular analysis of incHI1 antimicrobial resistance plasmids from *Salmonella* serovar Typhi strains associated with typhoid fever. Antimicrob Agents and Chemotherapy 2003; 47(9):2732–2739.

Wajid M, Awan AB, Saleemi MK, Weinreich J, Schierack P, Sarwar Y, et al. Multiple drug resistance and virulence profiling of *salmonella enterica* serovars Typhimurium and *enteritidis* from poultry farms of Faisalabad, Pakistan. Microbial Drug Resistance 2019; 25(1):133–142.

White PA, Iver CJMC, Rawlinson WD. Integrons and Gene Cassettes in the Enterobacteriaceae. Antimicrobial Agents and Chemotherapy 2001; 45(9):2658–2661.

Xu Z, Li L, Shirtliff ME, Peters BM, Li B, Peng Y, et al. Resistance class 1 integron in clinical methicillin-resistant *Staphylococcus aureus* strains in southern China, 2001-2006. Clinical Microbiology and Infection 2011; 17(5):714–718.

Zhao X, Yang J, Zhang B, Sun S, Chang W. Characterization of integrons and resistance genes in *Salmonella* isolates from farm animals in Shandong province, China. Frontiers in Microbiology 2017; 8:1–10.

Zhao X, Ju Z, Wang G, Yang J, Wang F, Tang H, et al. Prevalence and Antimicrobial resistance of *salmonella* isolated from dead-in-shell chicken embryos in Shandong, China. Frontiers in Veterinary Science 2021; 8:581946.

Zhou ZC, Feng WQ, Han Y, Zheng J, Chen T, Wei YY, et al. Prevalence and transmission of antibiotic resistance and microbiota between humans and water environments. Environment International 2018; 121(7):1155–1161.

Zhu Y, Lai H, Zou L, Yin S, Wang C, Han X, et al. Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. International Journal of Food Microbiology 2017; 259(10):43–51.