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VETERINARY MICROBIOLOGY - RESEARCH PAPER

The rise of multidrug resistant *Salmonella* isolates in healthy chickens in Brazil by successful establishment of plasmid IncHI2A carrying several antibiotic resistance genes

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Abstract

Salmonella spp. is an important global issue in food-producing animals. The present study evaluated antimicrobial resistance and virulence profiles in *Salmonella* spp. isolates from chickens in Brazil. Identification of serotypes, virulence and antimicrobial resistance genes, and plasmid profiles were performed. Three different serovars were found, *S*. Schwarzengrund, *S*. Newport and *S*. Kentucky. All isolates were considered Multidrug- resistance (MDR). Among the 32 *Salmonella* spp. isolates analysed, 29 isolates carried $bla_{\text{CTX-M-2}}$ gene and showed the insertion sequence IS*CR1* and a class 1 integron structure upstream from $bla_{\text{CTX-M-2.}}$ This gene was harboured in large IncHI2A plasmids with approximately 280kb. Furthermore, 30 isolates harboured *tet*A and *tet*B genes and 25 also harboured *qnr*B. The virulence genes *inv*A, *mis*L, *orf*L, *spi*C and *pip*D were detected in all isolates. The study shows a high prevalence of MDR *Salmonella* isolates disseminated in poultry farms. The association of the replicon IncHI2A with the resistance genes found, elevate the risk of foodborne disease outbreaks.

Keywords Brazil · ESBL · poultry · food-producing animals

Introduction

Salmonella spp. is a frequent etiological agent of foodborne infections. The intestinal tract of food-producing animals can be a reservoir of *Salmonella* spp., which may consequently lead to contamination of diverse derived food products. Foodborne salmonellosis has a high global impact in human health [1, 2]. The increasing global trade of food, especially unprocessed or raw meat and vegetables, new issues might arise regarding salmonellosis control. The presence of *Salmonella* spp. in healthy poultry represents a high risk factor, considering that poultry may be an important carrier for the transmission to humans through the consumption of contaminated derived food [3]. The combination of the previous factors to the presence of antimicrobial resistance genes on this bacteria genus aggravates the outcome of infections in both animals and humans [4].

Although there are more than 2600 different *Salmonella* serovars described, there is a tendency over time that few serovars stablish higher epidemiological relevance. In the Unites States, the CDC reported that only 5 serovars, including *S*. Enteritidis and *S*. Typhimurium, are responsible for more than 40% of all human salmonelosis cases [5, 6], during the last decade. The emergence of novel serovars with higher epidemiological relevance in the poultry production systems has ocurred throughout this period, and many factors are involved in this relevance dynamics, such as virulence factors, environmental adaptability or resistance to antimicrobials. Among these factors, the use of antimicrobials is the only influenced by humans, and it is important to control the use to minimize the election of resistant *Salmonella* isolates and also to control the

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spread of MDR clones by improving biosecurity measures throughout the food production chain and guarantee the quality of globally traded food [4].

Currently, the resistance genes that have been found in *Salmonella* isolates of animal origin, show that these genes are mostly associated to mobile genetic elements and confer resistance to important classes available for both humans and animal therapies. These genes are not *Salmonella* spp. specific, but can be aquired by a wide range of Enterobacteriaceae and Nonfermenting Gram-Negative Bacilli [7]. The dissemination of antimicrobial resistance often occurs via mobile genetic elements such as plasmids, transposons and gene cassettes harboured in integrons [8]. The most common integrons involved in antimicrobial resistance are class 1 integrons that are abundant in the genomes of many bacterial species [9]. The emergence and spread of *Salmonella* isolates presenting resistance to different antimicrobials is concerning because these drugs are crucial to the successful treatment of invasive and complicated infections [4, 10].

Currently, a large percentage of *Salmonella* isolates from domestic animals, isolated from clinical or non-clinical origin carry antibiotic resistance genes and resistance profile against antimicrobials, commonly used in hospital care. Food-production animals, especially those in intensive farming systems, such as poultry, swines and dairy cattle, have shown the presence of relevant resistance genes in pathogenic or microbiota bacteria [11]. Resistance genes for 3rd and 4th generation beta-lactams, which are crucial antibiotics in human medicine, are increasinlgy described in these animals. Resistance to these antibiotics are mediated by the *Amp*C and Extended Spectrum Beta-lactamase (ESBL) families and these are often found in *E. coli* and *Salmonella* spp. [12].

The spread and the global persistence of serotype *S.* Kentucky reflect a particular situation related to the increased globalization of travel and the food/animal trade in different geographical regions. This serotype has been recovered from several poultry farms worldwide, and has shown a high potential to cause human infection [13, 14]. The acquisition of an *E. coli* ColV virulence plasmid by *S.* Kentucky was also associated with enhanced colonization ability in chickens [15].

The increasing concern with antimicrobial resistance and food safety has been recognized by various international organizations [9, 16]. Considering the complex epidemiology of this issue, the investigation of genes, elements and sources involved in the dissemination are highly relevant at this moment to overcome or manage this problem. Thus, the present study evaluated antimicrobial resistance and virulence profile in different *Salmonella* isolates from chickens in Brazil, to determine the prevalence of resistance elements and characterize the resistance profile of these bacteria.

Material and methods

Bacterial isolates

In 2013, drag swabs were collected from 23 chicken farms, located in Goias, Brazil. Thirty-two *Salmonella* spp. isolates were obtained. Isolates were characterized with biochemical tests using API 20E strips (bioMérieux, France) and the serotyping method follow the Kaufmann-White Le Minor scheme.

Antimicrobial susceptibility

The antimicrobial susceptibility of the isolates was determined by using the disk diffusion methods [17], and the results were interpreted according to recommendations of the Clinical and Laboratory Standards Institute [18]. Antimicrobial agents were tested: amoxicillin-clavulanic acid (AMC), piperacillin/tazobactam (TZP), cefotaxime (CTX), ceftazidime (CAZ), ceftiofur (EFT), cefoxitin (FOX), cefepime (FEP), aztreonam (ATM), ertapenem (ETP), nalidixic acid (NAL), ciprofloxacin (CIP), enrofloxacin (ENR), tetracycline (TET), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT) and chloramphenicol (CHL). ESBL-producing isolates were screened by double disk synergism (DDS) using CTX and CAZ with AMC.

Pulsed-field gel electrophoresis (PFGE)

Genetic relationship among 32 MDR isolates was determined by analysis of *Xba*I-digested genomic DNA on pulsed field gel electrophoresis (PFGE), performed in CHEF DRIII System (Bio-Rad, USA) [19]. Gels were analysed with the BioNumerics fingerprinting software (Applied Maths, Belgium) and the normalized profiles were compared using the Dice similarity index. The dendrogram was constructed using the unweighted-pair group method using average linkage algorithm (UPGMA).

Investigation of resistance and virulence genes

The investigation of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$ (groups 1, 2, 8, 9 and 25), PMQR genes and *tet* genes (A, B, C, D, E and G) was carried out by PCR and sequencing [20–23]. The DNA sequences and translated amino acid sequences obtained were compared with references found in the LAHEY home page (http://www.lahey.org/Studies/). The presence of virulence genes *inv*A (invasion protein), *mis*L (involved in intramacrophage survival), *orf*L (adhesin/autotransporter), *spi*C (type III secretion system) and *pip*D

(type III secretion effector associated with SPI-1 system) was evaluated by PCR [24, 25].

Characterization of genetic environment of resistance

After identification of β-lactamase genes in resistant isolates, the genetic environment of β-lactamase genes was determined by PCR of upstream and downstream regions, as described previously [26]. Plasmids were investigated and characterized by the PBRT scheme, as previously described [27]. Southern blot and hybridization methods were performed to locate the replicon type carrying the ESBL-encoding gene. Briefly, plasmid DNA was digested with *S1* nuclease and analysed on PFGE gels (*S1*-PFGE). The PFGE gel was transferred to the nylon membrane (Hybond-N+, GE Healthcare Life Sciences, USA) and hybridization with specific probes for detection of the resistance gene and the incompatibility (Inc) groups. The identification of the replicon type harbouring the resistance gene was determined when these probes hybridized in the same position.

Results

Bacterial isolates

Salmonella spp. isolates were serotyped and classified among three different serovars *S*. Schwarzengrund (17/32), *S*. Newport (13/32) and *S*. Kentucky (2/32) (Table 1).

Antimicrobial susceptibility

In the present study, among the 32 isolates analysed, 29 (90.6%) showed ESBL phenotype. These same 29 *Salmonella* spp. isolates showed resistance to CTX, EFT and ATM. Additionally, some isolates also showed resistance to other β-lactams tested, including 34% resistance to AMC, 80% to CAZ and 87% to FEP. However, 100% were susceptible to FOX, TZP and ETP. Furthermore, 81% (26/32) of the isolates were also resistant to the non-beta-lactam antibiotics NAL, CIP and ENR, 72% to TET and 97% to SXT and 9% were resistant to CHL (Table 1). Thus, all isolates were considered MDR, not-susceptible to at least one agent in three or more antimicrobial categories [28]. The MDR *Salmonella* spp. were isolated in all farms (Table 1).

Pulsed-field gel electrophoresis (PFGE)

All 32 MDR *Salmonella* isolates were successfully typed by PFGE, 17 *S. Schwarzengrund* isolated from different farms belonged to the same PFGE-type A, 13 *S*. Newport isolated were classified as PFGE-type B and two *S*. Kentucky isolated belonged to PFGE-type C (Table 1).

Investigation of resistance and virulence genes

Investigation of β-lactamase groups by PCR revealed that all 29 ESBL-producing isolates (100%) carried the $bla_{\text{CTX-M-2}}$ gene (Table 1). The *qnr*B gene was found in 25 isolates (Table 1) and 30 isolates harboured *tet*A and *tet*B genes (Table 1).

The genes *inv*A, *mis*L, *orf*L, *spi*C and *pip*D were evaluated and all these genes were detected in all isolates,

Characterization of genetic environment of resistance

The genetic environment characterized in CTX-M-producing isolates showed the insertion sequence IS*CR1* and a class 1 integron structure with 7 kb upstream (5' region) from $bla_{\text{CTX-M-2}}$ in all encoding isolates (Table 1). The IS*CR1* was found upstream of the $bla_{\text{CTX-M-2}}$ and the resistance gene was associated with sul-1 type integron structure.

Plasmids replicons from groups IncHI2A were identified in 97% (31/32) of the isolates (31/32). Replicons IncP and IncA/C were found in 3% (1/32) of the isolates (Table 1). The hybridization showed that 29 *Salmonella* spp. isolates harboured $bla_{\text{CTX-M-2}}$ gene in large IncHI2A plasmids with approximately 280kb to 300kb (Table 1).

Discussion

These findings bring important information about the maintenance and dissemination of the resistance genes in animal farms. The association of $bla_{\text{CTX-M-2}}$ with IS*CR1* and class 1 integron, increases the co-selection and maintenance risks of resistant bacteria, once these mobile genetic elements carries a resistance gene to quaternary ammonium disinfectants. Thus, the use of this antimicrobial alone, in poultry facilities and farms as a biosafety measure, may interfere in bacterial population in this environment.

Fluoroquinolones are extensively used as therapeutic drugs in veterinary medicine and the continuous use of subinhibitory antibiotic concentrations, in the poultry environment, may promote the genetic recombination and select resistant *Salmonella* isolates with reduced susceptibility [29, 30]. The quinolone resistance detected may be related to chromosomal mutations in *quinolone*-resistance-determining region (QRDR) or associated with other resistance mechanisms (*e.g.* porin deficiencies, overexpression of efflux systems). However, the presence of *qnr* genes in plasmids increases the risk of horizontal dissemination of co-resistance among enterobacteria [31]. In the present

Table 1 Microbiological and molecular characteristic of MDR *Salmonella* isolates from poultry farms

Isolates	Serovar	Farm	Phenotype of resistance	Resistance genes	Plasmid content (Inc/kb)	Genetic environment	PFGE type
12406	Newport	1	CTX,FEP,ATM,EFT,NAL,CIP,ENR, TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
12407	Schwarzengrund	2	CTX,CAZ,FEP,ATM,EFT,NAL,CIP,E NR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A,NT/280, 60	Int class1+IS*CR1*	A
12408	Newport	2	AMC, CTX,CAZ,FEP,ATM,EFT,TET,SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A,NT/280, 40	Int class1+IS*CR1*	B
12410	Newport	3	AMC, CTX,CAZ,FEP,ATM,EFT,TET,SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A,NT/280, 40	Int class1+IS*CR1*	B
12411	Newport	4	CTX,CAZ,FEP,ATM,EFT,TET SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
12416	Schwarzengrund	5	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
12818	Schwarzengrund	6	CTX,CAZ,FEP,ATM,EFT,NAL,CIP,E NR,TET,SXT	$bla_{CTX-M-2}$, qnrB5, tetA, tetB	HI2A/280	Int class1+IS*CR1*	A
12819	Schwarzengrund	6	CTX,CAZ,FEP,ATM,EFT,NAL,CIP,E NR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
12824	Schwarzengrund	7	CTX,CAZ,FEP,ATM,EFT,NAL,CIP,E NR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A ,*tet*B	HI2A/280	Int class1+IS*CR1*	A
12825	Schwarzengrund	7	CTX,FEP,ATM,EFT,NAL,CIP,ENR, TET,SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
12828	Schwarzengrund	8	TET,CHL,SXT	*tet*A, *tet*B	HI2A/280	ND	A
13206	Newport	9	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
13207	Newport	9	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
13210	Schwarzengrund	10	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5	HI2A/280	Int class1+IS*CR1*	A
13211	Schwarzengrund	10	CTX,CAZ,FEP,ATM,EFT,TET,SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
13213	Schwarzengrund	11	NAL,CIP,ENR,TET,SXT,CHL,FLO	*qnr*B5, *tet*A	HI2A,A-C/280, 150	ND	A
13214	Newport	11	CTX,ATM,EFT,TET,SXT	$bla_{CTX-M-2}$, *tet*A, *tet*B	HI2A,P/280, 25	Int class1+IS*CR1*	B
13766	Schwarzengrund	12	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, tetB	HI2A/280	Int class1+IS*CR1*	A
13771	Schwarzengrund	13	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
14289	Schwarzengrund	14	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	A
14290	Schwarzengrund	15	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/300	Int class1+IS*CR1*	A
14292	Newport	16	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A,NT/280, 35	Int class1+IS*CR1*	B
14296	Schwarzengrund	17	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/300	Int class1+IS*CR1*	A
14297	Schwarzengrund	18	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A,NT/280, 60	Int class1+IS*CR1*	A
14298	Schwarzengrund	18	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280,35	Int class1+IS*CR1*	A
14302	Newport	19	NAL,CIP,ENR,TET,CHL	*qnr*B5	NT/200	ND	B
14995	Newport	20	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/300	Int class1+IS*CR1*	B
14996	Newport	20	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
14998	Kentucky	21	CTX,CAZ,FEP,ATM,EFT,NAL,CIP, ENR,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/300	Int class1+IS*CR1*	C
14999	Kentucky	21	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	C
15007	Newport	22	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B
15009	Newport	23	AMC,CTX,CAZ,FEP,ATM,EFT,NAL, CIP,ENR,TET,SXT	$bla_{CTX-M-2}$, *qnr*B5, *tet*A, *tet*B	HI2A/280	Int class1+IS*CR1*	B

AMC amoxicillin-clavulanic acid, *CTX* cefotaxime, *CAZ* ceftazidime, *FEP* cefepime, *ATM* aztreonam, *EFT* ceftiofur, *NAL* nalidixic acid, *CIP* ciprofloxacin, *ENR* enrofloxacin, TET: tetracycline, *CHL* chloramphenicol, *SXT* trimethoprim-sulfamethoxazole. *NT* plasmids non-typeable by PBRT, *ND* not determined

study, 81% (26/32) of the isolates were resistant to the most relevant therapeutic options, within these classes: NAL, CIP and ENR. Moreover, 25 of these isolates carried plasmidial *qnr*B5 gene, which could be easily transferred and contribute to reduce susceptibility to these antimicrobials.

Although the preventive use of tetracycline was banned in Brazil since 1998, in animal feed, this antimicrobial agent remains one of the most frequently used in therapeutics because it is easily accessible [32], which may explain the high levels of resistance found in our study. Both *tet*A and *tet*B genes were found in 30/32 (94%) of the *Salmonella* spp. isolates. This was similar to the findings in others studies reported [33].

The impacting data obtained herein show an alarming high rate of MDR, where 90% of the *Salmonella* spp. isolates from broilers were ESBL-producers. This results show that the resistant isolates circulating in the sampled flocks, have been successfully established within the poultry production sites. The gene $bla_{\text{CTX-M-2}}$ was harboured in plasmids that simultaneously carried resistance genes to other antimicrobial classes. The large sized plasmids found, carrying different resistance genes, also belonged to the same replicon. The fact that these were present in different serovars relates to the relevant role of this plasmid for bacterial survival and persistence, to the point where it has been disseminated horizontally to different bacteria. Antimicrobial co-resistance to several drugs is concerning, considering the drastic reduction in therapeutic options available to treat such infections. Even though the risk of foodborne infections is not easily estimated and non-typhoidal *Salmonella* are most related to intestinal infections, MDR isolates could increase morbidity and mortality especially in susceptible more hosts [34].

The *invA* is conserved among *Salmonella* serotypes and is a useful marker for molecular detection of this pathogen by PCR. However, the presence of other additional virulence genes in all 3 different serovars studied, highlighted the virulence potential and showed the risk of *Salmonella* isolates to cause persistent infections in poultry and contaminate derived food. These genes have already been reported in *Salmonella* isolates from chickens and humans in Brazil and South Africa [33].

These data showed that antimicrobial resistance is still growing and evolving, considering characteristics and the level of dissemination of the mobile genetic elements found. The high rate of CTX resistant isolates (90.6%) revealed in the present study, demonstrated the successful establishment of isolates with improved survival and dissemination characteristics. In a previous study, among 83 *Salmonella* isolates from 2009 to 2012, 21.7% were considered MDR, and 13.5% carried $bla_{\text{CTX-M-2}}$ or $bla_{\text{CMY-2}}$ genes [35]. However, the present study showed an increased MDR rate and $bla_{\text{CTX-M-2}}$ dissemination, reaching 100% (32/32) and 90% (29/32) of the isolates, respectively. Co-resistance to several drugs and virulence determinants may be present in the same plasmid, which may be co-selected by antibiotic pressure [36] resulting in increased morbidity, mortality, systemic infections and hospitalization rates with resistant non-typhoid *Salmonella isolates* [37]. Overall, the cooperation and the exchange of information between the sectors of agriculture, veterinary, food production and public health has become essential for the constant surveillance and control of antimicrobial resistance worldwide. The genetic mobile elements involved in the dissemination of antimicrobial resistance are able to spread not only among animal *Salmonella* spp. isolates, but in favourable environments the exchange of these genes may occur with other Enterobacteriaceae from animals and humans.

Conclusion

The molecular features presented by the *Salmonella* spp. isolates studied herein, showed the capacity of these pathogenic MDR bacteria to persist and disseminate resistance in animal environment. Thus, this study shed light on the high dissemination potential of resistance among different bacteria, allowing successful establishment of a mobile genetic element (MGE). The frequent association of the replicon IncHI2A with $bla_{\text{CTX-M-2}}$, with slight variations on size and additional resistance genes co-inserted, found in 90% of the isolates from different farms has been shown to be established since 2009 and this fact may elevate the risk of outbreaks of foodborne infections, considering its prevalence.

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Declaration

Competing interest The authors have no competing interests to declare

References

1. Crump JA, Sjolund-Karlsson M, Gordon MA, Parry CM (2015) Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. Clin Microbiol Rev 28:901–937

2. Barrow PA, Jones MA, Smith AL, Wigley P (2012) The long view: *Salmonella*--the last forty years. Avian Pathol 41:413–420
3. Barilli E, Bacci C, StellaVilla Z et al (2018) Antimicrobial resistance, biofilm synthesis and virulence genes in *Salmonella* isolated from pigs bred on intensive farms. Ital J Food Saf 7:7223
4. Antunes P, Mourao J, Campos J, Peixe L (2016) Salmonellosis: The role of poultry meat. Clin Microbiol Infect 22:110–121
5. Centers for Disease Control and Prevention (2014) National *salmonella* surveillance annual report, 2012. US Department of Health and Human Services, CDC, Atlanta, Georgia
6. Centers for Disease Control and Prevention (CDC) (2018) National *Salmonella* surveillance annual report, 2016. US Department of Health and Human Services, CDC, Atlanta, Georgia
7. Michael GB, Schwarz S (2016) Antimicrobial resistance in zoonotic nontyphoidal *Salmonella*: An alarming trend? Clin Microbiol Infect 22:968–974
8. Popowska M, Krawczyk-Balska A (2013) Broad-host-range incp-1 plasmids and their resistance potential. Front Microbiol 4:44
9. Maka L, Popowska M (2016) Antimicrobial resistance of Salmonella spp. isolated from food. Rocz. Panstw. Zakl. Hig 67:343–358
10. Parry CM, Threlfall EJ (2008) Antimicrobial resistance in typhoidal and nontyphoidal *Salmonella*. Curr Opin Infect Dis 21:531–538
11. Machado E, Coque TM, Canton R, Sousa JC, Peixe L (2008) Antibiotic resistance integrons and extended-spectrum {beta}-lactamases among enterobacteriaceae isolates recovered from chickens and swine in portugal. J Antimicrob Chemother 62:296–302
12. Zhao S, White DG, Friedman SL et al (2008) Antimicrobial resistance in *Salmonella* enterica serovar heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl Environ Microbiol 74:6656–6662
13. Wasyl D, Kern-Zdanowicz I, Domanska-Blicharz K, Zajac M, Hoszowski A (2015) High-level fluoroquinolone resistant salmonella enterica serovar Kentucky st198 epidemic clone with inca/c conjugative plasmid carrying *bla*(ctx-m-25) gene. Vet Microbiol 175:85–91
14. Westrell T, Monnet DL, Gossner C, Heuer O, Takkinen J (2014) Drug-resistant *Salmonella* enterica serotype Kentucky in europe. Lancet Infect Dis 14:270–271
15. Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC (2011) Population dynamics of *Salmonella* enterica serotypes in commercial egg and poultry production. Appl Environ Microbiol 77:4273–4279
16. World Health Organization (2011) The regional office for europe: tackling antibiotic resistance from a food safety perspective in europe. World Health Organization Regional Office for Europe, Copenhagen, p 65. Available online at: http://www.euro.who.int/__data/assets/pdf_file/0005/136454/e94889.pdf
17. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. Clinical and Laboratory Standards Institute (CLSI), CLSI document M100-S22, Wayne, PA
18. CLSI (2016) Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. CLSI document M100-S26. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA
19. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ (2006) Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, *Salmonella*, and Shigella for PulseNet. Foodborne Pathog Dis 3(1):59–67
20. Saladin M, Cao VT, Lambert T et al (2002) Diversity of ctx-m beta-lactamases and their promoter regions from enterobacteriaceae isolated in three parisian hospitals. FEMS Microbiol Lett 209:161–168
21. Cattoir V, Nordmann P (2009) Plasmid-mediated quinolone resistance in gram-negative bacterial species: An update. Curr Med Chem 16:1028–1046
22. Minarini LA, Poirel L, Cattoir V, Darini AL, Nordmann P (2008) Plasmid-mediated quinolone resistance determinants among enterobacterial isolates from outpatients in brazil. J Antimicrob Chemother 62:474–478
23. Bae D, Cheng CM, Khan AA (2015) Characterization of extended-spectrum beta-lactamase (esbl) producing non-typhoidal Salmonella (nts) from imported food products. Int J Food Microbiol 214:12–17
24. Cortez AL, Carvalho AC, Ikuno AA, Burger KP, Vidal-Martins AM (2006) Identification of *Salmonella* spp. Isolates from chicken abattoirs by multiplex-pcr. Res Vet Sci 81:340–344
25. Odjadjare EC, Olaniram AO (2015) Prevalence of antimicrobial resistant and virulent *Salmonella* spp. In treated effluent and receiving aquatic milieu of wastewater treatment plants in durban, south africa. Int J Environ Res Public Health 12:9692–9713
26. Dhanji H, Murphy NM, Doumith M et al (2010) Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the uk. J Antimicrob Chemother 65:2534–2537
27. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by pcr-based replicon typing. J Microbiol Methods 63:219–228
28. Magiorakos AP, Srinivasan A, Carey RB et al (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268–281
29. Lopez-Cadenas C, Sierra-Vega M, Garcia-Vieitez JJ, Diez-Liebana MJ, Sahagun-Prieto A, Fernandez-Martinez N (2013) Enrofloxacin: Pharmacokinetics and metabolism in domestic animal species. Curr Drug Metab 14:1042–1058
30. Lee KE, Jung JH, Jung BY, Park YH, Lee YH (2011) Characterization of nalidixic acid-resistant and fluoroquinolone-reduced susceptible *Salmonella* Typhimurium in swine. J Food Prot 74:610–615
31. Canton R, Ruiz-Garbajosa P (2011) Co-resistance: An opportunity for the bacteria and resistance genes. Curr Opin Pharmacol 11:477–485
32. Roberts MC (2005) Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 245:195–203
33. Zishiri OT, Mkhize N, Mukaratirwa S (2016) Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. Isolated from commercial chickens and human clinical isolates from south africa and brazil. Onderstepoort J Vet Res 83:e1–e11
34. Kuang D, Zhang J, Xu X et al (2018) Emerging high-level ciprofloxacin resistance and molecular basis of resistance in *Salmonella* enterica from humans, food and animals. Int J Food Microbiol 280:1–9
35. Penha Filho RAC, Ferreira JC, Kanashiro AMI, Berchieri Junior A, Darini A (2019) Emergent multidrug-resistant nontyphoidal *Salmonella* serovars isolated from poultry in brazil coharboring *blac*tx-m-2 and *qnr*b or *blac*my-2 in large plasmids. Diagn Microbiol Infect Dis Diagn Microbiol Infect Dis 95(1):93–98
36. Herrero A, Rodicio MR, Echeita MA, Mendoza MC (2008) *Salmonella* enterica serotype Typhimurium carrying hybrid virulence-resistance plasmids (puo-stvr): A new multidrug-resistant group endemic in spain. Int J Med Microbiol 298:253–261
37. Varma JK, Greene KD, Ovitt J, Barrett TJ, Medalla F, Angulo FJ (2005) Hospitalization and antimicrobial resistance in salmonella outbreaks, 1984-2002. Emerg Infect Dis 11:943–946