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# Multidrug resistant and ESBL-producing *Salmonella* spp. isolated from poultry

# *Salmonella* spp. produtora de ESBL e multirresistente a drogas isolada de frangos

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## Abstract

*Salmonella* spp. is one of the main agents responsible for foodborne infection in humans, and products of poultry origin are the most common infection sources. Studies have shown the occurrence of antimicrobials resistant *Salmonella* spp. in animal products. The Extended Spectrum *β*-Lactamase (ESBL) are enzymes that confer to bacteria the ability to hydrolyze cephalosporin with an oximino side chain and monobactams. This study aimed to investigate antimicrobial resistance profile, identify phenotypes and genotypes for multiple drug resistance (MDR) and that produce ESBL from isolates of *Salmonella* spp. in the broiler production chain. We used samples of *Salmonella* spp. (n=11) isolates from poultry, poultry products and poultry-source environment from the state of Maranhão - Brazil. The isolates of *Salmonella* spp. assessed showed genotypical and phenotypical characteristics of MDR. The results show that 72.72% (08/11) of the strains presented the phenotypic profile for ESBL production. The isolates showed positivity to at least 13.64% (03/22) of the genes studied and the highest frequencies were observed in genes $sul_1$ (73%), $dfr_{A12}$ (55%), $bla_{CTX\text{-}M}$ (55%), $tet_A$, $tet_B$ and $tet_C$, with 45%. In conclusion, the strains of *Salmonella* spp. isolates present genotypic and phenotypic characteristics for MDR and ESBL production, demonstrating the dissemination risk of these microorganisms through the food chain.

**Key words**: Zoonosis. Broiler Chicken. Antimicrobials. Resistance Genes.

## Resumo

*Salmonella* spp. é um dos principais agentes responsáveis por infecção de origem alimentar em humanos, sendo os produtos de origem avícola apontados como as fontes de infecção mais frequentes. Estudos demonstraram a ocorrência de *Salmonella* spp. resistente a antimicrobianos em produtos de origem animal. As *β*-lactamases de espectro extendido (ESBL) são enzimas que conferem às bactérias a capacidade de hidrolisar cefalosporinas com uma cadeia lateral oximino e monobactâmicos. Este estudo objetivou investigar o perfil de resistência a antimicrobianos, identificar fenótipos e genótipos de multirresistência a drogas (MDR), e produção de ESBL em isolados de *Salmonella* spp. provenientes da

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cadeia produtiva de frangos de corte. Foram utilizadas amostras de *Salmonella* spp. (n=11) isoladas de aves, produtos e ambiente de origem avícola do estado do Maranhão - Brasil. Os isolados de *Salmonella* spp. avaliados apresentaram resistência múltipla a drogas tanto genotípica quanto fenotipicamente. Os resultados mostram que 72,72% (08/11) das cepas apresentaram perfil fenotípico de produção de ESBL. Os isolados apresentaram positividade a pelo menos 13,64% (03/22) dos genes pesquisados, sendo as maiores frequências observadas nos genes $sul_1$ (73%), $dfr_{A12}$ (55%), $bla_{CTX\text{-}M}$ (55%), $tet_A$, $tet_B$ e $tet_C$, com 45%. Conclui-se que as cepas de *Salmonella* spp. isoladas apresentam características genotípicas e fenotípicas de multirresistência a drogas e produção de ESBL, demonstrando o risco de disseminação destes microrganismos ao longo da cadeia alimentar.
**Palavras-chave**: Zoonose. Frango de Corte. Antimicrobianos. Genes de Resistência.

## Introduction

*Salmonella* spp. is one of the main agents responsible for Foodborne Diseases (FBD) in humans, both in developing and developed countries (ABD-ELGHANY et al., 2015). In the United States alone, the agent accounts for 896 outbreaks, 23.662 illnesses, 3.168 hospitalization and 29 deaths by FBD confirmed cases, between 2009-2015 (DEWEY-MATTIA et al., 2018).

Products of poultry origin are accounted as the most common sources of infection (MELENDEZ et al., 2010). Studies have shown the occurrence of *Salmonella* spp. resistant to antimicrobials in animal products and consequently the potential for transmission of the agent along the food chain (WANG et al., 2013; ZIECH et al., 2016). Samples of multiresistant *Salmonella* spp. are considered a public health issue, as they limit therapeutic options for salmonellosis treatment in humans (ZISHIRI et al., 2016).

Extended Spectrum *β*-Lactamase (ESBL) are enzymes that confer to the bacteria the ability to hydrolyze cephalosporin with an oximino side chain (ceftriaxone, ceftazidime, cefotaxime) and monobactams (aztreonan). This complex group of enzymes is transported by plasmids of rapid development, increasing challenges in the treatment of hospitalized patients, from cases of urinary tract infections to sepsis (RAWAT; NAIR, 2010).

Currently, more than 400 ESBLs are described. The CTX-M group encompasses at least 168 variations (LAHEY CLINIC, 2017). Bacteria can acquire resistance to antibiotics mainly by chromosomal mutation and acquisition of mobile genetic elements such as plasmids by horizontal gene transfer (MILLAN, 2018). Plasmids are extrachromosomal DNA molecules that replicate independently of the chromosome and can carry resistance to other drugs, such as aminoglycosides, trimethoprim, sulfonamides, tetracycline and chloramphenicol (CARATTOLI, 2013; PITOUT et al., 2005).

This study aimed to determine the antimicrobial resistance profile, identify phenotypic and genotypic characteristics for multiple drug resistance (MDR) and for ESBL production in isolates of *Salmonella* spp. from different sources of the broiler production chain.

## Material and Methods

### *Ethical aspects*

The present work was carried out after approval by the Ethics Committee on the Use of Animals (CEUA - in Portuguese) - UEL, registered under Protocol No. 178/2014.

### *Bacterial isolates*

We used samples of *Salmonella* spp. (n=11) isolates from poultry, poultry products and poultry-source environment from the state of Maranhão, Brazil, five isolates of carcasses from artisanal slaughterhouses, four from the environment (drag swab and boot swab, from poultry shed) and two from broiler chickens (cloaca swabs). The samples were processed between 2013 to 2014, at the Microbiology Laboratory from the Instituto Federal de Educação do Maranhão (IFMA), according to normative instruction No. 8, MAPA (MAPA, 1995), and serotyping by Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil. The serovars found are described in Table 1.

**Table 1.** *Salmonella* spp. strains isolated in this study.

| Serovar | N | Source |
|---|---|---|
| *S.* Albany | 01 | Carcasses |
| *S.* Schwarzengrund | 09 | Environment (03); broiler chickens (02); Carcasses (04) |
| *S.* Typhimurium | 01 | Environment |
| **Total** | **11** | |

*Antimicrobial susceptibility test*

The Disk Diffusion test (BAUER et al., 1966) was used to determine the antimicrobial susceptibility profile, according to protocol of Clinical and Laboratory Standards Institute (CLSI, 2017). Strains of *Escherichia coli* ATCC 25922 and *Salmonella* Enteritidis ATCC 13076 were used as control. The antimicrobials tested are described in Table 2. Isolates with resistance to three or more classes of antimicrobials simultaneously were considered a phenotype with multiple drug resistance (MDR) (MAGIORAKOS et al., 2012).

**Table 2.** Antimicrobials tested and therapeutic application in human and veterinary medicine.

| | Antimicrobial class | Antimicrobial | Therapeutic application | | |
|---|---|---|---|---|---|
| | | | **Only human medicine** | **Only veterinary medicine** | **Human and veterinary medicine** |
| $\beta$-lactams | Carbapenens | imipenem - 10µg | | | X |
| | cephalosporin of first generation | cefazolin - 30µg | | | X |
| | cephalosporin of second generation | cefoxitin 30µg | | | X |
| | cephalosporin of third generation | cefotaxime 30µg | | | X |
| | | ceftazidime 30µg | | | X |
| | | ceftiofur 30µg | | X | |
| | | ceftriaxone 30µg | | | X |
| | cephalosporin of fourth generation | cefepime 30 µg | | | X |
| | Monobactams | aztreonam - 30µg | | | X |
| | Penicillin | amoxicillin - 10µg | | | X |
| | | amoxicillin 20µg + acid clavulanic 10µg | | | X |
| | | ampicillin - 10µg | | | X |
| | Aminoglycosides | gentamicin - 10µg | | | X |
| | | streptomycin - 300µg | | | X |
| | Fenicoles | chloramphenicol - 30µg | X | | |
| | | florfenicol - 30µg | | | X |
| | inhibitors of folates | sulfonamide - 300µg; | | | X |
| | | trimethoprim - 5µg | | | X |
| | Nitrofurans | nitrofurantoin - 300µg | X | | |
| | Quinolones | acid nalidixic - 30µg | | | X |
| | | ciprofloxacin - 5µg | | | X |
| | | norfloxacin - 10µg | | | X |
| | Tetracycline | tetracycline - 30µg | | | X |

Brazil's legislation - prohibit chloramphenicol and nitrofurans as growth promoters and therapeutic use (MAPA, 2003); tetracyclines, penicillins, cephalosporins, quinolones and sulfonamides are prohibit to be used as growth promoters, only the therapeutic use is available (MAPA, 2009).

*Genotypic profile of antimicrobial resitance and ESBL production*

We determined resistance to beta-lactams (($bla_{CTX\text{-}M}$, $bla_{CTX\text{-}M1}$, $bla_{CTX\text{-}M2}$, $bla_{CTX\text{-}M15}$, $bla_{OXA}$, $bla_{SHV}$, $bla_{TEM}$ - ESBL production) and $bla_{CMY\text{-}2}$ - production of AmpC), inhibitors of folates (trimethoprim $dfr_{A1}$, $dfr_{A7}$, $dfr_{A12}$, $dfr_{A14}$, $drf_{B}$; and to sulfonamides $sul_1$, $sul_2$, $sul_3$) and to tetracycline ($tet_A$, $tet_B$, $tet_C$, $tet_D$, $tet_E$ and $tet_G$). The reactions were prepared as follows: 80 ng of DNA template, Buffer 1x (Invitrogen), 50 mM of MgCl2, 2.5 mM of each dNTP, 1uL of each primer (10 µM), 1uL of taq DNA Polymerase (5 U/µL) (Invitrogen). The PCR Protocol consisted of an initial denaturation cycle at 94° C for 2 min, followed by 30 cycles of denaturation at 94° C for 1 min, hybridization for 1 min and extension at 72° C for 1 min, and final extension at 72° C for 10 min. Table 3 shows the sequence of every gene primer tested.

**Table 3.** Primers used in this study.

| Antimicrobial Class | Gene | Sequence (5'-3') | PCR product size (bp) | Reference |
|---|---|---|---|---|
| beta-lactams | $bla_{CMY-2}$** | F: TGGCCGAACTGACAGGCAAA<br>R: TTTCTCCTGAACGTGGCTGGC | 870 | Chen et al. (2004) |
| | $bla_{CTX-M}$* | F: TTTGCGATGTGCAGTACCAGTAA<br>R: CGATATCGTTGGTGGTGCCATA | 544 | Silva et al. (2011) |
| | $bla_{CTX-M1}$* | F: GACGATGTCACTGGCTGAGC<br>R: AGCCGCCGACGCTAATACA | 499 | Silva et al. (2011) |
| | $bla_{CTX-M2}$* | F: GCGACCTGGTTAACTACAATC<br>R: CGGTAGTATTGCCCTTAAGCC | 351 | Silva et al. (2011) |
| | $bla_{CTX-M15}$* | F: CACACGTGGAATTTAGGGACT<br>R: GCCGTCTAAGGCGATAAACA | 564 | Silva et al. (2011) |
| | $bla_{OXA}$* | F: ACCAGATTCAACTTTCAA<br>R: TCTTGGCTTTTATGCTTG | 598 | Gallardo et al. (1999) |
| | $bla_{SHV}$* | F: ATGCGTTATATTCGCCTGTG<br>R: GTTAGCGTTGCCAGTGCTCG | 573 | Silva et al. (2011) |
| | $bla_{TEM}$* | F: ATGAGTATTCAACATTTCCGTG<br>R: TTACCAATGCTTAATCAGTGAG | 861 | Silva et al. (2011) |
| inhibitors of folates (molecule - trimethoprin and sulfonamide) | $drf_{A1}$ | F:GTGAAACTATCACTAATGG<br>R: TTAACCCTTTTGCCAGATTT | 474 | Navia et al. (2003) |
| | $dfr_{A7}$ | F: TTGAAAATTTCATTGATTG<br>R: TTAGCCTTTTTTCCAAATCT | 474 | Navia et al. (2003) |
| | $dfr_{A12}$ | F: GGTGSGCAGAAGATTTTTCGC<br>R: TGGGAAGAAGGCGTCACCCTC | 319 | Navia et al. (2003) |
| | $dfr_{A14}$ | F:GAGCAGCTICTITTIAAAGC<br>R:TTAGCCCTTTIICCAATTTT | 393 | Navia et al. (2003) |
| | $dfr_{B}$ | F: GATCACGTGCGCAAGAAATC<br>R:AAGCGCAGCCACAGGATAAAT | 141 | Navia et al. (2003) |
| | $Sul_1$ | F: TTTCCTGACCCTGCGCTCTAT<br>R: GTGCGGACGTAGTCAGCGCCA | 425 | Ma et al. (2007) |
| | $Sul_2$ | F: CCTGTTTCGTCCGACACAGA<br>R: GAAGCGCAGCCGCAATTCAT | 435 | Ma et al. (2007) |
| | $Sul_3$ | F: ATGAGCAAGATTTTTGGAATCGTAA<br>R: CTAACCTAGGGCTTTGGATATTT | 792 | Ma et al. (2007) |
| Tetracyclines (molecule - tetracycline) | $tet_A$ | F: TTGGCATTCTGCATTCACTC<br>R: GTATAGCTTGCCGGAAGTCG | 494 | Ma et al. (2007) |
| | $tet_B$ | F: CAGTGCTGTTGTGTCATTAA<br>R: GCTTGGAATACTGAGTGTAA | 571 | Ma et al. (2007) |
| | $tet_C$ | F: CTTGAGAGCCTTCAACCCAG<br>R: ATGGTCGTCATCTACCTGCC | 418 | Ma et al. (2007) |
| | $tet_D$ | F: GCTCGGTGGTATCTCTGCTC<br>R: AGCAACAGAATCGGGAACAC | 546 | Ma et al. (2007) |
| | $tet_E$ | F: TATTAACGGGCTGGCATTTC<br>R: AGCTGTCAGGTGGGTCAAAC | 544 | Ma et al. (2007) |
| | $tet_G$ | F: GCTCGGTGGTATCTCTGCTC<br>R: CAAAGCCCCTTGCTTGTTAC | 550 | Ma et al. (2007) |

(**) - gene that also indicate Amp-C production. (*) - gene that also indicate ESBL production. F: forward, R: reverse

*Phenotypic profile for ESBL production*

Production of Extended Spectrum *β*-Lactamase (ESBL) was determined by the disk approximation test, according to Clinical and Laboratory Standards Institute (CLSI, 2017). The antimicrobial discs used were: amoxicillin + clavulanic acid (AMC 30 µg), aztreonam (ATM 30 µg), ceftazidime (CAZ 30 µg), ceftriaxone (CRO 30 µg), cefotaxime (CTX 30 µg). The AMC disk was applied at the plate center containing agar Mueller Hinton (MH) and the others at 20 mm from the edge of the central disk. The sample was considered positive when there was an increase in inhibition zone, with deformation in a halo of antibiotics prepared around the central disk of AMC. We also perform the phenotypic profile for ESBL production because the fact that bacteria has an ESBL gene does not means that it is able to express the resistance.

## Results

The 11 isolates of *Salmonella* spp. assessed showed phenotypical multiple resistance to drugs, and all of them showed genotypical resistance to at list two classes of antimicrobials (Table 4). A sample of *S.* Schwarzengrund (isolate No. 05, no-ESBL producing), from the environment, presented the lowest MDR to 5 from 13 (38.46%) classes of antimicrobials tested. The highest MDR was observed in two samples of the same serovar (isolates No. 07 and 08, both ESBL-producing), however, from broiler chickens with resistance to 09/13 (69.23%) of the antimicrobials classes tested.

Regarding the phenotypic profile for ESBL production, only 27.27% (03/11) of the isolates were negative, all belonging to the serovar *S.* Schwarzengrund, two samples were isolates from the carcass and one from the environment, 72.73% (08/11) of the isolates were positive for ESBL production. Regarding ESBL isolates origin 37.5% (03/08) were from the carcass, 25% (02/08) from broiler chickens and 37.5% (03/08) from the environment. AMO, AMP, CFZ, SUL, TET, and TRI were the antimicrobials with the largest resistance percentages (Figure 1).

The isolates showed positivity to at least 13.64% (03/22) of the genes studied. A sample of serovar *S.* Schwarzengrund from the carcass presented the highest percentage of positivity, 31.82% (07/22). The genes mostly found confer resistance the sulfonamides ($sul_1$) 72,73% (08/11), trimethoprim ($dfr_{A12}$) 54,54% (06/11), *β*-lactam ($bla_{CTX\ M}$) 54,54% (06/11) and tetracycline ($tet_A$, $tet_B$ and $tet_C$) 45,45% (05/11), resembling the phenotypic profile (Figure 2).

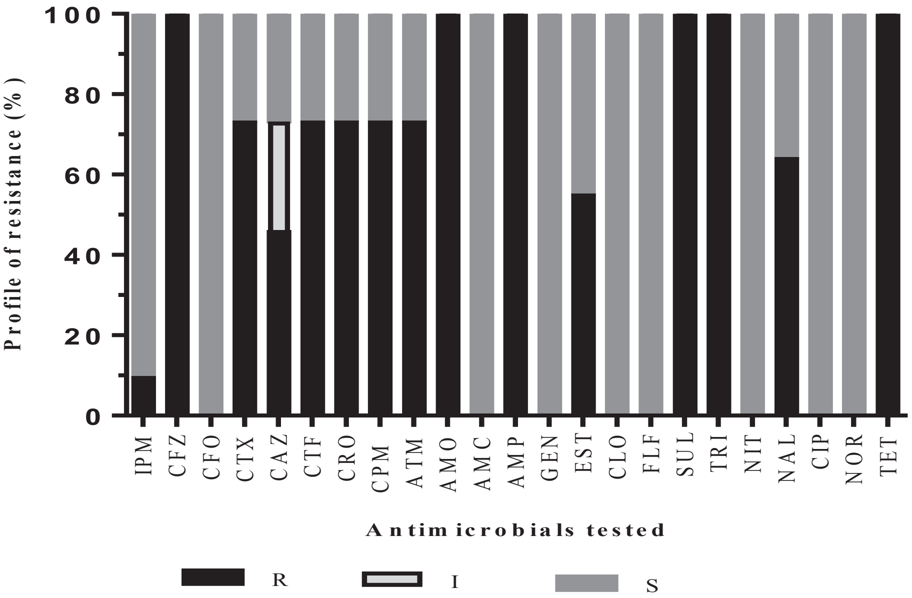
The comparison of genotypic with phenotypic profiles of the isolates showed that 63.64% (07/11) presented genes for ESBL production of which 28.57% (02/07, isolates No. 06 and 09) had the genes; however, they were not ESBL producers in the phenotypic evaluation. Another 27.27% (03/11, isolates No. 02, 04 and 11) presented phenotypic profile for ESBL production, despite the absence of genes that confer this capacity assessed in this study.

**Table 4.** Profile of phenotypic resistance to antimicrobials tested, genotypic resistance and ESBL production.

| Isolates | Serovar | Source | ESBL production | Antimicrobials | Phenotypic Resistance Percentage | Detected Genes | Genotypic Resistance Percentage |
|---|---|---|---|---|---|---|---|
| **01** | *S.* Schwarzengrund | carcasses | **Positive** | AMO, AMP, ATM, CAZ(I), CFZ, CRO, CTF, CTX, CPM, SUL, TET, TRI | 52.17% (12/23) | $bla_{CTX-M}$*; $dfr_{A12}$; $sul_1$ | 13.64% (03/22) |
| **02** | *S.* Schwarzengrund | environment | **Positive** | AMO, AMP, ATM, CAZ(I), CFZ, CRO, CTF, CTX, CPM, SUL, TET, TRI | 52.17% (12/23) | $dfr_{A12}$; $sul_1$; $tet_B$; $tet_C$ | 18.18% (04/22) |
| **03** | *S.* Schwarzengrund | carcasses | **Positive** | AMO, AMP, ATM, CAZ, CFZ, CPM, CRO, CTF, CTX, EST, SUL, TET, TRI | 56.52% (13/23) | $bla_{CTX-M}$*; $bla_{CTX-M2}$*; $dfr_{A12}$; $sul_1$; $tet_A$; $tet_B$; $tet_E$ | 31.82% (07/22) |
| **04** | *S.* Schwarzengrund | environment | **Positive** | AMO, AMP, ATM, CAZ(I), CFZ, CRO, CTF, CTX, CPM, EST, SUL, TET, TRI | 56.52% (13/23) | $dfr_{A1}$; $sul_1$; $tet_A$; $tet_C$ | 18.18% (04/22) |
| 05 | *S.* Schwarzengrund | environment | Negative | AMO, AMP, CFZ, NAL, SUL, TET, TRI | 30.43% (07/23) | $dfr_{A1}$; $sul_1$; $tet_A$; $tet_C$ | 18.18% (04/22) |
| 06 | *S.* Schwarzengrund | carcasses | Negative | AMO, AMP, CFZ, EST, NAL, SUL, TET, TRI | 34.78% (08/23) | $bla_{CTX-M}$*; $bla_{CTX-M2}$*; $sul_1$; $tet_B$ | 18.18% (04/22) |
| **07** | *S.* Schwarzengrund | broiler chickens | **Positive** | AMO, AMP, ATM, CAZ, CFZ, CPM, CRO, CTF, CTX, EST, NAL, SUL, TET, TRI | 60.87% (14/23) | $bla_{CTX-M}$*; $bla_{CTX-M2}$*; $tet_A$ | 13.64% (03/22) |
| **08** | *S.* Schwarzengrund | broiler chickens | **Positive** | AMO, AMP, ATM, CAZ, CFZ, CPM, CRO, CTF, CTX, EST, NAL, SUL, TET, TRI | 60.87% (14/23) | $bla_{CTX-M}$*; $sul_1$; $sul_2$; $tet_A$ | 18.18% (04/22) |
| 09 | *S.* Schwarzengrund | carcasses | Negative | AMO, AMP, CFZ, EST, IMP, NAL, SUL, TET, TRI | 39.13% (09/23) | $bla_{CTX-M}$*; $bla_{SHV}$*; $dfr_{A1}$; $dfr_{A12}$; $tet_B$; $tet_C$ | 27.27% (06/22) |
| **10** | *S.* Albany | carcasses | **Positive** | AMO, AMP, ATM, CAZ, CFZ, CPM, CRO, CTF, CTX, NAL, SUL, TRI, TET | 56.52% (13/23) | $bla_{CTX-M2}$*; $dfr_{A12}$; $sul_1$; $tet_B$ | 18.18% (04/22) |
| **11** | *S.* Typhimurium | environment | **Positive** | AMO, AMP, ATM, CAZ, CFZ, CPM, CRO, CTF, CTX, NAL, SUL, TET, TRI | 56.52% (13/23) | $dfr_{A12}$; $tet_C$; $tet_E$ | 27,27% (06/22) |
| 11/11 | | | | | | | |

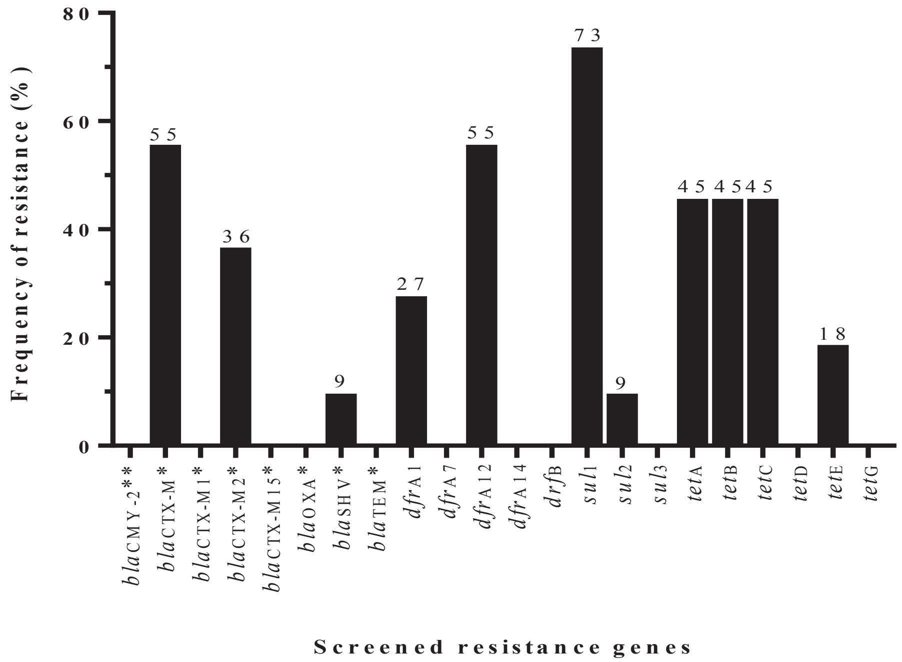
Antimicrobials tested: IPM-imipenem 10μg; CFZ-cefazolin 30μg; CFO-cefoxitin 30μg; CTX-cefotaxime 30μg; CAZ-ceftazidime 30μg; CTF-ceftiofur 30μg; CRO-ceftriaxone 30μg; CPM-cefepime 30 μg; ATM-aztreonam 30μg; AMO-amoxicillin 10μg; AMC-amoxicillin 20μg + ac. clavulanic 10μg; AMP-ampicillin 10μg; GEN-gentamicin 10μg; EST-streptomycin 300μg; CLO-chloramphenicol 30μg; FLF-florfenicol 30μg; SUL-sulfonamide 300μg; TRI-trimethoprim 5μg; NIT-nitrofuran 300μg; NAL-nalidixic acid 30μg; CIP-ciprofloxacin 5μg; NOR-norfloxacin 10μg; TET-tetracycline 30μg; $bla_{CMY-2}$**; $bla_{CTX-M}$*; $bla_{CTX-M1}$*; $bla_{CTX-M2}$*; $bla_{CTX-M15}$*; $bla_{OXA}$*; $bla_{SHV}$*; $bla_{TEM}$*; $dfr_{A1}$; $dfr_{A7}$; $dfr_{A12}$; $dfr_{A14}$; $drf_B$; $sul_1$; $sul_2$; $sul_3$; $tet_A$; $tet_B$; $tet_C$; $tet_D$; $tet_E$; $tet_G$. (**) - gene that also indicate Amp-C production, (*) - gene that also indicate ESBL production.

**Figure 1.** Antimicrobial resistance of *Salmonella* spp. isolates.




Antimicrobials tested: IPM - imipenem 10µg; CFZ - cefazolin 30µg; CFO - cefoxitin 30µg; CTX - cefotaxime 30µg; CAZ - ceftazidime 30µg; CTF - ceftiofur 30µg; CRO - ceftriaxone 30µg; CPM - cefepime 30 µg; ATM - aztreonam 30µg; AMO - amoxicillin 10µg; AMC - amoxicillin 20µg + ac. clavulanic 10µg; AMP - ampicillin 10µg; GEN - gentamicin 10µg; EST - streptomycin 300µg; CLO - chloramphenicol 30µg; FLF - florfenicol 30µg; SUL - sulfonamide 300µg; TRI - trimethoprim 5µg; NIT - nitrofuran 300µg; NAL - nalidixic acid 30µg; CIP - ciprofloxacin 5µg; NOR - norfloxacin 10µg; TET - tetracycline 30µg. R - Resistant; I - Intermediate; S - Susceptible.

**Figure 2.** Genotypic resistance (%) of *Salmonella* spp. isolates.




Screened genes: $bla_{CMY-2}$**; $bla_{CTX-M}$*; $bla_{CTX-M1}$*; $bla_{CTX-M2}$*; $bla_{CTX-M15}$*; $bla_{OXA}$*; $bla_{SHV}$*; $bla_{TEM}$*; $dfr_{A1}$; $dfr_{A7}$; $dfr_{A12}$; $dfr_{A14}$; $drf_{B}$; $sul_{1}$; $sul_{2}$; $sul_{3}$; $tet_{A}$; $tet_{B}$; $tet_{C}$; $tet_{D}$; $tet_{E}$; $tet_{G}$. (**) - gene that also indicate Amp-C production, (*) - gene that also indicate ESBL production.

## Discussion

Meat is the main protein source in human diet and chicken stands out, because it is the most consumed animal protein worldwide, reaching 13.5 kg/per capita, with an estimated increase in consumption to 14.1 kg/per capita in 2026 (OECD/FAO, 2017).

In the world, one in ten people become sick due to FBD (Foodborne Diseases), resulting in 33 million deaths/year and approximately 220 million cases of diarrhea in children under five years. The main etiological FBD agents include *Campylobacter* spp. and *Salmonella* spp., which can be present in products of animal origin derived from broilers, cattle, pigs, sheep and ostriches (WHO, 2017).

ESBL producing enterobacteriaceae pose a risk to human health, according to the World Health Organization (WHO, 2014), as in the treatment of infections by enterobacteriaceae in humans, the $\beta$-lactam antibiotics, especially third-generation cephalosporin are the drugs of choice (KOGA et al., 2015).

Studies have shown a high frequency of ESBL-producing bacterial isolates in farm animals and products of animal origin (KOGA et al., 2015; FISCHER et al., 2013; SCHILL et al., 2017). In our study, high levels of resistance were found against the $\beta$-lactam: AMO (100%); AMP (100%); monobactams: ATM (72.72%); first-generation cephalosporin: CFZ (100%); third-generation: CAZ (45.45%), CTX (72.72%), CTF (72.72%), CRO (72.72%) and fourth generation: CPM (72.72%). These isolates also showed resistance to other antimicrobials, such as sulfonamides, tetracycline and trimethoprim, and resistance to three classes of antimicrobials or more characterize features of MDR bacteria (MAGIORAKOS et al., 2012). MDR microorganisms limit therapeutic options, in some cases, cephalosporin are believed to be the latest drugs of choice (JINDAL et al., 2015).

Yaici et al. (2017) noted the risk of transmission of MRD microorganisms to human intestinal microbiota through cross-contamination of foods during processing. The authors found ESBL-producing bacteria in sandwiches sold on the streets in Algeria, indicating cross-contamination, since microorganisms such as *E. coli* and *Salmonella* spp. are inactivated after proper hygiene and cooking of food.

In this study, 72.72% (08/11) of the samples were ESBL-producing and 100% (11/11) MDR to the antimicrobials tested. Ziech et al. (2016) found positivity of 45% and 86% for ESBL production and MDR, respectively, in isolates of *Salmonella* spp. from cutting rooms of slaughterhouses in Brazil and reported on a risk of spreading phenotypes of MDR *Salmonella* spp. within the broiler industry, corroborated by the findings of this study.

Leverstein-Van Hall et al. (2011) compared the genotypic profile of ESBL in isolates of *E. coli* and *Salmonella* spp. of poultry origin with the profile of *E. coli* isolates from human patients. The authors reported that 19% of plasmid genes observed in human isolates were indistinguishable from those found in *E. coli* isolates from poultry origin, reinforcing the hypothesis of risk of transmission along the food chain.

Bacteria can become resistant by acquiring genes from other microorganisms (conjugation, transduction and transformation) of the same or different bacterial species by pressure of selection or mutations (TENOVER, 2006). Poole et al. (2017) demonstrated the capacity that isolates of MDR *E. coli* have to transfer genetic material via conjugation to receiving strains of *E. coli* DH5α and *Salmonella* Newport.

ESBL-producing microorganisms may present co-resistance to fluoroquinolones, tetracycline and trimethoprim (SALIU et al., 2017). In this study, 100% of the isolates were resistant to TET and TRI, which is a concerning fact since these active ingredients are therapeutic options for the treatment of various infections, including urinary tract infection (UTI) in humans.

The absence of resistance to Chloramphenicol and Nitrofurantoin can be justified by the decrease in pressure of selection after the prohibition of use of these active principles as growth promoters, both in treatment and in animal feed, in accordance with normative instruction No. 9, MAPA (MAPA, 2003). Based on the genotypic resistance profile of isolates of genes that confer resistance, cephalosporin (*bla*$_{CTXM}$), tetracycline (*tet*$_{A}$, *tet*$_{B}$ and *tet*$_{C}$), sulfonamides (*sul*$_{1}$) and trimethoprim (*dfr*$_{A12}$) presented the highest frequencies, corroborating the results of phenotypic resistance profile. The high frequency of resistance to these classes of antimicrobials may be a result of the wide use of these active ingredients in commercial poultry.

Although 100% of samples presented phenotypic resistance to Ampicillin, no isolate showed genotypic resistance to genes *bla*$_{TEM}$ and *bla*$_{OXA}$ and only 9% (1/11) to *bla*$_{SHV}$ gene, possibly because of the presence of other genes that were not investigated in this study. Studies point to *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ as the main genes responsible for ESBL production in bacterial isolates from poultry source (LEVERSTEIN-VAN HALL et al., 2011; SALIU et al., 2017). In this study *bla*$_{CTX-M}$ was the most frequent ESBL gene detected (55%), followed by *bla*$_{CTX-M2}$ and *bla*$_{SHV}$ corroborating with the literature.

## Conclusion

Isolates of *Salmonella* spp. from poultry products present genotypic and phenotypic characteristics of multiple drug resistance (MDR) and production of extended spectrum of beta lactamase (ESBL).

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