Prevalence and antimicrobial resistance profiles of *Salmonella* spp. in poultry meat

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Abstract

The spread of multidrug resistant (MDR) *Salmonella* strains, along the poultry supply chain, can represent a relevant threat to human health. This study aimed to evaluate the prevalence and antimicrobial resistance of *Salmonella* spp. isolated from poultry meat for human consumption. Between 2019 and 2021, 145 samples were analyzed according to ISO 6579-1:2017. The strains isolated were identified by using biochemical-enzymatic assays and serotyping, according to the Kauffmann-White-Le Minor scheme. The antibiotic susceptibility tests were determined using the Kirby-Bauer method. Forty *Salmonella* spp. strains were isolated and serotyping showed *Salmonella Infantis* to be predominant. 80% of the isolated strains were MDR and identified as *S. Infantis*. This study confirms the circulation of MDR *Salmonella* isolated from poultry meat and highlights the predominance of the *S. Infantis* serovar, which represents an emerging risk factor under the One Health holistic approach.

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Key words: poultry meat; Salmonella spp.; S. Infantis; AMR, MDR.

Contributions: all authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Availability of data and materials: data and materials are available by the authors.

Conference presentation: this paper was presented at the XXXI National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), 2022 September 22-23-24, Italy.

Received for publication: 30 December 2022.
Accepted for publication: 7 February 2023.

Italian Journal of Food Safety 2023; 12:11135
doi:10.4081/ijfs.2023.11135

Introduction

Foodborne diseases have constituted a growing public health concern worldwide. Despite being among the most widely consumed meat, chicken is also recognized as an important reservoir and disseminator of *Salmonella* spp. (Parvin *et al*., 2020). Bacteria of the genus *Salmonella* are found in the gastrointestinal tract of poultry and often contaminate carcasses during slaughter or processing; therefore, they can be transmitted to humans directly through contact with chickens or indirectly by consumption of contaminated poultry meat (Hoque *et al*., 2019). Indeed, the role played by poultry in the epidemiology of human *Salmonella* infections is recognized to be due to the development of intensive poultry production worldwide. The prevention of foodborne salmonellosis is currently a top priority for poultry producers, in the interest of public health. Moreover, the emergence of antimicrobial resistance (AMR) in *Salmonella* spp. has led to an increased hazard for human health because of the increased mortality of the infected patients (Sin *et al*. 2020). Several works show that *Salmonella* isolated from poultry meat can exhibit resistance to a wide range of antibiotic molecules (Hassena *et al*., 2019; Gambino *et al*. 2022). Overuse or abuse of antimicrobials in poultry production is an important factor that contributes to the emergence, selection, and spread of AMR in *Salmonella* spp. among the poultry population (Holmes *et al*., 2016). Antimicrobials are extensively used in poultry farming to treat and prevent poultry diseases as well as improve growth performance; this potentially results in the spreading of MDR *Salmonella* spp. (Page and Gautier, 2012). The emergence of multidrug resistant (MDR) *Salmonella* has been a growing public health concern around the world over the last 10 years (Hindermann *et al*., 2017). MDR *Salmonella* isolated from poultry meat were found to harbor β-lactams, aminoglycosides, tetracyclines, and sulfamides AMR genes, posing a significant threat to public health. Moreover, the emergence and dissemination of *Salmonella* spp. resistant to fluoroquinolones and third-generation cephalosporins limits the treatment with the currently available antibiotics such as carbapenems (Hindermann *et al*., 2017). The aim of the present study was to evaluate the prevalence and AMR profile of *Salmonella* spp. isolated from poultry meat samples, examined over three years, from 2019 to 2021.

Materials and Methods

Sampling collection, *Salmonella* detection and serotyping

From January 2019 to December 2021, a total of 145 poultry samples (119 chicken meat and 26 chicken carcasses) were analyzed. The samples, collected in the context of official controls provided by EU Regulation No 2073/2005 (European Commission, 2005), were tested for *Salmonella* according to ISO 6579-1 (2017). For pre-enrichment, 25 g of each sample, meat, and

neck skin of the carcasses respectively, were homogenized with 225 mL of buffered peptone water and incubated at 37°C for 18 to 24 hours. An aliquot of 0.1 mL of the culture was then mixed with 10 mL of rappaport-vassiliadis soy broth and incubated at 37°C for 24 hours. A loopful of culture broth was then streaked onto xylose lysine desoxycholate agar and incubated at 37°C for 24 hours. Typical colonies of *Salmonella* spp., with a black center and a slightly transparent zone of a reddish color, were streaked onto nutrient agar and incubated at 37°C for 24 hours. Finally, their characterization was performed by gram staining and biochemical assays, which included catalase, oxidase, indole, methyl red test, Voges-Proskauer test, and fermentation test using triple sugar iron agar or following the API 20E identification system (BioMerieux, Marcy l'Etoile, France). Serotyping was further performed using a standard agglutination test with anti-O and anti-H antisera. *Salmonella* spp. isolates were further serotyped by direct slide agglutination, using specific antisera (Statens Serum Institut, Copenhagen, Denmark), according to the Kaufmann-White-Le Minor scheme.

Antibiotic susceptibility test determination

The antibiotic susceptibility profile was determined using the Kirby-Bauer method, testing 17 antibiotics belonging to 6 different classes: kanamycin (K, 30μg), gentamicin (CN, 10μg), streptomycin (S, 10μg), tobramycin (TOB, 10μg), ampicillin (AMP, 10μg), amoxicillin/clavulanic acid (AMC, 30μg), cefotaxime (CTX, 30μg), ceftriaxone (CRO, 30μg), ceftazidime (CAZ, 30μg), imipenem (IMP, 10μg), nalidixic acid (NA, 30μg), ciprofloxacin (CIP, 5μg), enrofloxacin (ENR, 5μg), levofloxacin (LEV, 5μg), sulfamethoxazole/trimethoprim (SXT, 25μg), tetracycline (TE, 30μg) and chloramphenicol (C, 30μg). Interpretation of inhibition zones and classification of isolates as susceptible (S), intermediate (I), or resistant (R) was done according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2021). As per CLSI guidelines, isolates resistant to antibiotic molecules belonging to at least three different classes were reported as MDR (CLSI, 2021).

Results

Isolation and serotyping results

Salmonella isolates were recovered from 28% (confidence interval 95%=20.3-34.9) of the samples examined (40/145) among which 13% (6/45) in 2019, 22% (9/41) in 2020 and 42% (25/59) in 2021 (Table 1).

Serotyping revealed *S. Infantis* as the predominant serovar, accounting for 80% (32/40) of isolated strains. In particular, *S. Infantis* accounted for 67% of isolates (4/6) in 2019, 78% (7/9) in 2020, and 84% (21/25) in 2021. The 8/40 (20%) remaining serovars identified (Table 1) were 1/40 *S. Typhimurium* (2.5%), 1/40 *S. Newport* (2.5%), 4/40 *S. Kentucky* (10%), and 2/40 *S. Agona* (5%).

Antibiotic susceptibility results

Eighty-seven percent (35/40) of *Salmonella* isolates showed resistance to at least one antibiotic, with the following resistance percentages (Figure 1): K 47.5% (19/40), CN 5% (2/40), NA 72.5% (29/40), AMP 67% (27/40), TE 72.5% (29/40), S 30% (12/40), TOB 10% (4/40), AMC 15% (6/40), CTX 42.5% (17/40), CAZ 10% (4/40), CRO 25% (10/40), LEV 5% (2/40), SXT 67.5% (27/40). All strains were sensitive to IPM, CIP, C, and ENR. 32/40 strains (80%) were MDR, among which 3/32 (9%) were resistant to 3 different classes of antibiotics, 20/32 (64%) to 4 different classes of antibiotics, and 9/32 (27%) to 5 different classes of antibiotics (Table 2).

Discussion

Salmonella spp. is among the most frequent causes of foodborne illnesses and the growing presence of MDR strains is a further cause of concern (Franco *et al*., 2015; Hassena *et al*., 2019; Parvin *et al*., 2020; Proietti *et al*., 2020; Peruzy *et al*., 2020; Gargano *et al*., 2021; Lauteri *et al*., 2022). Between 2019 and 2021, 40 strains of *Salmonella* spp. were isolated from 145 samples of poultry meat, analyzed in the context of European Community Legislation, and their prevalence and antibiotic susceptibility profile was evaluated. As highlighted in other studies carried out in Italy (Franco *et al*., 2015; Proietti *et al*., 2020; Peruzy

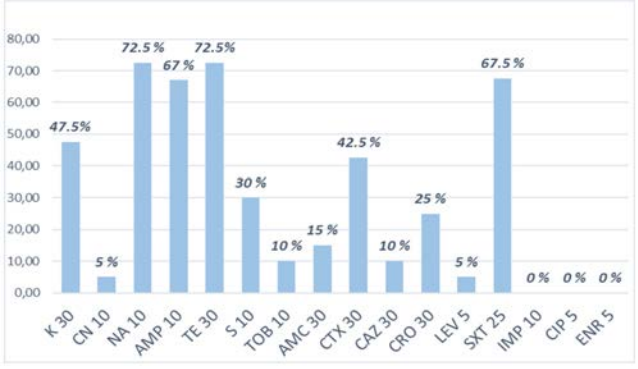

Figure 1. Phenotypic resistance (%).

Table 1. Prevalence of *Salmonella* serotypes.

Year	N° of samples analyzed	N° *Salmonella* spp. isolated (%)	Serovar (%)
2019	45	6 (13)	4 *S. Infantis* (66) 1 *S. Typhimurium (17)* *1* *S. Newport* (17)
2020	41	9 (22)	7 *S. Infantis* (78) 2 *S. Kentucky* (22)
2021	59	25 (42)	21 *S. Infantis* (84) 2 *S. Agona* (8) 2 *S. Kentucky* (8)

Table 2. Multidrug resistant pattern of the Salmonella isolates.

Serovar	Resistance phenotype	Resistance pattern (n. antibiotics classes)
S. Infantis	AMP, CTX, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	TE	Tetracyclines (1)
S. Infantis	K, AMP, CTX, NA, TE	Aminoglycosides, β-lactams, quinolones and tetracyclines (4)
S. Infantis	K, AMP, CTX, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	NA, SXT, TE	Quinolones, sulfonamides, tetracyclines (3)
S. Infantis	K, NA, SXT, TE	Aminoglycosides, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	S, AMP, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	S, NA, SXT, TE	Aminoglycosides, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, S, NA, SXT, TE	Aminoglycosides, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, S, NA, SXT, TE	Aminoglycosides, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, ST, AMP, CTX, NA, LEV, C	Aminoglycosides, β-lactams, quinolones, phenicoles (4)
S. Infantis	K, S, AMP, CTX, NA, LEV, C	Aminoglycosides, β-lactams, quinolones, phenicoles (4)
S. Infantis	K, SXT, TE	Quinolones, sulfonamides, tetracyclines (3)
S. Infantis	K, AMP, S, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	K, TOB, AMP, CTX, CRO, NA, SXT	Aminoglycosides, β-lactams, quinolones, sulfonamides (4)
S. Infantis	AMP, CTX, CRO, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	S, AMP, NA, TE	Aminoglycosides, β-lactams, quinolones and tetracyclines (4)
S. Infantis	K, CN, TOB, AMP, AMC, CTX, CRO, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	K, AMP, AMC, CTX, CRO, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, AMP, AMC, CTX, CRO, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	AMP, CTX, CAZ, CRO, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	AMP	β-lactams (1)
S. Infantis	AMP, AMC, CRO, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	TOB, AMP, AMC, CTX, CRO, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	AMP, AMC, CTX, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	CN, AMP, CTX, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	K, TOB, AMP, CTX, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	S, AMP, CTX, CAZ, NA, SXT, TE, C	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Infantis	AMP, CTX, CAZ, CRO, NA, SXT, TE	β-lactams, quinolones, sulfonamides, tetracyclines (4)
S. Infantis	K, S, AMP, NA, TE	Aminoglycosides, β-lactams, quinolones and tetracyclines (4)
S. Infantis	K, AMP, NA, TE	Aminoglycosides, β-lactams, quinolones and tetracyclines (4)
S. Newport	K, AMP, SXT, TE	Aminoglycosides, β-lactams, sulfonamides, tetracyclines (4)
S. Agona	S, AMP, SXT	Aminoglycosides, β-lactams, sulfonamides (3)
S. Agona	-	(0)
S. Kentucky	S, AMP, CAZ, CTX, CRO, NA, SXT, TE	Aminoglycosides, β-lactams, quinolones, sulfonamides, tetracyclines (5)
S. Kentucky	-	(0)
S. Kentucky	-	(0)
S. Kentucky	-	(0)
S. Typhimurium	-	(0)

AMP, ampicillin; CTX, cefotaxime; NA, nalidixic acid; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline; K, kanamycin; S, streptomycin; LEV, levofloxacin; C, chloramphenicol; TOB, tobramycin; CRO, ceftriaxone; AMC, amoxicillin/clavulanic acid; CAZ, ceftazidime; CN, Gentamicin.

et al., 2020), our data further confirm the key role played by poultry meat as a relevant source of *Salmonella* in Italy. Moreover, we observed a relevant increase in *Salmonella* prevalence along the three-year period taken into consideration, from 13% in 2019 to 42% in 2021. Furthermore, as per our data, *S*. *Infantis* (80%) turned out to be the prevalent serovar, with a relevant increase in isolation percentages, from 67% in 2019 to 84% in 2021.

This increase has been highlighted by other authors in other Italian and international studies, as regards both poultry meat and foods in general (Franco *et al*., 2015; Hindermann *et al*., 2017; Hassena *et al*., 2019; Parvin *et al*., 2020; Proietti *et al*., 2020; Peruzy *et al*., 2020; Gargano *et al*., 2021; Gambino *et al*., 2022; Lauteri *et al*., 2022). We revealed a high rate of AMR strains (80%). Among these, the highest rate of resistance was found against tetracyclines (72.5%), similar to what was reported in other studies carried out in Italy. In fact, Proietti *et al*. (2020) reported a 96% resistance to tetracyclines for *S*. *Infantis* from poultry meat. These values reflect a general spread of *Salmonella* strains resistant to tetracycline, which is also observed in other foods, even though with lower percentages compared to poultry. In fact, Peruzy *et al*. (2020) reported an 86% resistance to tetracycline in *Salmonella* isolated from poultry meat; this is due to a general abuse of these antibiotics over the past decades, especially in farms (EFSA, 2021; Gargano *et al*., 2021), even though in 2006 the European Union imposed a ban on the non-therapeutic use of antibiotics of human relevance, such as tetracyclines, in animal feed. This is an attempt to counteract the increasing spread of tetracycline antimicrobial resistance. Despite this, the spread of *Salmonella* spp. resistant to these drugs and isolated in poultry meat remains a relevant issue, especially from a One-Health perspective (EUCAST, 2017; Zhao *et al*., 2020).

Referring to fluoroquinolones, high resistance was observed only against nalidixic acid (72.5%), while low percentages of resistance were recorded against levofloxacin and none of the strains was resistant to enrofloxacin and ciprofloxacin. Our results are consistent with other authors' observations of this significant class of antibiotics in poultry in Italy (Peruzy *et al*., 2020; Proietti *et al*., 2020). In agreement with Peruzy *et al*. (2020) and Proietti *et al*. (2020), worryingly, we also observed a high percentage of resistance to sulfonamides (67.5%), a class of antibiotics commonly used against severe *Salmonella* infections in humans. None of the strains tested was resistant to imipenem, confirming the high sensitivity of *Salmonella* spp., isolated in foods and in particular in poultry meat, to this class of antimicrobials (Hindermann *at al*., 2017; Proietti *et al*., 2020; Gambino *et al*., 2022). Like others, we also observed low or moderate resistance to third-generation cephalosporins (10% ceftazidime, 25% ceftriaxone, and 42% cefotaxime) and the above percentages appear to be in line with those reported in other studies conducted in Italy, referring to foods in general and in particular to poultry meat (Peruzy *et al*., 2020; Proietti *et al*., 2020; Lauteri *et al*., 2022). The high percentages of MDR strains observed represent alarming data, not only considering the real risk that consumers may run into if they were infected by an MDR strain but also because many of these strains were resistant to classes of antibiotics commonly used in human medicine such as β-lactams (Hassena *et al*., 2019). The most frequent MDR profiles were: resistance to β-lactams, in particular to third-generation cephalosporins, fluoroquinolones, sulfonamides, and tetracyclines.

The results obtained should be taken into great account, especially considering that third-generation cephalosporins and fluoroquinolones are included among the antimicrobials of critical importance and that sulfonamides are considered the antimicrobials of choice for the treatment of human salmonellosis (European Medicine Agency, 2020).

Conclusions

In conclusion, the present study confirmed the circulation of AMR and especially MDR strains among zoonotic bacteria in poultry meat, such as *Salmonella* spp. This evidence presents a well-known emerging risk, especially in the One-Health holistic approach. Furthermore, our results highlighted the predominance, in these food matrices, of the serovar *S*. *Infantis*, which is the fourth most frequent cause of human salmonellosis in Europe and referring to which recent studies show the emergence of MDR clones, highlighting the importance of continuous monitoring.

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