TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2023) 47: 19-25
© TÜBİTAK
doi:10.55730/1300-0128.4264

Phenotypic and genotypic characterization of antimicrobial resistance in commonly isolated *Salmonella* serovars from chickens

Seyyide SARIÇAM İNCE*, Mehmet AKAN
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Received: 03.09.2022 • **Accepted/Published Online:** 24.01.2023 • **Final Version:** 10.02.2022

Abstract: Salmonellosis caused by *Salmonella* agents is the second most common zoonotic infection in humans. In recent years, *Salmonella*'s increasing antimicrobial resistance (AMR) has been a concern. The major transmission route of *Salmonella* is consumption of contaminated poultry products. Therefore, monitoring of antimicrobial resistance in chicken-originated *Salmonella* is critically important. This study investigated AMR in four commonly isolated *Salmonella* serovars from chickens, namely *Salmonella* Enteritidis (*S.* Enteritidis), *Salmonella* Infantis (*S.* Infantis), *Salmonella* Kentucky (*S.* Kentucky), and *Salmonella* Typhimurium (*S.* Typhimurium). A total of 133 isolates were examined by phenotypic and genotypic AMR characterization. Resistance to 14 different antimicrobials and eight resistance genes were investigated in all isolates. The AMR test indicated that there was no resistant isolate to all antimicrobials while 14.3% were susceptible to all antimicrobials. The highest resistance was to sulfonamides (57.1%), nalidixic acid (48.1%), and tetracycline (39.1%). The highest susceptibilities were to cefotaxime (86.5%), cefoxitin (92.5%), ceftazidime (78.2%), and ceftriaxone (97%). *S.* Infantis and *S.* Kentucky isolates had higher resistance to all antimicrobials than *S.* Enteritidis and *S.* Typhimurium isolates. Significantly high multidrug-resistance (MDR) was detected in 50.4% of all isolates, although MDR prevalence varied widely between serovars: 78.7% of all *S.* Infantis isolates were MDR whereas only 18.8% of *S.* Enteritidis isolates were MDR. The most prevalent resistance genes were *tetA* (35.2%) and *sul1* (31.6%), with 12.5% and 3.1% of *S.* Enteritidis isolates being positive for *tetA* and *sul1*, respectively, whereas 17.4% and 8.7% of *S.* Typhimurium isolates were positive. These rather low prevalence rates are probably due to effective monitoring of these serovars by control programs in Türkiye. The nondetection of *mcr1* and *mcr2* can be explained by the rare use of colistin in chicken flocks in Türkiye. The obtained findings emphasize the importance of AMR monitoring for *Salmonella* and the risks of chicken-originated isolates to humans.

Key words: Antimicrobial resistance, chicken, MDR, resistance gene, *Salmonella*, *S.* Infantis

1. Introduction

Salmonella is the second most common zoonotic agent worldwide. According to the European Food Safety Authority (EFSA) zoonoses report, *Salmonella* agents caused 90,105 human salmonellosis cases in 2019. *Salmonella* spp. consists of two species, *Salmonella enterica* and *Salmonella bongori*, with approximately 2700 serovars. The most prevalent serovars in human salmonellosis are *S.* Enteritidis (50.3%), *S.* Typhimurium (11.9%), *S.* Infantis (2.4%), and *S.* Kentucky (0.07%). However, control programs generally focus on *S.* Enteritidis and *S.* Typhimurium as they cause over 70% of human cases. *S.* Infantis is the most frequent serovar in broilers while *S.* Kentucky is another common serovar that spreads to humans via food products [1, 2].

Antimicrobial therapy is commonly used to treat bacterial infections in both humans and animals. However, improper use of antimicrobials has led to treatment failure, increased costs and mortality, and the spread of resistant pathogens [3]. According to the World Health Organization's (WHO) surveillance report, the spread of AMR and MDR nontyphoidal *Salmonella* is a global challenge [4]. WHO has included *Salmonella* in its high-priority pathogen list due to increasing AMR [5]. In recent years, AMR in *Salmonella* has increased in food-producing animals [6, 7]. Many studies conclude that resistant *Salmonella* of animal origin poses a risk to humans [8]. Humans are exposed to resistant *Salmonella* through the consumption of contaminated foods, particularly contaminated poultry products (eggs and chicken meat). Thus, the monitoring of AMR in chicken-originated *Salmonella* is critical for detecting cases and reducing the risk to public health [9].

Accordingly, this study determined the phenotypic and genotypic AMR in the most common *Salmonella* serovars isolated from chickens. The findings provide valuable

* Correspondence: s.saricam-92@hotmail.com

This work is licensed under a Creative Commons Attribution 4.0 International License.

information regarding AMR in commonly isolated *Salmonella* serovars, based on evaluation of serovars, antimicrobials, resistance genes, and breeding types.

2. Materials and methods

2.1. *Salmonella* isolation and identification

Salmonella isolates were obtained from litter/feces samples of broiler and layer chicken flocks. *Salmonella* isolation was performed by the ISO 6579-1:2017 procedure [10]. Serotyping was performed with specific somatic and flagella antisera (Biorad, France), using the Kauffmann–White–Le Minor scheme [11]. A total of 133 isolates were used, including 32 *S.* Enteritidis, 47 *S.* Infantis, 31 *S.* Kentucky, and 23 *S.* Typhimurium (Table 1). The 20% glycerol stocks were prepared to store bacterial cultures until molecular characterization.

2.2. Antimicrobial resistance test

All isolates were tested for AMR using the Kirby-Bauer Disc Diffusion method on Mueller Hinton agar (Oxoid, UK) as defined in the Clinical and Laboratory Standards Institute (CLSI) manual. The following antimicrobials were used (Oxoid, UK): ampicillin (AMP: 10 µg), cefotaxime (CTX: 30 µg), cefoxitin (FOX: 30 µg), ceftazidime (CAZ: 30 µg), ceftriaxone (CRO: 30 µg), chloramphenicol (C: 30 µg), ciprofloxacin (CIP: 5 µg), gentamycin (CN: 10 µg), meropenem (MEM: 30 µg), nalidixic acid (NA: 2 µg), sulfonamides (S3: 300 µg), tetracycline (TE: 30 µg), trimethoprim (W: 5 µg) and trimethoprim-sulfamethoxazole (SXT: 25 µg). *Escherichia coli* ATCC25922 strain was preferred as the quality control strain. The colony suspensions were adjusted equivalent to a 0.5 McFarland turbidity standard on a densitometer (Biosan, Latvia). After incubation at 36 °C ± 1 °C for 16–18 h, the zone diameters (mm) were evaluated as resistant, intermediate, or susceptible based on CLSI criteria [12]. *Salmonella* isolates resistant to three or more antimicrobial classes were considered to exhibit MDR [9].

2.3. Bacterial DNA extraction

Bacterial DNA was obtained from all isolates using the conventional boiling method. The bacterial suspensions were respectively incubated at 100 °C for 10 min and on ice for 5 min. DNA concentrations and qualities were checked using NanoDrop equipment (Thermo Scientific, USA).

2.4. Molecular characterization of resistance

The antimicrobial resistance genes to ampicillin (bla_{TEM}), colistin (*mrc1*, *mrc2*), fluoroquinolones (*qnrB*), sulfonamides (*sul1*), tetracyclines (*tetA, tetB*) and trimethoprim (*dfrA1*) were amplified by polymerase chain reaction (PCR). The PCR analyses were performed using specific primers as previously reported (Table 2). The reactions were conducted in a total of 25 µL of mixture volume containing 0.2 µL of Taq polymerase (2U/µL) (Thermo Scientific, USA), 0.5 µL of 10 mM dNTPs, 1 µL of each 10 mM primer, 2.5 µL of 10X buffer, 3 µL of $MgCl_2$, 14.8 µL of nuclease-free water, and 2 µL of template DNA. Amplifications were performed as follows: initial denaturation for 5 min at 95 °C, 34 cycles of denaturation for 20 s at 94 °C, annealing for 20 s at a defined temperature, extension for 20 s at 72 °C, and final extension for 5 min at 72 °C. Positive and negative controls were included for each reaction. The amplicons were analyzed with 1.5% agarose gel electrophoresis (Thermo Scientific, USA). The samples were visualized by G: Box Chemi UV transillumination (SynGene, India).

3. Results

The AMR test findings indicated that 14.3% (19/133) of the *Salmonella* isolates were susceptible to all tested antimicrobials. There was no resistant isolate to all antimicrobials while 78.9% (105/133) were resistant to at least one. Resistance to sulfonamides 57.1% (76/133) was the most common while high resistances were also found to nalidixic acid 48.1% (64/133), tetracycline 39.1% (52/133), and ampicillin 37.6% (50/133). The highest intermediate resistance was to ciprofloxacin at 48.1% (64/133). The highest susceptibilities were found to cephalosporin group antimicrobials, cefotaxime 86.5% (115/133), cefoxitin 92.5% (123/133), ceftazidime 78.2% (104/133), and ceftriaxone 97% (129/133) (Figure 1).

The antimicrobial resistance rates based on the serovars are shown in Figure 2. The highest resistances were in *S.* Infantis isolates for sulfonamides (85.1%) and nalidixic acid (78.7%). All *S.* Enteritidis isolates were susceptible to ceftriaxone and ciprofloxacin whereas *S.* Typhimurium isolates were susceptible to cefoxitin, ceftriaxone and meropenem. A significantly high MDR rate of 50.4% (67/133) was detected in all isolates. Moreover, 55.2%

Table 1. Distribution of *Salmonella* isolates by serovar and breeding type.

Breeding type	***S.* Enteritidis**	***S.* Infantis**	***S.* Kentucky**	***S.* Typhimurium**
Broiler	20	38	27	21
Layer	12	9	4	2
Total	32	47	31	23

Table 2. Primers, sequences, and annealing temperatures for the resistance genes.

Antimicrobial class	Resistance genes	Primer sequence (5'-3')*	Size (bp)	Annealing temperature (°C)	Reference
Beta-lactams	*bla*$_{TEM}$	F: GCACGAGTGGGTTACATCGA R: GGTCCTCCGATCGTTGTCAG	310	60	[26]
Colistin	*mrc1* *mrc2*	F: AGTCCGTTTGTTCTTGTGGC R: AGATCCTTGGTCTCGGCTTG F: CAAGTGTGTTGGTCGCAGTT R: TCTAGCCCGACAAGCATACC	320 715	58 58	[27]
Fluoroquinolones	*qnrB*	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	53	[28]
Sulfonamides	*sul1*	F: TCGGATCAGACGTCGTGG R: CCAGCCTGCAGTCCGCCT	258	60	[29]
Tetracyclines	*tetA* *tetB*	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTGATGACTCTT	577 634	55 55	[30]
Folate pathway inhibitors	*dfrA1*	F: GGAGTGCCAAAGGTGAACAGC R: GAGGCGAAGTCTTGGGTAAAAAC	367	55	[31]

*F, forward; R, reverse.

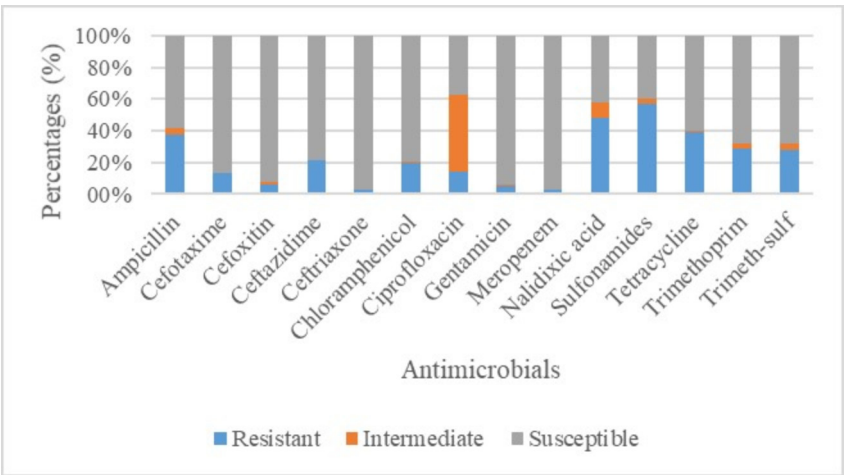

Figure 1. Distribution of resistant, intermediate, and susceptible isolates by the antimicrobials.

(37/67) of all MDR isolates were *S.* Infantis. At 78.7% (37/47), *S.* Infantis isolates had higher MDR rates than the other serovars.

The antimicrobial resistance rates based on the breeding types were shown on Figure 3. The highest resistance rates were to the same antimicrobials in both breeding types. The broiler isolates showed the highest resistance to sulfonamides (59.4%) and nalidixic acid (53.8%) compared to 48.1% and 25.9%, respectively, of the layer isolates that were resistant. All layer isolates were susceptible to four different agents, namely cefotaxime, ceftazidime, ceftriaxone, and chloramphenicol.

The *Salmonella* isolates for these four serovars were investigated for antimicrobial resistance genes. None of the isolates had *mrc1* and *mrc2* genes. In all isolates, the most commonly detected genes were in *tetA* (35.2%), *sul1* (31.6%), and *bla*$_{TEM}$ (15.0%). Prevalences were low for all other genes. Regarding the prevalence of resistance genes in terms of serovars, *S.* Infantis had the highest prevalence, particularly *sul1* (72.3%) and *tetA* (70.2%) (Figure 4).

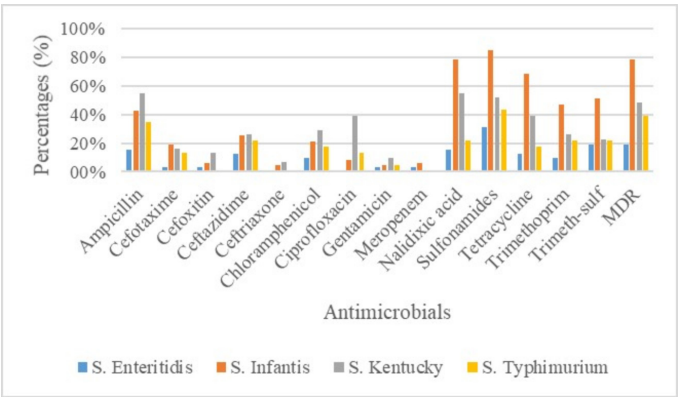

Figure 2. Distribution of resistant and MDR isolates by the serovars.

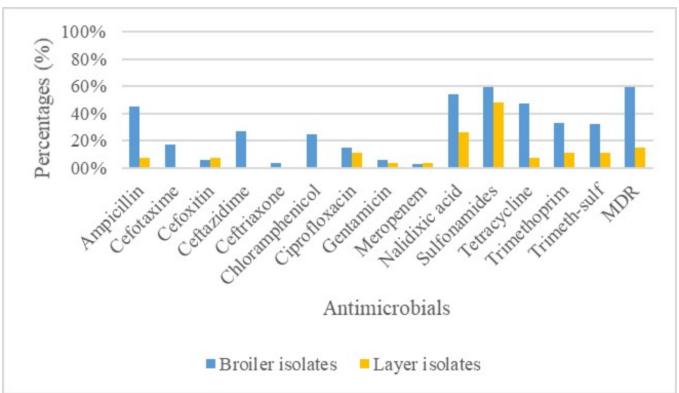

Figure 3. Distribution of resistant and MDR isolates by breeding types.

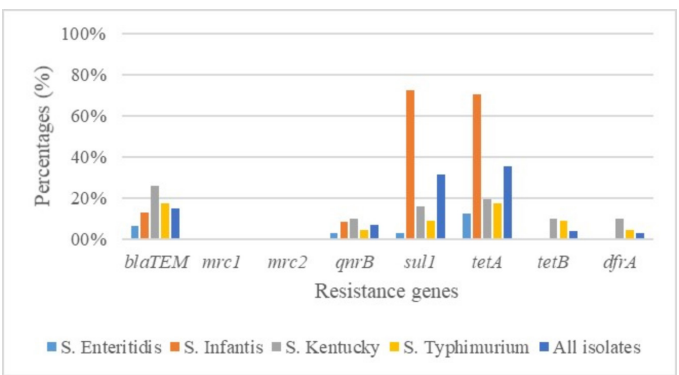

Figure 4. Distribution of antimicrobial resistance genes among the isolates.

In addition, phenotypic and genotypic findings were compared for the most prevalent resistance genes. Fifty-two isolates were found *tetA*- or *tetB*-positive. Similarly, fifty-two isolates were resistant to tetracycline also by the disc diffusion test. Seventy-six isolates were resistant to sulfonamides, with the presence of *sul1* in forty-two isolates while fifty isolates were resistant to ampicillin, with the presence of bla_{TEM} in twenty isolates.

4. Discussion

Salmonellosis is one of the most common foodborne infections worldwide, transmitted to humans through infected animals and consumption of contaminated food. Being very effective vectors, contaminated poultry animals and poultry products are the major source of *Salmonella* transmission to humans. Due to cross-contamination, humans are exposed to antimicrobial resistant strains

of *Salmonella* [13], which poses a serious risk to public health. Many studies have reported increasing prevalence and spread of antimicrobial resistant *Salmonella,* especially MDR *Salmonella*. Accordingly, WHO has added *Salmonella* to its priority pathogens list for global challenge. Therefore, monitoring of AMR *Salmonella* in chickens is recommended to control AMR [9, 14].

In this study, we detected that the highest resistance was to sulfonamides (57.1%), nalidixic acid (48.1%), tetracycline (39.1%), and ampicillin (37.6%). This is consistent with the resistance rates reported by Thi et al. (2020) to sulfonamides (75.86%), tetracycline (51.72%), and ampicillin (31.03%). These high resistance levels may be due to wide use of sulfonamides and nalidixic acid in chickens. Tetracycline and ampicillin have been used as antimicrobial agents in recent years, although their effectiveness has been decreasing in veterinary implementation. Our findings are thus in line with previous reports detailing increasing resistance of *Salmonella.*

Many studies have reported significantly high resistance to ciprofloxacin in recent years. Utrarachkij et al. (2016), Fardsanei et al. (2018), Güran et al. (2020), and Jiang et al. (2021) reported high ciprofloxacin resistance, such as levels of 51.1%, 90.9%, 100%, and 57.6%, respectively [3, 15-17]. In contrast, we found low resistance (14.3%) and high intermediate resistance (48.1%) to ciprofloxacin. Given that ciprofloxacin is a fluoroquinolone antimicrobial recommended as a first choice for treating human salmonellosis [4], ciprofloxacin-resistant *Salmonella* in contaminated food presents a serious risk to humans by hindering effective treatment. We therefore recommend monitoring resistance trends in ciprofloxacin.

The tested serovars were compared in terms of antimicrobial resistance rates. Significant differences were detected in the distribution of resistant isolates. *S.* Infantis and *S.* Kentucky isolates had higher resistance to almost all antimicrobials than *S.* Enteritidis and *S.* Typhimurium isolates. Compared with other *Salmonella* serovars, *S.* Infantis isolates had the highest resistance rates to the most of tested antimicrobials, such as ampicillin (42.6%), nalidixic acid (78.7%), sulfonamides (85.1%), and tetracycline (68.1%). The next highest rates were for *S.* Kentucky, such as ampicillin (54.8%), nalidixic acid (54.8%), sulfonamides (51.6%), and tetracycline (38.7%). Abdel-Maksoud et al. (2015) also found high rates of resistance to ampicillin (97%), and nalidixic acid (94%), sulfonamides (100%), and tetracycline (97%) [18]. However, one *S.* Kentucky isolate was resistant to at least 12 of the 14 tested antimicrobials and had five of the eight tested resistance genes. This finding is compatible with a previous study that reported particularly high resistance in *S.* Kentucky [19].

In our study, all isolates showed high susceptibility to cephalosporins. These low rates of cephalosporin resistance are in line with the findings of Abdel-Maksoud et al. (2015), who reported low resistance to cephalosporins among poultry-originated *Salmonella* isolates [18]. None of the layer isolates was resistant to cefotaxime, ceftazidime, ceftriaxone, or chloramphenicol while layer isolates showed only limited resistance to all tested antimicrobials except nalidixic acid and sulfonamides. These findings are compatible with Pande et al. (2015) [20], who reported low antimicrobial resistance in layer-originated most common *Salmonella* serovars.

While we found a high rate of MDR *Salmonella* (50.4%), this is lower than that reported in some previous studies. Wei et al. (2019) and Queslati et al. (2021), for example, reported high MDR rates (respectively 81% and 87.5%) in chickens [21, 22] whereas we detected MDR *S.* Enteritidis in only 18.8% of *S.* Enteritidis isolates, which contrasts with some previous studies. For example, Medeiros et al. (2011), Lu et al. (2014), and Asif et al. (2017) reported high rates of MDR *S.* Enteritidis isolates from chickens, at 63.9%, 92.6%, and 54.8%, respectively [14, 23, 24].

Regarding resistance genes, the highest prevalences were for *tetA* (35.3%) and *sul1* (31.6%), which encode tetracycline and sulfonamide resistance, respectively, in *Salmonella*. These findings are supported by the high positivity for *tetA* and *sul1* reported by Lu et al. (2014) and Thi et al. (2020). Comparing *tetA* and *sul1* rates by serovars, 12.5% and 3.1% of *S.* Enteritidis isolates were positive for *tetA* and *sul1*, respectively, while 17.4% and 8.7% of *S.* Typhimurium isolates were positive. These rates are significantly lower than those previously reported [24], which may reflect effective monitoring of *S.* Enteritidis and *S.* Typhimurium serovars with control programs in Türkiye. These low prevalences indicate the risk that resistance genes may be acquired by horizontal transfer remains low. We did not detect *mcr1* and *mcr2* in any isolate, which, we believe, is because colistin is rarely used in chicken flocks. In addition, colistin is only used in salmonellosis cases after resistance has been detected to commonly used antimicrobials. The low resistance to colistin can be explained with this approach [21].

Finally, we found no correlation between phenotypic resistance and genotypic resistance among the serovars. Some resistance genes were not detected in AMR-positive isolates, which could be due to silent genes, the presence of other genes, nonintegrated genes, or lack expression of existing genes in *Salmonella* isolates [21, 25].

Our findings provide valuable information about AMR in commonly isolated *Salmonella* serovars from chickens. We investigated AMR in *S.* Enteritidis, *S.* Infantis, *S.* Kentucky, and *S.* Typhimurium isolates by phenotypic and genotypic characterization. The findings were then evaluated in terms of serovars, antimicrobials, resistance genes and breeding type.

Funding
This research received no grant from any funding agency/ sector.

Conflict of interest
The authors declare no conflict of interests.

References

1. EFSA. The European Union one health 2019 zoonoses report. EFSA Journal 2021; 19 (2): 31-77.
2. Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG et al. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science 2015; 8 (3): 284-293. https://doi.org/10.1080/21553769.2015.1051243
3. Jiang ZH, Anwar TM, Peng XQ, Biswas S, Elbediwi M et al. Prevalence and antimicrobial resistance of Salmonella recovered from pig-borne food products in Henan, China. Food Control 2021; 121: 1-8. https://doi.org/10.1016/j.foodcont.2020.107535
4. Dominguez JE, Vinas MR, Herrera M, Moroni M, Gutkind GO et al. Molecular characterization and antimicrobial resistance profiles of Salmonella Heidelberg isolates from poultry. Zoonoses Public Health 2021; 68 (4): 309-315. https://doi.org/10.1111/zph.12819
5. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infectious Diseases 2018; 18 (3): 318-327. https://doi.org/10.1016/S1473-3099(17)30753-3
6. Thi HN, Pham TTT, Turchi B, Fratini F, Ebani VV et al. Characterization of Salmonella spp. Isolates from Swine: Virulence and Antimicrobial Resistance. Animals 2020; 10 (12): 1-14. https://doi.org/10.3390/ani10122418
7. Dahshan H, Shahada F, Chuma T, Moriki H, Okamoto K. Genetic analysis of multidrug-resistant Salmonella enterica serovars Stanley and Typhimurium from cattle. Veterinary Microbiology 2010; 145 (1-2): 76-83. https://doi.org/10.1016/j.vetmic.2010.02.035
8. Mthembu TP, Zishiri OT, El Zowalaty ME. Molecular detection of multidrug-resistant Salmonella isolated from livestock production systems in south africa. Infection and Drug Resistance 2019; 12: 3537-3548.
9. Shang K, Wei B, Cha SY, Zhang JF, Park JY et al. The occurrence of antimicrobial-resistant Salmonella enterica in hatcheries and dissemination in an integrated broiler chicken operation in Korea. Animals 2021; 11 (1): 1-14. https://doi.org/10.3390/ani11010154
10. ISO 6579-1:2017 (E). Microbiology of the food chain -Horizontal method for the detection, enumeration and serotyping of Salmonella Part 1: Detection of Salmonella spp. Geneva, Switzerland: ISO; 2017.
11. Grimont PAD, Weill FX. Antigenic formulae of the Salmonella serovars. 9th ed. Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella, Institute Pasteur; 2007.
12. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, in M100. 28th ed. Wayne, USA: Clinical and Laboratory Standards Institute; 2018.
13. Wang X, Biswas S, Paudya N, Pan H, Li X et al. Antibiotic resistance in Salmonella Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. Frontiers Microbiology 2019; 10: 1-12. https://doi.org/10.3389/fmicb.2019.00985
14. Asif M, Rahman H, Qasim M, Khan TA, Ullah W et al. Molecular detection and antimicrobial resistance profile of zoonotic Salmonella Enteritidis isolated from broiler chickens in Kohat, Pakistan. Journal of the Chinese Medical Association 2017; 80 (5): 303-306. https://doi.org/10.1016/j.jcma.2016.11.007
15. Guran HS, Ciftci R, Gursoy NC, Ozekinci T, Alali WQ. Prevalence of antibiotic-resistant Salmonella in retail organic chicken. British Food Journal 2020; 122 (4): 1238-1251. https://doi.org/10.1108/BFJ-10-2019-0790
16. Fardsanei F, Soltan Dallal MM, Douraghi M, Memariani H, Bakhshi B et al. Antimicrobial resistance, virulence genes and genetic relatedness of Salmonella enterica serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran. Journal of Global Antimicrobial Resistance 2018; 12: 220-226. https://doi.org/10.1016/j.jgar.2017.10.005
17. Utrarachkij F, Nakajima C, Siripanichgon K, Changkaew K, Thongpanich Y et al. Genetic diversity and antimicrobial resistance pattern of Salmonella enterica serovar Enteritidis clinical isolates in Thailand. Journal of Infection and Chemotherapy 2016; 22 (4): 209-215. https://doi.org/10.1016/j.jiac.2015.12.011
18. Mohammed AM, Rania AK, Atef EG, Rawia FG, Hemmat MA et al. Genetic characterisation of multidrug-resistant Salmonella enterica serotypes isolated from poultry in Cairo, Egypt. African Journal of Laboratory Medicine 2015; 4 (1): 1-7. https://doi.org/10.4102/ajlm.v4i1.158
19. Xiong Z, Wang S, Huang Y, Gao Y, Shen H et al. Ciprofloxacin-resistant Salmonella enterica serovar Kentucky ST198 in broiler chicken supply chain and patients, China, 2010-2016. Microorganisms 2020; 8 (1): 1-9. https://doi.org/10.3390/microorganisms8010140
20. Pande VV, Gole VC, McWhorter AR, Abraham S, Chousalkar KK. Antimicrobial resistance of non-typhoidal Salmonella isolates from egg layer flocks and egg shells. International Journal of Food Microbiology 2015; 203: 23-26. https://doi.org/10.1016/j.ijfoodmicro.2015.02.025

21. Oueslati W, Rjeibi MR, Benyedem H, Mamlouk A, Souissi F et al. Prevalence, risk factors, antimicrobial resistance and molecular characterization of Salmonella in northeast tunisia broiler flocks. Journal of Veterinary Science 2021; 9 (1): 1-18. https://doi.org/10.3390/vetsci9010012

22. Wei XY, You L, Wang D, Huang H, Li SJ et al. Antimicrobial resistance and molecular genotyping of Salmonella enterica serovar Enteritidis clinical isolates from Guizhou province of Southwestern China. Plos One 2019; 14 (9): 1-14. https://doi.org/10.1371/journal.pone.0221492

23. Medeiros MA, Oliveira DC, Rodrigues DP, Freitas DR. Prevalence and antimicrobial resistance of Salmonella in chicken carcasses at retail in 15 Brazilian cities. Revista Panamericana de Salud Pública 2011; 30 (6): 555-560.

24. Lu Y, Zhao H, Sun J, Liu Y, Zhou X et al. Characterization of multidrug-resistant Salmonella enterica serovars Indiana and Enteritidis from chickens in Eastern China. Plos One 2014; 9 (5): 1-6. https://doi.org/10.1371/journal.pone.0096050

25. Anjum MF, Choudhary S, Morrison V, Snow LC, Mafura M et al. Identifying antimicrobial resistance genes of human clinical relevance within Salmonella isolated from food animals in Great Britain. Journal of Antimicrobial Chemotherapy 2011; 66 (3): 550-559. https://doi.org/10.1093/jac/dkq498

26. Carlson SA, Bolton LF, Briggs CE, Hurd HS, Sharma VK et al. Detection of multiresistant Salmonella Typhimurium DT104 using multiplex and fluorogenic PCR. Molecular and Cellular Probes 1999; 13 (3): 213-222.

27. Rebelo AR, BortolaiaV, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveillance 2018; 23 (6): 1-11. https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672

28. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nature Medicine 2006; 12 (1): 83-88. https://doi.org/10.1038/nm1347

29. Guerri ML, Aladuena A, Echeita A, Rotgeret R. Detection of integrons and antibiotic-resistance genes in Salmonella enterica serovar Typhimurium isolates with resistance to ampicillin and variable susceptibility to amoxicillin-clavulanate. International Journal of Antimicrobial Agents 2004; 24 (4): 327-333. https://doi.org/10.1016/j.ijantimicag.2004.04.009

30. Ng LK, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes. Molecular and Cellular Probes 2001; 15: 209-215. https://doi.org/10.1006/mcpr.2001.0363

31. Torkan S, Khamesipour F, Anyanwu MU. Detection of virulence and antibacterial resistance genes in Salmonella isolates from diarrhoeic dogs in Iran. Revue de Medecine Veterinaire 2015; 166 (7-8): 221-228.