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Characterization of antimicrobial resistance in *Salmonella enterica* strains isolated from Brazilian poultry production

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Abstract Antimicrobial resistance profiles and presence of resistance determinants and integrons were evaluated in *Salmonella enterica* strains from Brazilian poultry. The analysis of 203 isolates showed that those from the poultry environment (88 isolates) were significantly more resistant to antimicrobials than isolates from other sources, particularly those isolated from poultry by-product meal (106 isolates). Thirty-seven isolates were resistant to at least three antimicrobial classes. Class 1 integrons were detected in 26 isolates, and the analysis of the variable region between the 5′ conserved segment (CS) and 3′ CS of each class 1 integron-positive isolate showed that 13 contained a typical 3′ CS and 14 contained an atypical 3′ CS. One *Salmonella* Senftenberg isolate harbored two class 1 integrons, showing both typical and atypical 3′ CSs. The highest percentage of resistance was found to sulfonamides, and *sul* genes were detected in the majority of the resistant isolates. Aminoglycoside resistance was detected in 50 isolates, and *aad*A and *aad*B were present in 28 and 32 isolates, respectively. In addition, *str*A and *str*B were detected in 78.1 and 65.6 % isolates resistant to streptomycin, respectively. Twenty-one isolates presented reduced susceptibility to β-lactams and harbored bla_{TEM}, bla_{CMY}, and/or $bla_{CTX\text{-}M}$. Forty isolates showed reduced susceptibility to tetracycline, and most presented *tet* genes. These results highlight the importance of the environment as a reservoir of resistant *Salmonella*, which may enable the persistence of resistance determinants in the poultry production chain, contributing, therefore, to the debate regarding the impacts that antimicrobial use in animal production may exert in human health.

Keywords *Salmonella enterica* · Poultry by-product meal · Poultry environment · Antimicrobial resistance · Poultry production chain

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Introduction

Salmonella enterica is an important pathogen involved in foodborne diseases that are mostly derived from the consumption of foods of animal origin, particularly poultry products. This microorganism is responsible for tens of millions of illnesses and more than hundred thousand deaths annually worldwide (WHO 2013) and has become a major concern due to the emergence of *S. enterica* isolates that are resistant to antimicrobials (Van et al. 2012).

The dissemination of multidrug-resistant (MDR) *Salmonella* has been associated with the broad use of

antimicrobials, particularly as growth promoters in food-producing animals, enhancing the selection of resistance in bacteria (Molbak 2005). In this context, Brazil, which is the main exporter and the third producer country of chicken meat (ABEF 2011), has adopted restrictive practices regarding the use of antimicrobials as feed additives. Many antimicrobials have been forbidden since 1998 (avoparcin, chloramphenicol, nitrofurantoin, tetracycline, β-lactams, quinolones, systemic sulfonamides, spiramycin, and erythromycin) (MAPA 1998, 2003, 2009, 2012).

The characterization of antimicrobial resistance usually includes the detection of resistance genes that may be harbored in mobile genetic elements, such as integrons, and can therefore be spread by lateral genetic transfer (Rodríguez et al. 2006). Class 1 integrons are the most common in *S. enterica* and have been often associated with MDR (Kim et al. 2011). Class 1 integrons contain a recombination site (*att*I) and an integrase gene (*int*I) in the 5′ conserved segment (CS). The 3′ CS end frequently presents the *sul*1 gene (*sul* genes encode resistance to sulfonamides) and possesses the *qacE*Δ1 gene that encodes quaternary ammonium resistance. However, an atypical 3′ CS in a class 1 integron may present *sul*3 in lieu of *sul*1 (Wannaprasat et al. 2011). The *sul*2 gene has been found in plasmids carried by *S. enterica* (Hur et al. 2011), not inserted in integrons, and is usually associated with *str*AB genes that confer resistance to aminoglycosides (Yau et al. 2010). The presence of different resistance gene cassettes, including the *aad*, *dfr*, and *bla* genes encoding aminoglycoside adenyltransferases (resistance to aminoglycosides), dihydrofolate reductases (resistance to trimethoprim), and β-lactamases (resistance to β-lactams), respectively (Firoozeh et al. 2012; Glenn et al. 2013), has been described in the variable region of class 1 integrons located between the 5′ CS and 3′ CS. A complex class 1 integron has been found in the chromosomal *Salmonella* Genomic Island 1, which usually carries genes encoding resistance to β-lactams, tetracycline, sulfonamides, aminoglycosides, and chloramphenicol (Hur et al. 2011).

Therefore, considering that *S. enterica* is a zoonotic pathogen that presents an important economic impact to the poultry production chain, this study aimed to contribute to the surveillance of antimicrobial resistance and the investigation of genetic resistance determinants found in Brazilian poultry isolates.

Materials and methods

Bacterial isolates

A total of 203 *S. enterica* isolates were recovered from 2002 to 2012 in Southern Brazil by culture on selective media and biochemical identification following the protocol described by Oliveira et al. (2002). Most isolates were serotyped at the National Reference Centre, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil. The isolates were from several samples from broilers bred: 106 from poultry by-product meals (meat, $n = 38$; feathers, $n = 21$; meat and bones, $n = 9$; viscera, $n = 25$; blood, $n = 9$; mixed poultry by-product meals, $n = 4$); 88 from the poultry environment (drag swabs from broiler houses, $n = 76$; disposable shoe covers, $n = 11$; and swabs from feed factory environment, $n = 1$); and nine from other poultry samples (pipped egg, $n = 1$; cloacal swab, $n = 2$; poultry carcass, $n = 1$; and poultry organs, $n = 5$). These last nine isolates were designated as general poultry samples. For all analyses, the isolates were grown in trypticase soy broth (TSB) (BioBras, Brazil) at 37 °C for 24 h and stored with 20 % glycerol at −80 °C.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates was evaluated by the disk diffusion method according to the CLSI guidelines (CLSI 2008, 2013). The antimicrobials tested were nalidixic acid, amikacin, ampicillin, cefaclor, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, spectinomycin, sulfonamides, trimethoprim/sulfamethoxazole, tetracycline, and tobramycin (Sensifar, Brazil). Additionally, the susceptibility to ceftiofur, enrofloxacin, florfenicol, and neomycin was determined by the agar disk diffusion method and interpreted according to the manufacturer's instructions (Cefar, Brazil) (Supplementary Table 1).

Isolates that presented reduced susceptibility to ciprofloxacin, sulfamethoxazole, trimethoprim/sulfamethoxazole, chloramphenicol, nalidixic acid,

ampicillin, and tetracycline, as determined through the disk diffusion method, were evaluated to determine the minimal inhibitory concentrations (MIC) for these drugs by the microdilution method in duplicate (CLSI 2008, 2013). MIC results were analyzed visually and by spectrophotometry at 620 nm. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for antibiotic quality control in all antimicrobial susceptibility tests.

Molecular determinants of resistance

The presence of integrons and genes encoding resistance determinants to sulfonamides, β-lactams, tetracycline, and aminoglycosides was evaluated. An initial screening was performed to determine the presence of integrons targeting integrases 1, 2, and 3 (White et al. 2000). The integron-positive isolates were then analyzed to specifically detect class 1 and 2 integrons (White et al. 2001; Su et al. 2006). Primers targeting the 5′ and 3′ CSs were used to amplify the variable region of class 1 integrons (White et al. 2000). To determine the presence of an atypical 3′ CS in a class 1 integron, PCR targeting *qac*H (Chuanchuen et al. 2008a) and *sul*3 (Chuanchuen and Padungtod 2009) was performed. All isolates resistant to sulfamethoxazole were evaluated regarding the presence of *sul*1 (Grape et al. 2003), *sul*2 (Kerrn et al. 2001), and *sul*3 (Chuanchuen et al. 2008b). The presence of $bla_{CTX\text{-}M}$ (Edelstein et al. 2003), bla_{CMY} (Winokur et al. 2001), and bla_{TEM} (Carlson et al. 1999) was verified in isolates presenting reduced susceptibility to β-lactams. Isolates with reduced susceptibility to aminoglycosides and only to streptomycin were tested for the presence of *aad*A (Madsen et al. 2000) and *aad*B (Frana et al. 2001) and of *str*A and *str*B (Gebreyes and Thakur 2005), respectively. The *tet*A, *tet*B, and *tet*C genes were investigated in isolates with reduced susceptibility to tetracycline (Aarestrup et al. 2003). All primers used in this study are listed in Supplementary Table 2.

Genomic DNA extraction

The bacterial genomic DNA was extracted as previously described (Rademaker and de Bruijin 1997). The DNA obtained was quantified and evaluated spectrophotometrically (at A260 nm and by the A260 nm/A280 nm ratio, respectively), diluted to 100 ng/μL, and stored at −20 °C.

PCR amplification

Amplification mixtures were prepared in 25 μL containing 0.2 mM of each deoxynucleotide (dNTP) (Invitrogen, USA), 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.2 U *Taq* DNA polymerase (Invitrogen), 0.8 μM of each primer (IDT, USA), and 4 ng/μL DNA template. Amplifications were performed in duplicate in a thermocycler (Veriti Thermal Cycler, Applied Biosystems, USA). The $MgCl_2$ concentration and the annealing temperature used for each primer are shown in Supplementary Table 2. The cycling parameters were 94 °C for 5 min, 30 cycles of 94 °C for 1 min, annealing for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. Positive and negative controls were included in all reactions. The amplicons were visualized by electrophoresis on an agarose gel stained with ethidium bromide (0.5 μg/mL) and analyzed using a Gel Doc L-Pix image system (Loccus Biotecnologia, Brazil). A 100-base-pair (bp) DNA ladder (Ludwig Biotecnologia, Brazil) was used as molecular marker.

DNA sequencing

Amplicons from variable regions of class 1 integrons with a typical 3′ CS were purified using ammonium acetate and sequenced using an automated DNA sequencer (ABI 3130 XL Genetic Analyzer XL, Applied Biosystems, USA). The sequences obtained were compared to sequences deposited in the GenBank DNA database using BLAST 2.0 (http://www.ncbi.nlm.nih.gov/BLAST) and aligned with the sequences of GenBank accession numbers FN432031.1, LN794247.1, and JX566770.1 using MEGA 5.0 (http://www.megasoftware.net). At least one amplicon from each resistance gene was also sequenced to evaluate the specificity of the primers.

Statistical analysis

The statistical differences between percentages of resistance to all antimicrobials were evaluated by Cochran test. The Chi-Square test was used to analyze the differences of resistance between groups of

Table 1 Antimicrobial resistance in *Salmonella enterica* isolated from poultry-related samples

Samples (number of isolates)		Resistance to antimicrobial drugs (%)																
		AMI	ESP	EST	GEN	NEO	TOB	AMP	CFC	CTF	NAL	CIP	ENO	SUL	SUT	CLO	FLF	TET
Poultry meal ($n = 106$)	R	1.8	4.7	5.6	1.8	1.8	0	0	0	0	6.6	0	0	28.3	0	0	0	0
	IR	0.9	12.2	19.9	2.8	76.4	2.8	0	1.8	1.8	0	1.8	1.8	0	0	0	0	0
Poultry ($n = 9$)	R	0	11.1	11.1	0	0	0	11.1	0	0	55.5	0	0	44.4	11.1	11.1	11.1	11.1
	IR	11.1	0	0	0	77.7	0	0	0	0	0	11.1	11.1	0	0	0	0	11.1
Poultry environment ($n = 8$)	R	6.8	19.3	28.4	4.5	1.1	13.6	13.6	12.5	12.5	27.2	1.1	1.1	51.1	38.6	7.9	7.9	43.1
	IR	4.5	9.1	11.3	1.1	0	2.8	1.1	0	0	0	13.6	13.6	0	0	4.5	4.5	2.2

R Resistant, *IR* intermediate resistance, *AMI* amikacin, *ESP* spectinomycin, *EST* streptomycin, *GEN* gentamicin, *NEO* neomycin, *TOB* tobramycin, *AMP* ampicillin, *CFC* cefaclor, *CTF* ceftiofur, *NAL* nalidixic acid, *CIP* ciprofloxacin, *ENO* enrofloxacin, *SUL* sulfonamides, *SUT* trimethoprim/sulfamethoxazole, *CLO* chloramphenicol, *FLF* florfenicol, *TET* tetracycline

isolates (poultry by-product meals, poultry environment, and poultry samples), and to verify the correlation between resistance phenotypes and genes. Both Fisher's exact and Student's *t* tests were used to compare MIC values obtained from the triplicates. The analyses were performed using SPSS 18.0 (IBM), and *p* values <0.05 were considered statistically significant for all tests (95 % confidence or 5 % significance).

Results

The percentages of 203 *S. enterica* isolates tested that were resistant to 17 different antimicrobials are summarized in Table 1. The MIC values for chloramphenicol, ampicillin, ceftazidime, ciprofloxacin, nalidixic acid, tetracycline, sulfamethoxazole, and trimethoprim/sulfamethoxazole are shown in Fig. 1. Forty (19.7 %) isolates were susceptible to all antimicrobials; among these, 62.5 % were from poultry environment, 32.5 % from poultry by-product meals, and 5 % from poultry organs. Isolates from the poultry environment were significantly more resistant to antimicrobials, with the exception of nalidixic acid, compared with the other isolates ($p < 0.05$), particularly those isolated from poultry by-product meal ($p < 0.001$). The highest percentage of resistance was found to sulfonamides, but a higher percentage of resistance to nalidixic acid was detected in the poultry samples compared with the meal and environmental samples. Eighty-nine different patterns of antimicrobial resistance were found (Table 2 and Supplementary Table 3): 163 (80.3 %) isolates showed reduced susceptibility to at least one antimicrobial, 37 (18.2 %) of which were MDR (resistant to at least three classes of drugs). Four MDR isolates (*Salmonella enterica* serovar Heidelberg (*S.* Heidelberg), *Salmonella* Cerro, and two *Salmonella* Senftenberg) showed the penta-resistant phenotype ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline). One *S.* Heidelberg isolate with ACSSuT resistance profile was also resistant to seven other antimicrobials.

Class 1 integrons were present in 26 isolates (12.7 %), 21 (80.8 %) of which were MDR, whereas class 2 integrons were not detected. None of the isolates from poultry by-product meal showed the presence of integrons. 25 of the 26 isolates (96.1 %)

Antimicrobials	Drug concentration (μg/mL)																
	0.03	0.06	0.12	0.25	0.5 (0.5/9.5)	1 (1/19)	2 (2/38)	4 (4/76)	8 (8/152)	16 (16/304)	32 (32/608)	64 (64/1,216)	128 (128/2,432)	256	512	1,024	2,048	4,096
Ampicillin								1	1	1	2	3	2	2	2	2		
Ceftazidime						34		1	3	1	4	3	1		2			
Chloramphenicol						2	1	1	1	4	5	3						
Ciprofloxacin	4		6	7	2	2												
Nalidixic acid							12	14	2	4	4		1	7	6	11	4	2
Sulfamethoxazole									21			6	19	24	16	11	28	
Trimethoprim/ sulfamethoxazole					10			2	6	10	11	8						
Tetracycline							4	1	4		1	27	6	2				

Fig. 1 Minimum inhibitory concentrations (MIC) to the antimicrobial agents tested against intermediate-resistant or resistant *Salmonella enterica* isolates previously evaluated by the disk diffusion method. The concentration of trimethoprim/sulfamethoxazole is shown in *parentheses*, and the concentration range used for each antimicrobial is shown in *gray*. The *solid lines* represent the breakpoints established by CLSI (2013)

with integrons were isolated from the poultry environment. The variable regions between 5′ CS and 3′ CS of class 1 integrons were analyzed by PCR, and the results showed that 13 isolates presented the typical 3′ CS and that 14 contained an atypical 3′ CS linked to the *qacH–sul3* domain in the absence of the *sul*1 gene. The amplification and sequencing of the variable region between 5′ CS and the typical 3′ CS showed 10 isolates with 1.7-kb fragments presenting the *aad*A1 and *dfr*A1 genes (GenBank accession numbers KJ848440 to KJ848449), whereas three isolates showed fragments of approximately 1 kb that contained only *aad*A1 (GenBank accession numbers KJ756515 to KJ756517) (Table 2). *S.* Senftenberg isolated from the environment harbored two class 1 integrons: one integron had a typical 3′ CS and a variable 1.7-kb-long region, and the other had an atypical 3′ CS linked to *qacH–sul3*.

Fifty (24.6 %) isolates were resistant to aminoglycosides, and 45 of these (90 %) harbored at least one gene encoding resistance to this antimicrobial class. The *aad*A and *aad*B genes were detected concomitantly in 17 (34 %) isolates resistant to aminoglycosides, whereas each gene alone was observed in 11 (22 %) and 15 (30 %) *S. enterica* isolates, respectively. Additionally, two and three intermediate-resistant isolates presented *aadA* and *aadB* alone, respectively (Table 2). On what concerns to resistance to streptomycin, the 32 resistant isolates were tested for *str*A and *str*B, showing their concomitant presence in 17 (53.1 %) isolates, and each gene alone in eight (25 %) and four (12.5 %) isolates. The *str*A and *str*B genes were also found in three and one isolates with intermediate resistance to streptomycin, respectively (Table 2).

Resistance to sulfonamide was significantly associated with the presence of *sul* genes ($p < 0.05$) because 64.4 % of the sulfonamide-resistant isolates presented at least one *sul* gene (Table 2, Supplementary Table 4). The *sul*1, *sul*2, and *sul*3 genes were detected alone in three (4.1 %), eight (11 %), and 17 (23.3 %) isolates, respectively. Concomitance of *sul*2 with *sul*1 or *sul*3 was detected in 11 (15.1 %) and seven (9.6 %) isolates, respectively, and the *S.* Senftenberg isolate that harbored two class 1 integrons presented both *sul*1 and *sul*3. The comparison of the MIC values presented by the isolates with *sul* genes verified that 20 (71.4 %) isolates with an MIC of 2,048 μg/mL contained the *sul*3 gene (Supplementary Table 4). Thirteen isolates carrying *sul*1 (86.7 %) also harbored a class 1 integron. However, integrons were not found in two *sul*1-positive and 11 *sul*3-positive isolates.

Among the 21 isolates with reduced susceptibility to β-lactams, 85.7 % (18) presented bla_{TEM}, which was significantly associated with the phenotype of resistance to these antimicrobials ($p < 0.05$), five (23.8 %) presented bla_{CMY}, and two (9.5 %) harbored $bla_{CTX\text{-}M}$ (Table 2, Supplementary Table 5).

Forty (19.7 %) *S. enterica* isolates showed reduced susceptibility to tetracycline. The genes *tet*A, *tet*B, and *tet*C alone were detected in 24 (60 %), two (5 %), and

Table 2 Antimicrobial resistance pattern, presence of integron, and resistance genes in *Salmonella enterica* isolated from poultry-related samples

Isolates (identification number)	Origins	Resistance patterns	Integron and resistance genes
S. enterica (S107)	Drag swab	**EST**	*str*A
S. Schwarzengrund (S141)	Drag swab	AMP	bla_{CMY}, bla_{TEM}
S. enterica (S134)	Drag swab	**TET**	*tet*C
S. enterica (S122)	Drag swab	**ESP**, EST	*aad*B, *str*A, *str*B
S. Gafsa (S97)	Viscera Meal	ESP, **NEO**	*aad*B
S. enterica (O:4,5) (S163)	Blood meal	ESP, SUT	*sul*2, *aad*A
S. enterica (S143)	Drag swab	EST, SUL	*sul*1, *aad*B
S. Anatum (S83)	Meat Meal	EST, **NEO**	*aad*B, *str*B
S. Anatum (S76)	Meat Meal	EST, **NEO**	*aad*B
S. Mbandaka (S93)	Meat Meal	EST, NEO	*aad*B
S. Cerro (S82)	Meat Meal	EST, NEO	*aad*B
S. Infantis (S100)	Blood Meal	**EST**, **NEO**	*str*B
S. Adelaide (S39)	Viscera Meal	GEN, **ESP**	*aadB*
S. enterica (S158)	Drag swab	**TOB**, SUL	*sul*2, *aad*B
S. enterica (S142)	Drag swab	CFC, CTF	bla_{TEM}
S. Senftenberg (S153)	Poultry organs	NAL, SUL	*sul*1
S. enterica (S174)	Poultry organs	NAL, SUL	*sul*2
S. enterica (S200)	Drag swab	NAL, SUL	*sul*2, *sul*3
S. Senftenberg (S128)	Drag swab	ESP, SUL, SUT	*int*I1[d], *sul*3, *aad*A, *aad*B
S. Senftenberg (S10)	Meat Meal	EST, **NEO**, SUL	*aad*B, *str*B
S. Senftenberg (S124)	Drag swab	EST, SUL, TET	*int*I1[d], *sul*3, *str*A, *str*B, *tet*A
S. enterica (S182)	Drag swab	EST, AMP, TET	*aad*B, *str*A, *str*B, bla_{TEM}, *tet*B
S. Infantis (S156)	Drag swab	AMP, CFC, CTF	bla_{CMY}
S. enterica (S127)	Drag swab	AMP, CFC, CTF	bla_{TEM}
S. enterica (S103)	Feather Meal	**CFC**, **CTF**, SUL	bla_{TEM}
S. Senftenberg (S184)	Drag swab	SUL, SUT, TET	*sul*3, *tet*A
S. Gallinarum (S193)	Poultry organs	SUL, SUT, **TET**	*int*I1[d], *sul*2, *sul*3, *tet*A
S. enterica (S195)	Drag swab	SUL, SUT, TET	*sul*3, *tet*A
S. enterica (S202)	Drag swab	SUL, SUT, TET	*int*I1[d], *sul*3, *tet*A
S. enterica (S102)	Blood Meal	AMI, **ESP**, EST, **NEO**	*aad*A, *aad*B
S. Infantis (S179)	Drag swab	ESP, EST, SUL, TET	*sul*2, *aad*B, *str*A, *tet*A
S. Worthington (S139)	Drag swab	**ESP**, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*B, *tet*A
S. enterica (S203)	Drag swab	**ESP**, SUL, SUT, TET	*sul*3, *aad*B, *tet*A
S. Senftenberg (S164)	Drag swab	EST, GEN, SUL, TET	*sul*2, *aad*A, *aad*B, *str*B, *tet*A
S. Senftenberg (S148)	Drag swab	AMP, CFC, CTF, NAL	bla_{CMY}, bla_{TEM}
S. Adelaide (S40)	Viscera Meal	AMI, ESP, **NEO**, **GEN**, SUL	*aad*B
S. Senftenberg (S54)	Viscera Meal	**ESP**, **NEO**, **CFC**, **CTF**, SUL	*aad*A, bla_{TEM}
S. Senftenberg (S177)	Drag swab	ESP, EST, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*B, *str*A, *tet*A
S. enterica (S118)	Drag swab	ESP, **EST**, SUL, SUT, TET	*sul*3, *aad*A, *str*A
S. enterica (S196)	Drag swab	**ESP**, **EST**, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*B, *str*A, *tet*A
S. enterica (O:13,23) (S180)	Drag swab	EST, AMP, SUL, SUT, TET	*sul*2, *aad*B, *str*A, *str*B, bla_{TEM}, *tet*B
S. enterica (O:4,5:l,v:-) (S169)	Disposable shoes covers	NAL, **CIP**, **ENO**, SUL	*sul*3
S. enterica (O:4,5) (S108)	Drag swab	AMP, CFC, CTF, NAL, SUL	*sul*2, bla_{CMY}

Table 2 continued

Isolates (identification number)	Origins	Resistance patterns	Integron and resistance genes
S. enterica (S120)	Drag swab	AMI, NEO, NAL, **CIP**, **ENO**, SUL	*aad*B
S. Senftenberg (S167)	Drag swab	**AMI**, ESP, EST, SUL, SUT, TET	*int*I1[d], *sul*2, *sul*3, *aad*A, *aad*B, *str*A, *tet*A
S. Senftenberg (S129)	Drag swab	ESP, EST, **NEO**, SUL, SUT, TET	*sul*3, *aad*A, *aad*B, *str*A, *str*B
S. Infantis (S130)	Drag swab	ESP, EST, **NEO**, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*A, *aad*B, *str*A, *str*B, *tet*A
S. Senftenberg (S112)	Drag swab	ESP, EST, **TOB**, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*A, *str*A, *tet*A
S. Montevideo (S205)	Drag swab	ESP, EST, AMP, SUL, SUT, TET	*sul*2, *sul*3, *aad*A, *aad*B, *str*A, *str*B, bla_{CTX-M}
S. Senftenberg (S114)	Drag swab	AMI, ESP, **EST**, GEN, SUL, SUT, TET	*int*I1[d], *sul*3, *aad*A, *aad*B, *str*A, *tet*A
S. Senftenberg (S171)	Drag swab	**AMI**, ESP, **EST**, **NEO**, SUL, SUT, TET	*sul*3, *aad*A, *aad*B, *tet*A
S. enterica (S119)	Drag swab	**ESP**, TOB, CFC, CTF, **CIP**, **ENO**, NAL	*aad*A, *aad*B, bla_{TEM}
S. Senftenberg (S138)	Disposable shoes covers	**ESP**, **EST**, SUL, SUT, CLO, FLF, TET	*int*I1[a,c,d], *sul*1, *sul*3, *aad*A1, *dfr*A1, *str*A
S. enterica (O:4,5:l,v:-) (S187)	Drag swab	**ESP**, NAL, SUL, SUT, CLO, FLF	*sul*3
S. Cerro (S176)*	Drag swab	EST, AMP, SUL, SUT, CLO, FLF, TET	*sul*2, *aad*A, *aad*B, *str*A, *str*B, bla_{TEM}
S. Worthington (S192)	Drag swab	EST, NAL, SUL, SUT, **CLO**, **FLF**, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *aad*B, *str*A, *str*B, *tet*A
S. Worthington (S194)	Drag swab	EST, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *aad*B, *str*A, *str*B
S. Worthington (S204)	Drag swab	EST, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *aad*B, *str*A, *str*B
S. Heidelberg (S109)	Drag swab	AMI, **ESP**, **NEO**, NAL, **CIP**, **ENO**, SUL, TET	*sul*2, *tet*C
S. Senftenberg (S201)	Poultry carcass	**AMI**, ESP, EST, AMP, SUL, CLO, FLF, TET	*sul*2, *sul*3, *aad*B, *str*B, bla_{CMY}, bla_{TEM}, *tet*A, *tet*B
S. Senftenberg (S123)*	Drag swab	AMI, ESP, AMP, SUL, SUT, CLO, FLF, TET	*int*I1[d], *sul*2, *sul*3, *aad*A, *aad*B, bla_{TEM}, *tet*A
S. Worthington (S170)	Drag swab	**AMI**, EST, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *aad*B, *str*A, *tet*A
S. Worthington (S113)	Drag swab	EST, **TOB**, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *str*A, *str*B, *tet*A
S. Worthington (S146)	Drag swab	ESP, CFC, CTF, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, bla_{TEM}, *tet*A
S. Worthington (S185)	Drag swab	EST, NAL, **CIP**, **ENO**, SUL, SUT, **CLO**, **FLF**, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *str*A, *str*B, *tet*A
S. Senftenberg (S166)	Drag swab	EST, NAL, CIP, ENO, SUL, SUT, CLO, FLF	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, *str*A
S. Schwarzengrund (S140)	Drag swab	ESP, EST, CFC, CTF, NAL, **CIP**, **ENO**, SUL, SUT, TET	*int*I1[a,b], *sul*1, *sul*2, *aad*A1, *str*A, *str*B, bla_{TEM}, *tet*A, *tet*B

Table 2 continued

Isolates (identification number)	Origins	Resistance patterns	Integron and resistance genes
S. Heidelberg (S111)	Drag swab	ESP, EST, GEN, NEO, **TOB**, AMP, CFC, CTF, SUL, TET	*int*I1[a,b], *sul*1, *aad*A1, *str*A, *str*B, bla_{CTX-M}, bla_{TEM}, *tet*A, *tet*C
S. Senftenberg (S165)*	Drag swab	ESP, EST, **NEO**, AMP, CFC, CTF, SUL, SUT, CLO, FLF, TET	*int*I1[d], *sul*2, *sul*3, *aad*A, *aad*B, *str*A, *str*B, bla_{TEM}, *tet*A
S. Worthington (S147)	Drag swab	AMI, ESP, CFC, CTF, NAL, **CIP**, **ENO**, SUL, SUT, **CLO**, **FLF**, TET	*int*I1[a,c], *sul*1, *sul*2, *aad*A1, *dfr*A1, bla_{TEM}, *tet*A
S. Heidelberg (S110)*	Drag swab	ESP, EST, **GEN**, **NEO**, **TOB**, AMP, CFC, CTF, NAL, CIP, ENO, SUL, CLO, FLF, TET	*int*I1[a,b], *sul*1, *sul*2, *aad*A1, *str*A, *str*B, bla_{TEM}, *tet*A, *tet*C

NAL nalidixic acid, *AMI* amikacin, *AMP* ampicillin, *CFC* cefaclor, *CTF* ceftiofur, *CIP* ciprofloxacin, *CLO* chloramphenicol, *ENO* enrofloxacin, *ESP* spectinomycin, *EST* streptomycin, *FLF* florfenicol, *GEN* gentamicin, *NEO* neomycin, *SUL* sulfonamides, *SUT* trimethoprim/sulfamethoxazole, *TET* tetracycline, *TOB* tobramycin

The bolded drugs showed intermediate resistance

[a] Indicates 3′ conserved segment of class 1 integron

[b] Indicates approximately amplicon size for 5′ CS-3′ CS region of 1.0 kb

[c] 1.7 kb

[d] Indicates atypical 3′ conserved segment of class 1 integron with *sul*3 gene

* Indicates the presence of penta-resistant phenotype (ACSSuT)

two (5 %) of these isolates, respectively, whereas the presence of *tet*A plus *tet*B (two isolates) and *tet*A plus *tet*C (two isolates) were also found (Table 2, Supplementary Table 6).

Discussion

S. enterica is an important pathogen involved in foodborne diseases that is usually transmitted by poultry-derived products (Van et al. 2012). Moreover, the presence of resistance determinants to antimicrobials used in human medicine has made this microorganism a major threat to public health (Collignon et al. 2009). In this context, the characterization of antimicrobial resistance in *Salmonella* isolated from poultry samples may aid the understanding of the role of practices, supplies, devices, and/or outdoor and indoor environments in the re-introduction and maintenance of resistant isolates in poultry farms. The antimicrobial resistance in *Salmonella* from poultry has been extensively analyzed (Campioni et al. 2014; Hofacre et al. 2001; Hur et al. 2011; Li et al. 2012), although isolates from the environment of poultry houses and components of poultry feed have not been investigated to the same extent as those from other sources. Therefore, this study focused on isolates from poultry by-product meal and environment of poultry houses, in order to evaluate their relative significance as reservoirs of *Salmonella* in the poultry production chain.

Feed has been considered a potential source of *Salmonella* contamination in poultry farms (Ge et al. 2013; Sapkota et al. 2014), whose origin may be derived from its ingredients (Sapkota et al. 2007). Poultry meal has been described as an important feed ingredient that may contain *Salmonella* resistant to at least five antibiotics (Hofacre et al. 2001). Conversely, most *S. enterica* isolates from poultry by-product meal analyzed in this study were sensitive to all antimicrobials tested, although isolates presenting reduced susceptibility to ceftiofur, a third-generation-cephalosporin used in day-old chicks, were detected. In contrast, many MDR isolates were found in the poultry house environment, and these isolates presented resistance to the drugs of choice for the treatment of salmonellosis in humans (CDC 2013). Moreover, the ACSSuT phenotype, which is usually associated with

S. Typhimurium (Yu et al. 2008), was found in the serovars S. Senftenberg, S. Heidelberg, and S. Cerro, which may be due to the horizontal spread of the resistance determinants responsible for this phenotype (Dionisi et al. 2011). This may be even more troubling because resistance can be spread to commensal bacteria, which can act as a reservoir for resistance genes. Therefore, appropriate sanitation of the indoor environment and equipment is needed to avoid the persistence of MDR *Salmonella*. Furthermore, the improvement of biosecurity can decrease the prophylactic use of antimicrobials, as described in the pig production chain (Laanen et al. 2013). The use of antimicrobials as growth promoters or for therapeutic purposes in veterinary medicine can exert a selective pressure favoring resistant isolates (Kempf et al. 2013) that cannot be overcome rapidly. Indeed, eight isolates resistant to chloramphenicol were identified in 2011 (data not shown), although this antimicrobial has not been used in Brazilian animal production for over 10 years (MAPA 2003). This resistance was possibly co-selected with other antimicrobials under use in animal production, whose determinants of resistance would be carried by the same mobile genetic elements. Alternatively, the maintenance of chloramphenicol resistance can be due to the cross-resistance with other antibiotics and biocides (Chuanchuen et al. 2008b).

A high proportion of isolates resistant to sulfonamide was found, even in the isolates from poultry by-product meal, which may be due to the wide use of sulfonamide over many years (Oliveira et al. 2005). Sulfonamide resistance was associated with *sul* genes in the majority of the tested isolates, and *sul*2 and/or *sul*3 were more prevalent, as previously described for *Salmonella* isolated from poultry (Hur et al. 2011). The remaining isolates may be resistant due to mutation of the chromosomal gene (*fol*P) encoding dihydropteroate synthase, as already described for other bacteria (Mohd-Zain et al. 2013; Skold 2001). The low prevalence of *sul*1 is in accordance with the presence of class 1 integrons in only 12.3 % of the isolates because this gene has been associated with a typical 3′ CS. However, two *sul*1-positive isolates did not carry integrons. A previous study has shown that *sul*1 is not always present on the class 1 integron and can also be carried by a plasmid (Han et al. 2012). The presence of *sul*3 has been associated with an atypical 3′ CS in the absence of *sul*1 (Machado et al. 2013), as was observed in half of our isolates that harbored class 1 integrons. Additionally, one S. Senftenberg isolate presented two distinct class 1 integrons and both *sul*1 and *sul*3, which were associated with typical and atypical 3′ CSs, respectively. This concomitant presence of more than one class 1 integron presenting *sul* genes has already been shown in other *Salmonella* serovars isolated from human and poultry (Firoozeh et al. 2012; Lee et al. 2009). However, 44 % of the *sul*3-positive isolates did not carry integrons, and this gene was thus likely inserted into plasmids (Han et al. 2012). In contrast, *sul*2 showed a lower association with integrons, which is in accordance with its integron-independent plasmid origin (Antunes et al. 2005).

Aminoglycosides and tetracyclines have been widely used in veterinary medicine (Schwarz et al. 2001), which likely led to the selection of resistant isolates to these antimicrobials. In this study, it was investigated the presence of some genetic determinants of enzymatic inactivation of aminoglycosides, which were found alone or associated in the majority of the isolates resistant to these antimicrobials. In this context, *str* genes were detected in most streptomycin-resistant isolates presenting a higher prevalence when compared to other studies (Anjum et al. 2011; Glenn et al. 2013). Additionally, it must be emphasized that mechanisms involving the efflux of the agent out the bacterial cell and/or alteration of the molecular target of the antibiotic, as well as other enzymes responsible for the inactivation of aminoglycosides, could be found concomitantly in the isolates with *aad* and/or *str* genes, or in those negative for the presence of these genes (Jana and Deb 2006). In relation to tetracycline resistance, the *tet* genes were detected in most isolates resistant to this antimicrobial, particularly *tet*A, which is in accordance with previous findings from the analysis of *S. enterica* from poultry (Glenn et al. 2013). The remaining isolates with reduced susceptibility to tetracycline may present other *tet* genes also found in bacteria from food animals (Frye and Jackson 2013).

In accordance with the findings from other studies (Costa et al. 2013; Jong et al. 2014), resistance to β-lactams was found in only a few of the tested isolates. However, the presence of third-generation-cephalosporin resistance per se is a cause for concern because it limits the therapeutic options for the treatment of human salmonellosis. All β-lactam-resistant isolates showed at least one of the *bla* genes investigated, which corroborates that β-lactamase production is the

main mechanism of resistance to β-lactams in *Salmonella* (Bush and Jacoby 2010).

The surveillance of antimicrobial resistance in *Salmonella* contributes to the debate concerning the impacts that antimicrobial use in animal production and the consequent selection of resistant isolates can exert in human health. Additionally, our results highlight the importance of the environment as a reservoir of resistant *Salmonella*. The persistence of resistance determinants in the poultry production chain reinforces the need for alternative strategies to prevent infectious diseases that may compensate, at least partially, for the loss of productivity in the poultry industry due to the possible banning of antimicrobials as growth promoters.

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