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The poultry chain in Sardinia (Italy): monitoring of *Salmonella* contamination and antibiotic resistance profiles

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Abstract
This study assessed the occurrence of *Salmonella* along the poultry production chain in Sardinia and characterized the isolates by serotyping and antimicrobial resistance testing, providing preliminary insights into contamination routes and consumer risk. Sampling was carried out in four sessions during 2024 at one farm, one slaughterhouse, and at retail, covering different stages of the production chain: 32 farm environmental samples, 48 slaughterhouse samples, and 92 retail chicken meat samples (skin-on cuts, skinless cuts, and meat preparations) were collected overall. A total of 172 samples were tested, and *Salmonella* isolates were serotyped and subjected to antimicrobial susceptibility testing. *Salmonella* was not detected in any of the samples collected at either the farm or the slaughterhouse. The pathogen was detected in retail meat, with 21 positives out of 92 (22.8% prevalence). The highest prevalence was detected in samples of meat cuts with skin (11/40, 27.5%), followed by poultry meat preparations (3/12, 25%) and meat cuts without skin (7/40, 17.5%). Of the 21 isolates, 20 (95%) were identified as *Salmonella enterica* serovar Infantis, while one isolate belonged to the serovar Thompson. *S.* Thompson isolate was susceptible to all tested antibiotics, whereas all *S.* Infantis isolates (100%) showed resistance to agents from at least three classes (quinolones, sulfonamides, and tetracyclines), indicating a high level of multidrug resistance. Moreover, resistance to cefoxitin, colistin, azithromycin, and tigecycline was observed. Our findings highlight the role of the poultry production chain as an important reservoir of multidrug-resistant *Salmonella* Infantis and underline the need for strict control measures. Particular attention should be given to the retail stage and consumer handling, which remain critical points for the prevention of foodborne pathogen transmission.

Introduction

Salmonella remains one of the leading causes of foodborne illness worldwide and poses a major public-health burden, with foodborne transmission accounting for most human cases. Foods of animal origin are the main vehicles and, in 2023, the leading food vehicles for *Salmonella* in the EU were eggs, mixed foods, and broiler (*Gallus gallus*) meat (EFSA and ECDC, 2024). Notably, two of these vehicles originate from poultry, underscoring the central role of poultry in *Salmonella* epidemiology. Over the past two decades, the European Union (EU) has implemented comprehensive *Salmonella* control programs initiated by Regulation (EC) 2160/2003 (European Commission, 2003). These require Member States to monitor *Salmonella* in poultry flocks and carcasses and to set national reduction targets. These control programs have sharply reduced *Salmonella* in poultry and contributed to lowering the number of human cases; recent surveillance confirms the trend, with a 2023 average flock prevalence of 0.19% in broilers (EFSA and ECDC, 2024). Italy's National Control Plan for Salmonellosis in Poultry (PNCS) enforces EU rules through routine farm testing, official inspections, and strict corrective actions. Reports indicate that prevalence targets are consistently met and the situation remains stable and favourable (Ministero della Salute, 2025a). Nevertheless, broiler meat remains among the foods most frequently implicated in outbreaks: the latest EU zoonoses report identifies broiler meat as one of the main food vehicles associated with *Salmonella* and, in 2023, EFSA and ECDC described multi-country outbreaks linked to chicken meat and products (EFSA and ECDC, 2024). In fact, poultry populations are often colonized by *Salmonella* without observable symptoms. The pathogen spreads through horizontal and vertical routes, enabling persistence in flocks (Barrow *et al*., 2012; Cosby *et al*., 2015). During slaughter and meat processing, the intestinal carriage of *Salmonella* represents a critical hazard. Evisceration, defeathering and handling can facilitate the transfer of the pathogen to the carcass surface, where cross-contamination can occur. Once present on raw meat, *Salmonella* can survive under refrigeration and may persist throughout the distribution chain, ultimately reaching the consumer. Therefore, poultry meat represents a major source of *Salmonella* transmission to humans and contributes significantly to the burden of salmonellosis (EFSA, 2019). Effective *Salmonella* monitoring in poultry production requires sampling at multiple stages of the food chain. Farms and slaughterhouses are critical sampling points

for identifying contamination sources, whereas retail sampling provides data on the prevalence of *Salmonella* in consumer-ready products (EFSA, 2019). Together, these approaches allow an assessment of the overall microbiological quality of poultry meat available to consumers.
Various *Salmonella* serovars were observed in broiler production and, as for EU, in 2023, *Salmonella enterica* serovar Infantis was by far the dominant serovar in the 'broilers-broiler meat' source and ranked among the top four serovars across food-animal sources (EFSA and ECDC, 2024, 2025). In Italy, the serotypes most frequently found in broilers were *S.* Infantis, *S.* Enteritidis, *S.* Bredeney, *S.* Thompson and *S.* Hadar (EFSA, 2019; EFSA and ECDC, 2024).
In addition to its pathogenic potential, *Salmonella* is increasingly associated with antimicrobial resistance (AMR), which complicates treatment options and raises concerns about the spread of resistant strains through the food chain. Broiler-derived strains often display high resistance to multiple drug classes and several poultry-associated serotypes have been implicated in the global spread of multidrug-resistant (MDR) clones transmissible to humans (Antunes *et al*., 2016; EFSA and ECDC, 2025).
To our knowledge, no previous studies have investigated *Salmonella* distribution in poultry or poultry meat in Sardinia. This study represents an exploratory investigation of *Salmonella* occurrence along the poultry meat production chain in Sardinia, from farm to retail, aimed at providing an initial overview of *Salmonella* occurrence and antimicrobial resistance in the Sardinian poultry sector and an assessment of the potential risk to consumers at the point of purchase.

Materials and Methods

A total of 172 samples were collected during 2024 over four sampling sessions conducted throughout the year (January, May, July and October) and covering different stages of the production chain: farm, slaughter and at the retail level. Environmental sampling at the farm was conducted on the same day as slaughter, in the housing environment of the birds scheduled for slaughter that day. The same broilers were then followed through subsequent stages of the production chain and the following samples were collected: i) transport crate swabs from the same flock; and ii) neck skin samples collected from the same birds after slaughter.

Sampling at the farm

A broiler farm was selected for sampling. The farm consisted of four poultry houses and an integrated slaughterhouse, with a total of about 4,000 birds in production. The broilers reached about 3.0-3.5 kg live weight at 45-60 days and were reared in the same houses from the day of arrival (day 1) until slaughter (day 45-60). The farm applied biosecurity measures aimed at preventing the introduction and spread of *Salmonella*. These included closed poultry houses with no outdoor access for birds, restricted access to authorized personnel with mandatory changes of clothing and footwear, cleaning and disinfection of poultry houses and equipment between production cycles, and the implementation of rodent and insect control programs. During the four sampling times, a total of 32 environmental samples were collected from various locations at the same broiler house, including: feed (n=8), nipple drinkers (n=8), litter (n=8) and corridor floor (n=8). In detail, during each sampling, at least 100 g of feed was collected from the feeding troughs in a sterile plastic bag. The surfaces of the nipple drinkers were sampled with a sterile sponge pre-moistened with 10 mL of sterile buffered peptone water (BPW, 3M Health Care, Milan). At least 100 g of litter was collected in a sterile plastic bag. Sampling of the corridor floors was conducted using sterile boot swabs (Reg. EU 200/2012, Reg EU 2019/268; Technical Service Consultants Ltd, Heywood, UK) (European Commission, 2012, 2019), by walking over the floor, covering at least 20 m^2, including corners and areas around the feed and water supply. This method allows the collection of a mixture of dust, fecal material and other organic debris, providing a composite sample representative of the environmental contamination within the poultry house.

Sampling at the slaughterhouse

The slaughterhouse was directly connected to the broiler farm and processed only animals reared on-site, without receiving poultry from external farms. During the four sampling times at the slaughterhouse, 48 samples were collected. Specifically, 12 samples were taken from the surface of the cages used for animal transport (80×60×50 cm, holding approximately 12-14 broilers) using the sponge method in a sampling area of about 2 m². Moreover, 36 neck skin samples were collected after slaughter, following the Regulation (EC) 2073/2005 (European Commission, 2005).

Sampling at retail stores

Since it was not possible to obtain products originating from the farm and slaughterhouse after sectioning, separate sampling was conducted, and complementary samples were purchased from retail stores. Overall, 92 meat samples were purchased from six retail stores. Each sample corresponded to a single retail package, consisting of expanded polystyrene trays sealed with plastic film. The samples included 40 samples of portioned meat with skin, 40 samples of portioned meat without skin, and 12 samples of meat-based preparations.

Microbiological analysis and antimicrobial susceptibility testing

In total, 172 samples were analyzed for the detection of *Salmonella* spp., according to ISO 6579-1:2017 (ISO, 2017). For species identification, the isolates were tested by mass spectrometry, using matrix-assisted laser desorption/ionization-time of flight. Phenotypic serotyping of *Salmonella* isolates was conducted using commercial polyclonal O and H antisera (Statens Serum Institut, Copenhagen, Denmark) by the slide agglutination method according to the White-Kauffmann-Le Minor scheme (ISO, 2014). When necessary, phase inversion was performed following standard procedures to identify both flagellar phases. The detected antigenic structures and the serovars names were expressed according to the White-Kauffmann-Le Minor formula.

For each positive sample, one *Salmonella* isolate was selected as representative of the *Salmonella* strain and subjected to antimicrobial susceptibility testing. Antimicrobial susceptibility was tested according to the EUCAST guidelines (2025) using the broth microdilution method with the Sensititre automated system (Thermo Fisher Scientific, Monza, Italy) according to the producer's instructions. The panel of antimicrobials tested was selected according to the specifications of Commission Implementing Decision (EU) 2020/1729, ensuring harmonization with the European monitoring standards. The EUVSEC3 and EUVSEC2 AST plates (Thermo Fisher Scientific, Monza, Italy) were used for testing. Specifically, the EUVSEC3 plate was employed for general antimicrobial susceptibility profiling in *Enterobacterales*, while the EUVSEC2 plate was specifically used for the detection of extended-spectrum β-lactamase (ESBL) production, in accordance with the manufacturer's instructions and EUCAST guidelines. Isolates were classified as MDR if they were resistant to at least one agent in three or more antimicrobial classes (Magiorakos *et al.*, 2012).

Results and Discussion

Salmonella was not detected in any of the samples collected at either the farm or the slaughterhouse. Our findings differ from the national data showing widespread *Salmonella* detection. The Italian monitoring programs confirmed the occurrence of the pathogen in samples collected at poultry farms (Enter-Vet, 2024), and positive samples have also been reported at slaughterhouses in Italy, with a prevalence ranging from 8.7 to 51.85% (Peruzy *et al.*, 2022; Rosamilia *et al.*, 2025), and in the Member States (EFSA and ECDC, 2024). The absence of *Salmonella* in farm and slaughterhouse samples suggests effective control measures. The absence of detection might be partly explained by the strict biosecurity measures implemented on the farm, which included restricted access to authorized personnel, mandatory hygiene procedures for staff, regular cleaning and disinfection, and effective rodent and insect control programs. Such measures were consistent with EU *Salmonella* control recommendations (EFSA, 2019) and may have contributed to preventing environmental contamination. However, a potential drawback of our study is that sampling was limited to one broiler

farm, the limited number of sampled production sites represents a constraint for the generalization of the findings. This limitation mirrors the structure of Sardinia's broiler industry, which is composed of a small number of holdings (fewer than 20 in total) and an overall population of approximately 120,000 birds. Only 16 farms keep more than 250 animals, confirming the region's relatively modest level of intensive poultry production (Ministero della Salute, 2025b).

Salmonella was detected in 21 of 92 retail meat samples (22.8%). Detection exclusively at retail is plausible due to post-slaughter factors that can introduce or amplify *Salmonella* downstream of the slaughterhouse, including (i) cutting/portioning and handling practices, (ii) equipment and surface hygiene (*e.g*., cutting boards, knives, work tables), (iii) packaging/processing environments and (iv) temperature management during distribution and sale (EFSA, 2011; Thames *et al*., 2020). In addition, the frequent mixing of meat from different batches and slaughterhouses during cutting, processing, and distribution (events that are neither predictable nor reproducible) further weakens the correlation between hygiene at the farm and slaughterhouse levels and contamination detected at retail. This interpretation is consistent with international guidance (Codex CXG 78-2011) and with studies documenting cross-contamination from raw poultry to utensils, surfaces, and foods, persistence on processing equipment, and growth under temperature-abuse scenarios (Obe *et al*., 2020; Noviyanti *et al*., 2024). The *Salmonella* prevalence in our samples was highest in meat cuts with skin (11/40, 27.5%), followed by poultry preparations (3/12, 25%) and cuts without skin (7/40, 17.5%). These findings align with reports of a high burden of contamination in retail products in Italy and Europe. In Italy, a 2021-2023 study found *Salmonella* in 32.6% of retail chicken products (Di Taranto *et al.*, 2025). At the EU level in 2023, "fresh poultry meat" showed the highest contamination ratio at the distribution stage (9.0%), followed by "meat products made from poultry meat intended to be eaten cooked" (8.1%) (EFSA and ECDC, 2024). A meta-analysis of 78 European studies across 21 countries estimated an overall 7.1% prevalence in retail poultry meat (Gonçalves-Tenório *et al*., 2018).

Data directly comparing skin-on versus skinless products at retail are scarce and sometimes inconsistent. For example, a survey by the Food Safety Authority of Ireland reported a *Salmonella* prevalence of 0.4% (1/225) in skin-on products compared with 1.8% (7/379) in skinless products, although the low number of positive samples did not allow any statistically reliable interpretation of a causal relationship (FSAI, 2016). In contrast, studies from Spain and the USA reported markedly higher *Salmonella* prevalence in chicken parts with skin (Capita *et al*., 2007; Guran *et al*., 2017; USDA, 2024). Overall, multiple lines of evidence support the role of skin as a surface likely to harbour or mask *Salmonella* contamination. This conclusion is also supported by experimental inoculation studies, which have shown that once *Salmonella* Typhimurium adheres to the poultry skin, it is difficult to eliminate during processing (Yang *et al.*, 2001).

Regarding serotyping results, 20 of the 21 isolates (95%) were identified as *Salmonella enterica* serovar Infantis (antigenic formula 6,7:r:1,5), while one isolate belonged to the serovar Thompson (antigenic formula 6,7:k:1,5). *S.* Infantis has been consistently reported as the fourth most frequent serovar from humans in Europe and is by far the most common serovar in the 'broilers' animal-food source (EFSA and ECDC, 2024). Since 2010, the prevalence of *S.* Infantis has increased markedly and from 2014 onwards it has become the leading serovar isolated from broilers in many European countries (Montoro-Dasi *et al.*, 2023; EFSA and ECDC, 2024). Several hypotheses have been proposed to explain this trend. One likely factor is the implementation of vaccination and control programs specifically targeting *S.* Enteritidis and *S.* Typhimurium. Commission Regulation (EC) 1086/2011, amending Regulation (EC) 2073/2005, set microbiological safety criteria for fresh poultry meat restricted to these two serovars (European Commission, 2011), so that the targeted control of *S.* Enteritidis and *S.* Typhimurium may have allowed the creation of an ecological niche favourable to the diffusion of *S.* Infantis in the broiler chain (Montoro-Dasi *et al.,* 2023). Moreover, *S.* Infantis exhibits several genetic traits that enhance its epidemiological success, including the acquisition and dissemination of antimicrobial resistance, tolerance to heavy metals, and strong biofilm-forming capacity (Alba *et al.,* 2020).

S. Thompson, on the other hand, is not among the main serovars associated with the poultry production chain in Europe. It has been sporadically detected in broilers and poultry products, although with much low prevalence (EFSA and ECDC, 2024).
In humans, *S.* Infantis and *S.* Thompson typically cause self-limiting gastroenteritis or mild colitis and are less invasive than *S.* Typhimurium. Extraintestinal disease is uncommon and usually confined to immunocompromised individuals (Nygård *et al*., 2008; Aviv *et al*., 2019). Nevertheless, they are recognized as emerging serovars, linked to global spread and multidrug resistance, posing a growing public-health concern (EFSA and ECDC, 2025).
The AMR analysis results of our investigation are in support of this observation. In this regard, the *S.* Thompson isolate was susceptible to all the antimicrobials tested, whereas all *S.* Infantis isolates (100%) were resistant to ciprofloxacin, nalidixic acid, sulfamethoxazole, and tetracycline. Being all *S.* Infantis isolates resistant to at least three antimicrobial classes, they can be classified as MDR (Magiorakos *et al.,* 2012). In addition, resistance was observed for trimethoprim (16/20, 80%), cefoxitin (7/20, 35%), tigecycline (6/20, 30%), azithromycin, and colistin (1/20, 5%). A heatmap showing AMR results in *S.* Infantis isolates is presented in Figure 1. Photographs of the test results of an isolate have been incorporated (Figure 2).
These concerning results are in line with European patterns. Resistance to ciprofloxacin, nalidixic acid, sulfamethoxazole and tetracycline are typical of the clone of *S.* Infantis prevalent in broilers in Europe (Alba *et al*., 2020) and this profile is further confirmed by recent European surveillance data, which reported that, in 2023, *S.* Infantis isolates from broilers displayed the highest resistance levels to ciprofloxacin and nalidixic acid among *Salmonella* serovars (EFSA and ECDC, 2025).
The widespread dissemination of MDR observed in this study highlights a concerning scenario, particularly given the high frequency of resistance to antimicrobials that are considered essential for both veterinary and human medicine. Resistance to colistin, azithromycin and tigecycline is especially alarming, since these agents are classified as critically important (WHO, 2019). This finding is consistent with international surveillance data (EFSA and ECDC, 2025). The resistance to cefoxitin in 35% of isolates is another noteworthy result. Although cefoxitin resistance is not definitive evidence of AmpC β-lactamase production, it is frequently used as a phenotypic marker suggestive of this mechanism (Polsfuss *et al.*, 2011). The possible presence of AmpC-producing strains is of clinical and epidemiological concern and further molecular analyses are needed to confirm the presence of AmpC genes. Notably, none of the isolates displayed phenotypic profiles consistent with ESBL or carbapenemase production. This result suggests that, although multidrug resistance is widespread, the distribution of ESBL- or carbapenemase-producing strains may still be limited in the population analyzed. This contrasts with the increasing reports of ESBL-producing strains detected along the food chain of broiler meat production in Italy and Europe (Casagrande Proietti *et al*., 2020; EFSA and ECDC, 2025). Overall, the results highlight the relevance of surveillance and integrated approaches to understanding resistance dynamics and supporting effective control strategies in the broiler meat production chain.

Conclusions

The results of our investigation confirm that *Salmonella* Infantis is increasingly spreading in the poultry industry. The detection of *Salmonella* spp. in poultry meat at retail raises concern due to the risk of cross-contamination during handling and preparation. This risk is further exacerbated by the frequent multidrug resistance of the isolates, including resistance to critically important antimicrobials, thereby amplifying the potential public-health impact.
Our findings confirm the role of the poultry production chain, including poultry meat products, as an important reservoir of epidemic MDR clones and resistance genes that can spread to humans. Applying good manufacturing practices and strict hygiene measures during food preparation is fundamental to reducing the risk of foodborne outbreaks. The retail stage remains critical, as it represents the final step of the production chain. Moreover, a crucial challenge remains: The weakest link in the chain is represented by the consumer, who ultimately bears responsibility for implementing

proper practices to control the risk at the final stage of the food chain. It is therefore essential to provide consumers with proper information to cook these products safely, for instance, by including on the label clear cooking instructions tailored to the product's nature and composition, such as the recommendation to consume them only after thorough and complete cooking, reaching at least 75°C at the core of the product, as shown in DGISAN (2016) Note, prot.no. 1038.

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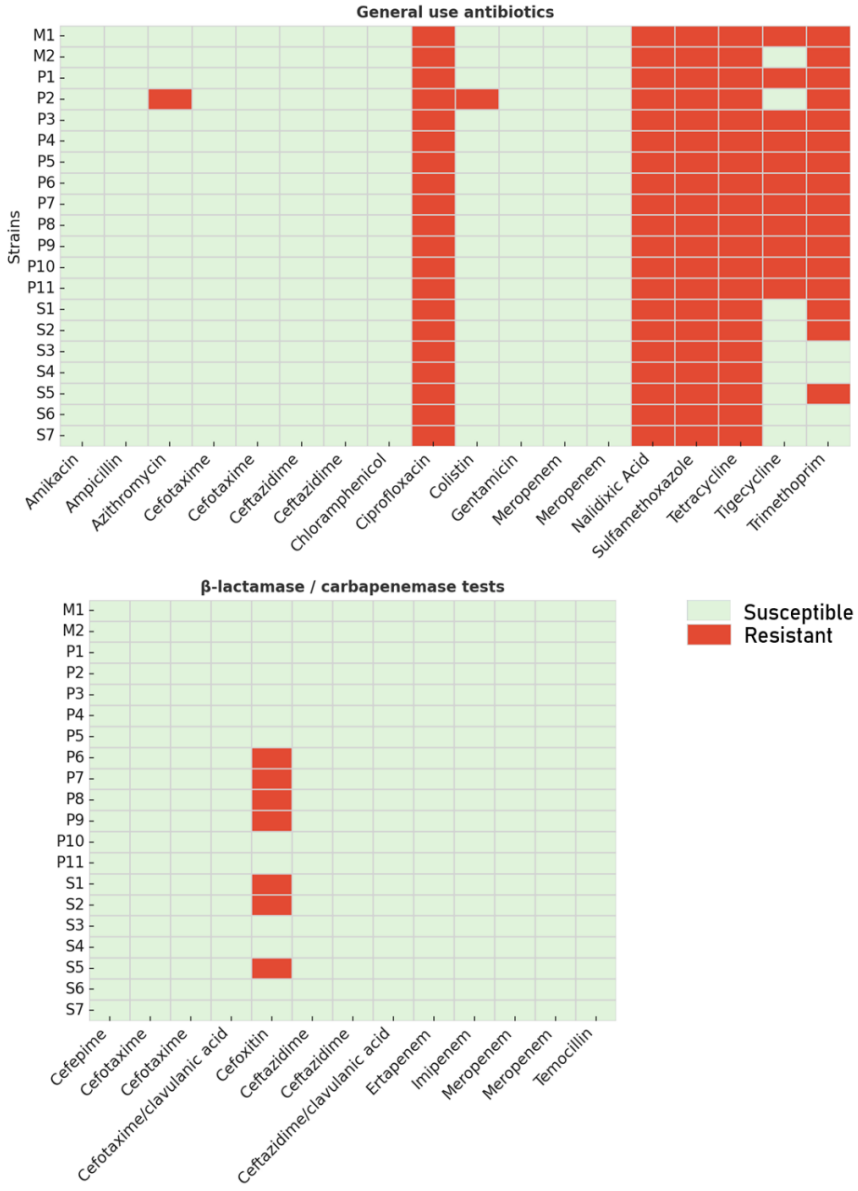

Figure 1. Heatmap showing antimicrobial resistance in *S.* Infantis isolates. M1-2: isolates collected from meat preparations samples, P1-11: isolates collected from meat cuts with skin samples, S:1-7: isolates collected from meat cuts without skin samples.

Figure 2. Test results of the Sensititre automated system (Thermo Fisher Scientific, Monza, Italy) for an ESBL-producing *Salmonella* isolate.