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Antimicrobial multiresistance and biofilm formation in *Salmonella enterica* isolated from broiler production chain[1]

Daniela A.P. Brito[2]*, Alexandre Oba[3], Fernanda G. Paião[4] and Bianca L. Ferreira[5]

ABSTRACT.- Brito D.A.P., Oba A., Paião F.G. & Ferreira B.L. 2024. **Antimicrobial multiresistance and biofilm formation in *Salmonella enterica* isolated from broiler production chain.** *Pesquisa Veterinária Brasileira 44:e07463, 2024*. Laboratório de Microbiologia, Departamento de Desenvolvimento Educacional, Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, Campus Maracanã, Av. dos Curiós s/n, Vila Esperança, São Luís, MA 65095-460, Brazil. E-mail: danielabrito@ifma.edu.br

Poultry and poultry products are considered the predominant sources of *Salmonella enterica* contamination and are important reservoirs of bacteria with antimicrobial resistance. The objective of this study was to identify *Salmonella* with multidrug resistance (MDR) phenotype, with the ability to form biofilms and elucidate the presence of genes that encode antimicrobial resistance in isolates from the broiler production chain in the state of Maranhão, Brazil. A total of 121 strains of *S. enterica* of different serovars were evaluated for antimicrobial susceptibility, and of these, 26 strains were used to detect the ability to form biofilms and identify resistance genes using PCR. Antimicrobial resistance was observed in 95 (78.5%) *Salmonella* isolates, and 57 (47.1%) showed MDR phenotype. The isolates showed greater resistance to the sulfonamide principles (58.7%), trimethoprim (48.8%), tetracycline (45.4%), nalidixic acid (44.6%), amoxicillin and ampicillin (26.4%), and cefazolin (22.3%). *Salmonella* Schwarzengrund (n=21/61.7%), Albany (n=15/62.5%), and Enteritidis (n=4/44.5%) showed the highest indices of MDR phenotype. The ability to form biofilms at 37°C was found in 13 of the 26 strains evaluated, which were considered poor producers. The resistance genes *bla*CTX-M, *bla*CTX-M2, *bla*SHV, *sul*1, *sul*2, *tet*A, *tet*B, *tet*C, *tet*E, *dfr*A12, and *dfr*A1 were observed in the serovars Schwarzengrund, Albany, Enteritidis, Heidelberg, and Typhimurium. The results showed a high occurrence of *S. enterica,* with multiple resistance to conventional antimicrobials and the ability to form biofilms in the poultry production chain.

INDEX TERMS: *Salmonella* spp., antimicrobials, biofilms, resistance genes, poultry.

RESUMO.- [**Multirresistência antimicrobiana e formação de biofilmes em isolados de *Salmonella enterica* da cadeia produtiva de frangos.**] As aves e os produtos de origem aviária são fontes de contaminação predominantes de *Salmonella enterica* e importantes reservatórios de bactérias com resistência antimicrobiana. Objetivou-se identificar *Salmonella* com fenótipos de multirresistência a drogas (MDR), com a capacidade de formação de biofilmes e a presença de genes que codificam resistência antimicrobiana em isolados da cadeia de frangos de corte, do estado Maranhão, Brasil. Avaliaram-se 121 cepas de *S. enterica* de sorovares diferentes quanto ao teste de suscetibilidade aos antimicrobianos e destas, 26 cepas para detecção da capacidade de formar biofilmes e genes de resistência pela técnica de PCR. Foram encontradas resistência antimicrobiana em 95 (78,5%) dos isolados de *Salmonella* e 57 (47,1%) apresentaram fenótipos MDR. Os isolados apresentaram maior resistência aos princípios sulfonamida (58,7%), trimetoprim (48,8%), tetraciclina (45,4%), ácido nalidíxico (44,6%), amoxicilina e

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[2] Departamento de Desenvolvimento Educacional (DDE), Instituto Federal de Educação, Ciência e Tecnologia do Maranhão (IFMA), Av. dos Curiós s/n, Vila Esperança, São Luís, MA 65095-460, Brazil. *Corresponding author: danielabrito@ifma.edu.br

[3] Departamento de Zootecnia, Universidade Estadual de Londrina (UEL), Londrina, PA, Brazil.

[4] Graduate Program in Food Technology, Universidade Tecnológica do Paraná, PR, Brazil.

[5] Agronomy Course, Instituto Federal de Educação, Ciência e Tecnologia do Maranhão (IFMA), São Luís, MA, Brazil.

ampicilina (26,4%) e cefazolin (22,3%). Os sorovares *Salmonella* Schwarzengrund (n=21/61.7%), Albany (n=15/62.5%) e Enteritidis (n=4/44.5%) apresentaram os maiores índices de fenótipos MDR. A capacidade de formar biofilmes foi encontrada em 13 cepas avaliadas, consideradas fracamente produtoras. Nos sorovares *S.* Schwarzengrund, Albany, Enteritidis, Heidelberg e Typhimurium foram detectados os genes de resistência *bla*CTX-M, *bla*CTX-M2, *bla*SHV, *sul*1, *sul*2, *tet*A, *tet*B, *tet*C, *tet*E, *dfr*A12 e *dfr*A1. Os resultados evidenciaram a elevada ocorrência de fenótipos de *S. enterica* com resistência múltipla a antimicrobianos convencionais, com capacidade de formar biofilmes, na cadeia produtiva de aves destinadas ao consumo humano.

TERMOS DE INDEXAÇÃO: *Salmonella* spp., antimicrobianos, biofilmes, genes de resistência, frangos.

INTRODUCTION

Salmonella infection is a leading cause of foodborne diarrheal illnesses in America and Europe (Ferrari et al. 2019, Jajere 2019, EFSA & ECDC 2023) The incidence of salmonellosis transmitted through the food production chain has increased significantly, with an estimated 94 million cases resulting in 155,000 deaths yearly (WHO 2014). The use of antimicrobials is a crucial measure for the treatment of patients with severe or systemic infections (WHO 2022). However, therapeutic effectiveness may be hampered by the increasing spread of *Salmonella* with multiple drug resistance (MDR), including clinically important antimicrobial agents (Cosby et al. 2015).

Infections caused by *Salmonella* MDR are generally transmitted through food, with farm animals serving as reservoirs and animal products as transmission routes for the resulting human diseases (Glenn et al. 2013). Birds, rearing environments, and poultry products are predominant sources of *Salmonella enterica* and are notable reservoirs of antimicrobial-resistant bacteria (Wang et al. 2015). The extensive use of antimicrobials in disease prophylaxis and as growth promoters in modern poultry farming has been identified as one of the reasons for the increased prevalence of MDR bacteria (Abreu et al. 2023). In several countries, MDR has resulted in outbreaks of human salmonellosis caused by the *S. enterica* serovars Enteritidis, Typhimurium, Heidelberg, and Schwarzengrund, which can be isolated from sources in the broiler production chain (Glenn et al. 2013, Mandelli et al. 2019, Ćwiek et al. 2020, EFSA & ECDC 2020, 2024).

The spread of different MDR serovars of *Salmonella* is rapid and mainly associated with the exchange and incorporation of numerous genes that encode antimicrobial resistance by conjugating mobile genetic elements (Abatcha et al. 2014, Cosby et al. 2015). In a shared habitat, these genes are easily mobilized between *Salmonella* and other bacteria of the Enterobacteriaceae family (Algarni et al. 2022, Baker et al. 2024). *Salmonella* spp. can act as receptors or donors of resistance genes, contributing to the dissemination of these elements in the human food chain (Baker et al. 2024, Orole et al. 2024).

The dissemination and persistence capacity of *Salmonella* spp. in chicken production chains is related to the pathogenicity mechanisms and virulence factors involved in host infection, as well as the process of adaptation to the environment outside the host (Iñiguez-Moreno et al. 2018). One of these strategies is the ability of *Salmonella* strains to form biofilms, which reduces the effectiveness of sanitation in poultry and slaughterhouses and, consequently, increases the risk of food contamination (Mackenzie et al. 2017, Borges et al. 2018). There is a correlation between the biofilm formation capacity of *Salmonella* spp. and its resistance to antimicrobials used in human treatments (Sereno et al. 2017, González et al. 2018, Mandelli et al. 2019). Therefore, from the perspective of infection, biofilm-producing *Salmonella* may have a greater degree of survival, adaptation, or dissemination (Mackenzie et al. 2017, Musa et al. 2024).

This study aimed to investigate the profile of *Salmonella* resistance to antimicrobial agents, the genetic determinants of resistance, and the capacity to form biofilms in isolates from different sources in the broiler production chain in Maranhão, in the Northeast region of Brazil.

MATERIALS AND METHODS

Ethical approval. Ethical approval for this study was obtained from Ethics Committee on Animal Use (CEUA) at "Universidade Estadual de Londrina" (UEL) (CEUA no. 15093.2014.96)

Bacterial strains. A total of 121 strains of *Salmonella enterica* were isolated from the chicken production chain in the northern mesoregion of the state of Maranhão from 2013 and 2014, of which 26 were from environmental samples (trawl swab, propé, and cecal feces), seven from poultry samples (cloacal swab), and 88 from slaughterhouse samples (broiler carcasses). The strains were previously isolated, biochemically characterized and serotyped by the "Instituto Oswaldo Cruz" (Fiocruz), Rio de Janeiro, Brazil. For the analysis, the isolates were cultivated in tryptone soy broth (TSB) at 37°C for 24 h and then preserved in 20% glycerol at -20°C.

Antimicrobial susceptibility test. The antimicrobial susceptibility profiles of *Salmonella* isolates were determined by the disc diffusion method (Bauer et al. 1966), using the protocol recommended by the Clinical and Laboratory Standards Institute (CLSI 2008, 2013). Antimicrobial discs representative of the classes of penicillin (amoxicillin, 10µg; ampicillin, 10µg), cephalosporins (cefazolin, 30µg), carbapenems (imipenem, 10µg), and quinolones (nalidixic acid, 30µg; ciprofloxacin, 5µg; ciprofloxacin, 5µg) were used. Norfloxacin (10µg), phenicols (chloramphenicol, 30µg; fluorphenicol, 30µg), aminoglycosides (streptomycin, 300µg; gentamicin, 10µg), folate inhibitors (sulfonamide, 300µg; trimethoprim, 5µg), tetracyclines (tetracycline, 30µg) and nitrofurans (nitrofurantoin, 300µg) were used. The reference strains *Escherichia coli* ATCC 25922 and *S.* Enteritidis ATCC 13076 were used for test validation. MDR phenotype was considered as isolates with simultaneous resistance to three or more classes of antimicrobials as defined by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) (Rodrigues et al. 2020a).

Molecular determinants of resistance. Twenty-six samples of *S. enterica,* belonging to the most prevalent serovars in the production cycle of broiler chickens, with the greatest relevance in public health and phenotypic resistance to two or more classes of antimicrobials, were selected in the present study. The evaluated serovars of *S. enterica* included Schwarzengrund (n=12), Albany (n=5), Enteritidis (n=5), Heidelberg (n=2), and Typhimurium (n=2).

The isolates were inoculated in xylose lysine deoxycholate (XLD) agar culture medium and incubated at 37°C for 24 h. After growth, a characteristic colony was transferred to Luria Bertani (LB) broth and incubated at 37°C for 18 h. The genomic DNA of the samples was extracted using a genomic DNA purification kit (Promega®), following the manufacturer's instructions. The samples were quantified using

the nanospectrum (KASVI®). The microtubes containing the genetic material were stored at -20°C until use.

PCR was performed, according to Paião et al. (2013), to confirm that the isolates belonged to *Salmonella*, using the primers for the *inv* A gene described by Fratamico (2003). This was followed by a search for genes that determine resistance to tetracyclines (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E and *tet*G), beta-lactams (*bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX-M, *bla*CTX-M1, *bla*CTX-M2, *bla*CTX-M15, *bla*CMY-2), sulfonamides (*sul*1, *sul*2, *sul*3), and trimethoprim (*dfr*A1, *dfr*A7, *dfr*A12, *dfr*A14), using previously described amplification primers and protocols (Ma et al. 2007, Ribeiro et al. 2011).

Biofilm formation test. The biofilm formation capacity of 26 strains of *Salmonella* spp. was evaluated using the microtiter method in a polystyrene plate at a growth temperature of 37°C, as described by Borges et al. (2018). Each well's optical density (OD) was measured using a microplate reader at 450nm. The OD of each strain was obtained from the arithmetic mean absorbance value of eight wells, and this value was compared with the mean absorbance of the negative controls (ODn). Strains were classified according to Stepanović et al. (2004) as follows: No biofilm producer (OD≤ODn), weak biofilm producer (ODn<OD<2× ODn), moderate biofilm producer (2× ODn≤OD<4× ODn) and strong biofilm producer (OD≥4× Odn).

RESULTS

In the present study, 78.5% (95/121) of *Salmonella enterica* isolates originating from the chicken production chain were resistant to antimicrobials. Resistance was observed against sulfonamide (58.7%), trimethoprim (48.8%), tetracycline (45.4%) and nalidixic acid (44.6%) (Fig.1). All the isolates were sensitive to norfloxacin, ciprofloxacin, and fluorfenicol.

Of the 121 samples of *S. enterica*, 57 (47.1%) were simultaneously resistant to three or more classes based on MDR phenotype (Table 1). The *S. enterica* serovars Schwarzengrund (n=21), Albany (n=15), Enteritidis (n=4), Heidelberg (n=2), Kentucky (n=3), Muenchen (n=3), Typhimurium (n=1), Hadar (n=1), Agona (n=1), Panama (n=1), Anatum (n=1), and Seftenberg (n=1) showed MDR phenotype. As for the isolation sources, *Salmonella* isolated from broiler chickens showed the highest frequency of MDR phenotypes (5/7 isolates), followed by the rearing environment (12/26 isolates) and carcasses (40/88 isolates).

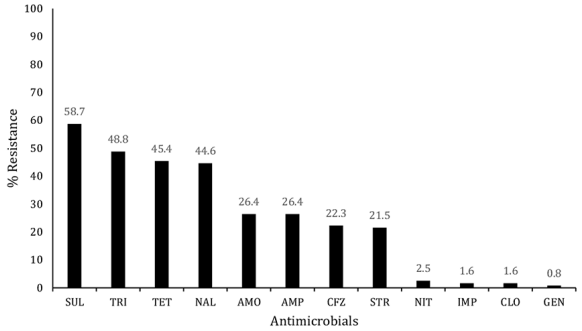

Fig.1. Frequency (%) of antimicrobial resistance in *Salmonella enterica* isolated in a poultry production chain in the state of Maranhão, Brazil. SUL = sulfonamide (300µg), TRI = trimethoprim (5µg), TET = tetracycline (30µg), NAL = nalidixic acid (30µg), AMO = amoxicillin (10µg), AMP = ampicillin (10µg), CFZ = cefazolin (30µg), STR = streptomycin (300µg), NIT = nitrofurantoin (300µg), IMP = imipenem (10µg), CLO = chloramphenicol (30µg), GEN = gentamicin (10µg).

Eleven of the 21 genes encoding resistance against sulfonamides, trimethoprim, beta-lactams, and tetracyclines were detected in the *S. enterica* serovars Schwarzengrund, Albany, Enteritidis, Heidelberg, and Typhimurium isolates (Table 2). In 11 isolates showing resistance phenotype against beta-lactams, amoxicillin, ampicillin, and cefazolin, the genes *bla*CTX-M, *bla*CTX-M2, and *bla*SHV were detected in eight (72.7%), four (36.3%), and three (27.2%) samples evaluated, respectively. The detection rate of resistance genes for sulfonamides was 15 (71.42%) of the 21 isolates with resistance phenotype, in which *sul*1 appeared in 11 (42.3%) and *sul*2 in three (14.28%) of the samples. The *tet* resistance genes were detected in all 22 isolates with tetracycline resistance phenotypes, with eight (36.3%) for *tet*A, 15 (68.1%) for *tet*B, 12 (54.5%) for *tet*C, and three (13.6%) for *tet*E. Regarding trimethoprim, nine (56.2%) of the 16 isolates showed phenotypes associated with the presence of genetic resistance determinants, of which eight (50%) had the *dfr*A12 gene and four (25%) the *dfr*A1 gene. The ability to form biofilms was detected in 13 (50%) isolates categorized as poor biofilm producers, with a resistance profile of one (n=1), two (n=3), three (n=4), four (n =4), and five (n=1) classes of antimicrobials (Table 2).

Table 1. Distribution of antibiotic resistance of *Salmonella* strains from different sources in the broiler production chain

Salmonella enterica	Isolates resistant to antimicrobial classes						Total
	0		1-2		≥3		
	n	%	n	%	n	%	
Schwarzengrund	5	14.7	8	23.5	21	61.7	34
Albany	4	16.6	5	20.8	15	62.5	24
Enteritidis	0	0.0	5	55.5	4	44.5	9
Heidelberg	3	33.3	4	44.4	2	22.2	9
Panama	0	0.0	5	83.3	1	16.6	6
Kentucky	0	0.0	2	40.0	3	60.0	5
Muenchen	2	40.0	0	0.0	3	60.0	5
Hadar	2	66.6	0	0.0	1	33.3	3
Agona	0	0.0	2	66.6	1	33.3	3
Typhimurium	0	0.0	1	50.0	1	50.0	2
Derby	2	100.0	0	0.0	0	0.0	2
Anatum	0	0.0	0	0.0	1	100	1
Seftenberg	0	0.0	0	0.0	1	100	1
Orion	1	100.0	0	0.0	0	0.0	1
Worthing	1	100.0	0	0.0	0	0.0	1
O:4.5*	3	33.3	3	33.3	3	33.3	9
O:6.8*	2	66.6	1	33.3	0	0.0	3
O:3.10*	1	50.0	1	50.0	0	0.0	2
O:4.5:I,v:-*	0	0.0	1	100.0	0	0.0	1
TOTAL	26	21.5	38	31.4	57	47.1	121
Sources of isolation							
Environment	5	19.2	9	34.6	12	46.1	26
Broiler	0	0.0	2	28.5	5	71.4	7
Carcass	20	22.7	28	31.8	40	45.4	88
TOTAL	25	20.6	39	32.2	57	47.1	121

* Unidentified flagellar structure.

DISCUSSION

The high infection rates of *Salmonella* spp. with multiple drug resistance from sources in the poultry production chain are similar to those in various parts of the world, including Brazil (85.7%) (Perin et al. 2020), Egypt (76.7%) (Elkenany et al. 2019), Cambodia, Thailand (45%) (Trongjit et al. 2017), India (100%) (Sharma et al. 2019), and China (60.5%) (Zhu et al. 2017). *Salmonella* isolated from broiler chickens and their products have higher antimicrobial resistance rates than those isolated from other domestic animal species (EFSA & ECDC 2020), which may reflect the high selection pressure suffered under the management practices of modern poultry. The antimicrobial resistance profiles according to the isolation sources of the study revealed that the isolates with MDR phenotype were present in the most varied points of the poultry production chain, with the poultry sources having the highest proportion of MDR phenotype (71.4%). These results reinforce the assertion that there is excessive use of antimicrobials in poultry farming, making these production animals an important reservoir of *Salmonella* with MDR, representing a risk for people who have direct contact as occupational workers or indirectly as consumers of food (Abreu et al. 2023). Furthermore, MDR Salmonella can spread through poultry waste from poultry farms and slaughterhouses, acting as a potential source of propagation of pathogens and antimicrobial resistance genes in the environment (Saraiva et al. 2022).

Sulfonamide was the antimicrobial principle that presented the highest resistance index in *Salmonella* isolated from different sources and has been extensively used in poultry farming for decades (Matiello et al. 2015). The wide dissemination and predominance of *sul*1 in the *S. enterica* isolated from birds and poultry products have been recorded in these serovars (Ribeiro et al. 2011, Brasil 2012, Glenn et al. 2013, Matiello et al. 2015). Its presence is generally associated with gene cassettes of class 1 integrons, which are responsible for the clonal or horizontal dissemination of multiple antimicrobial resistance genes in *S. enterica* (Fortes et al. 2012, Cosby et al. 2015).

The tetracycline group showed high resistance rates, particularly in Schwarzengrund (24/34) and Heidelberg (7/9) serovars. Countries like Brazil (64.6%) (Scur et al. 2014), United States (65.8%) (FDA 2012), and China (65.9%) (Wang et al. 2015) still record high levels of resistance against tetracyclines in *Salmonella* isolated from the poultry production chain. The spread of resistance is associated with numerous resistance genes (*tet)*, and the presence of the *tet* gene coincided with the resistance phenotype in all isolates. In *Salmonella* spp., the main resistance mechanism associated with these genes is the activation of efflux pumps, which prevents the drug from accumulating inside the cell at a concentration necessary for bacterial death (Hur et al. 2012). Unlike other studies that detected a predominance of the *tet*A gene in isolates from different poultry sources (Ribeiro et al. 2011, Glenn et al. 2013, Adesiji et al. 2014, Matiello et al. 2015, Rodrigues et al. 2020b), *tet*B was predominant in the isolates we studied.

In the beta-lactam group, three different types of *bla* genes were detected (*bla*CTX-M, *bla*CTX-M2, and *bla*SHV)

Table 2. Phenotypic profile of antimicrobial resistance, resistance genes and biofilm production of *Salmonella* spp. strains

n	Serovar	Source	Phenotypic resistance	Genotypic resistance	Biofilm production
1	Schwarzengrund	Carcass	Est Sul Tet	sul_2 tet_B	Yes
2	Schwarzengrund	Carcass	Est Nal Tet	tet_B	Yes
3	Schwarzengrund	Carcass	Est Nal Tet	tet_B	Yes
4	Schwarzengrund	Carcass	Est Nal Tet	tet_A tet_C	Yes
5	Schwarzengrund	Carcass	Est Nal Sul Tet	sul_1 tet_B tet_C tet_E	No
6	Schwarzengrund	Carcass	Amo Amp Cfz Est Nal Tet	*tet*A tet_B bla_{CTX-M}	No
7	Schwarzengrund	Carcass	Amo Amp Cfz Sul Tri Tet	*sul*1 bla_{CTX-M} dfr_{A12}	No
8	Schwarzengrund	Carcass	Amo Amp Cfz Est Sul Tri Tet	*sul*1 *tet*A tet_B *tet*E *bla*CTX-M bla_{CTX-M2} dfr_{A12}	No
9	Schwarzengrund	Carcass	Amo Amp Cfz Est Nal Sul Tri Tet	sul_1 tet_B bla_{CTX-M} bla_{CTX-M2}	No
10	Schwarzengrund	Cloaca	Amo Amp Cfz Est Nal Sul Tri Tet	tet_A bla_{CTX-M} bla_{CTX-M2}	No
11	Schwarzengrund	Cloaca	Amo Amp Cfz Est Nal Sul Tri Tet	sul_1 sul_2 tet_A bla_{CTX-M}	No
12	Schwarzengrund	Carcass	Amo Amp Cfz Est Imp Nal Sul Tri Tet	tet_B tet_C bla_{CTX-M} bla_{SHV} dfr_{A1} dfr_{A12}	No
13	Albany	Carcass	Nal Sul Tri	sul_1	Yes
14	Albany	Carcass	Nal Sul Tri	sul_1	Yes
15	Albany	Carcass	Est Nal Sul Tet	sul_1 tet_B	Yes
16	Albany	Carcass	Est Nal Sul Tet	tet_B tet_C	Yes
17	Albany	Carcass	Amo Amp Cfz Nal Sul Tri Tet	*sul*1 tet_B bla_{CTX-M2} dfr_{A12}	No
18	Enteritidis	Litter	Sul Tri Tet	sul_1 tet_A tet_C	Yes
19	Enteritidis	Cloaca	Nal Tet	tet_B tet_C	No
20	Enteritidis	Cloaca	Nal Nit Sul Tet	sul_1 tet_B tet_C	Yes
21	Enteritidis	Cloaca	Amo Amp Nal Nit Sul Tri	tet_B tet_C bla_{SHV} dfr_{A1} dfr_{A12}	No
22	Enteritidis	Cloaca	Amo, Amp, Cfz Nal Sul Tri Tet	tet_B tet_C bla_{CTX-M} bla_{SHV} dfr_{A1} *dfr*A12	Yes
23	Heidelberg	Carcass	Nal Sul Tri Tet	sul_1 tetA tet_C	No
24	Heidelberg	Carcass	Est Nal Sul Tri Tet	sul_1 tet_A tet_C dfr_{A1}	Yes
25	Typhimurium	Cloaca	Sul Tri	sul_2 dfr_{A12}	Yes
26	Typhimurium	Cloaca	Amo Amp Cfz Nal Sul Tri Tet	tet_C tet_E dfr_{A12}	No

that encode extended-spectrum beta-lactamases (ESBL). Enzymes belonging to the CTX-M family are predominant in enterobacteria in South America (Silva & Lincopan 2012), with detection record of *bla*CTX-M2 in *Salmonella* isolated from poultry and poultry products in Brazil (Silva et al. 2013, Costa et al. 2024).

Notably, two isolates of *Salmonella* ser. Enteritidis and one isolate of *Salmonella* Schwarzengrund showed the presence of *bla*SHV, which confers multiple resistance against six or more antibiotics. The *bla*SHV genes are considered rare in *Salmonella*. However, they have been detected in food-producing animals and human clinical cases of salmonellosis and have a high capacity for horizontal transfer between bacteria of the Enterobacteriaceae family through conjugative plasmids (Pouget et al. 2013, Orole et al. 2024).

The *bla*CTX-M and *bla*CTX-M2 genes had a greater association with the *sul*1, *tet*B, and *dfr*A12 gene determinants, whereas all samples with the *bla*SHV gene were associated with the *tet*B, *tet*C, *dfr*A1, and *dfr* genes A12. Several genetic determinants of antimicrobial resistance in some *Salmonella* isolates suggest a potential co-selection of resistance genes to distinct classes of antimicrobials conventionally used in poultry. Moreover, the dissemination of these resistance genes between bacteria from animal production along multiple paths of the food production chain results in the preservation of the complex gene cassettes expressing MDR (Marshall & Levy 2011). In the context of One Health, the presence of *Salmonella* paratyphoid, a zoonotic pathogen with several antimicrobial resistance genes often carried on mobile elements, may be an important source of genomic transfer of resistance to commensal bacteria from different hosts or the environment (Saraiva et al. 2022, Baker et al. 2024).

Salmonella Enteritidis is the predominant serovar responsible for human salmonellosis outbreaks worldwide, caused by the consumption of contaminated poultry products (Ferrari et al. 2019, Liu et al. 2023). The results of this study showed that all isolates with antimicrobial resistance originated from environmental sources. Among these, four showed MDR phenotype, with the presence of various genetic determinants for resistance and the ability to form biofilms. Therefore, *Salmonella* ser. Enteritidis may represent a risk owing to its zoonotic potential, ability to exchange antimicrobial resistance genes, and persistence and dissemination along the production chain.

Salmonella Schwarzengrund showed the highest rates of multiresistant phenotypes (21/61.7%) in isolates from different sources. The most common resistance pattern observed in phenotypes with resistance to ≥7 antimicrobials (eight isolates) was Amo, Amp, Cfz, Est, Nal, South, Tri, and Tet (four isolates), which corroborated with the findings of Chen et al. (2010). These results resemble the phenotypic pattern of non-typhoidal *Salmonella* multidrug resistance (ACSSuT) in hospitalized patients in Brazil (Reis et al. 2018). This phenotypic profile of multiresistance found in serovars Schwarzengrund, Enteritidis and Typhimurium (Table 2) in this study requires surveillance, as they are one of the few serovars that cause invasive diseases in humans and require immediate antimicrobial therapy (Zhan et al. 2019, Abreu et al. 2023). High levels of resistance pose a significant threat to public health, particularly for individuals with affected immune systems who are at increased risk of serious complications and mortality (WHO 2024). Circulation of *Salmonella* strains with a high frequency of MDR phenotype and the presence of several antimicrobial resistance genes in the poultry production chain can be considered a concern for public health because it limits the number of antimicrobials for treatment in cases of severe infections (Akiyama & Khan 2012).

In this study, strains of different multidrug-resistant serovars could form biofilms at 37°C, with low production and resistance to sulfonamides, tetracyclines, and trimethoprim (Table 2). These results indicate the risk of bacterial persistence in different environments of the chicken production chain from the contamination of utensils and equipment, which may share genes that encode resistance among biofilm bacteria (Sereno et al. 2017, Musa et al. 2024).

CONCLUSIONS

The present study demonstrated the high occurrence of *Salmonella enterica* with multidrug resistance (MDR) phenotype in the broiler production chain. MDR isolates from different sources showed a high frequency of phenotypic and genotypic resistance to the antimicrobial groups sulfonamides, trimethoprim, tetracyclines, and beta-lactams, considered antimicrobials of importance in veterinary and human medicine.

The *S. enterica* serovars Schwarzengrund and Enteritidis had the highest frequency of MDR phenotype associated with resistance genes and the ability to form biofilms.

Isolates from the production chain of poultry destined for human consumption pose a risk to public health and animal health owing to the possible spread of *Salmonella* MDR and the implications for the treatment of severe clinical conditions.

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