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Evaluation of Antimicrobial resistance on Salmonella strains isolated from food, animal and human samples, between 2017-2021 years in Southern Italy

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Abstract: *Salmonella enterica* is one of the most common cause of foodborne infection in the world and the first one in Italy. Italy is involved with the other EU Member States in the surveillance of the antimicrobial resistance of *Salmonella* on a large scale, but is a country where typical food production affects importantly the population diet. Be aware of the reality of the Puglia and Basilicata have provided a more focused point of view on the territory and anticipate what the data reported in future Italian reports could be. The antimicrobial resistance was detected using MIC detection method, with EUVSEC® plates, on the strains collected between 2017-2021 years. The results of serotyping showed that the *S. Infantis* is the serovar that has increased the most over time in veterinary samples, while *S. Tyhimurium* and its monophasic variant are the most isolated in human samples. The results of antimicrobial resistance study leading to agreement with European data: high resistance versus quinolones, tetracyclines, ampicillin, trimethoprim; low resistance versus colistin and cephems. The significant exception was that all strains were resistant to Sulphametoxazolo. The presence of MDRs, 84,95% on veterinary and 77,40% of human strains, which often include critically important antibiotics, is a sign that there is still much to study and do on managing the use of antibiotics.

Keywords: Salmonella; Italy; S. Kentucky; S. Typhimurium; S. Monophasic variant of Typhimurium; S. Derby; S. Infantis; Antimicrobial resistance; MDR

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1. Introduction

Salmonella enterica is the second most commonly reported zoonotic agent in the European Union, after *Campylobacter* spp.. Otherwise, *Salmonella* is the leading cause of foodborne disease in Italy with 3268 cases reported in 2019, instead of 1633 cases of campylobacteriosis (1). A recent review inferred that multidrug resistant non typhoidal *Salmonella* may have more serious human health implication compared to those of pan susceptible strains (1, 2). Apulia and Basilicata regions cover almost 10% of the territory and 7,6% of the population of Italy, representing a very important part of the national territory (Eurostat site http://dati-censimentipermanenti.istat.it/Index.aspx# 21/12/2021). The Food Microbiology Unit of the Istituto Zooprofilattico Sperimentale di Puglia e Basilicata (IZSPB) collects *Salmonella* strains isolated in the territory of the two regions, according to national surveillance and monitoring plans, as well as for research and collaboration with universities and other research bodies, thus playing a fundamental role in the epidemiological

surveillance. In collaboration whit the Department of Basic Medical Sciences, Neurosciences and Sense Organs of Bari University and the ENTER-NET local network, the aim of this study was to evaluate the antimicrobial resistance of *Salmonella* serotypes isolated from human, animal and food matrices in Apulia and Basilicata regions between 2017-2021. In particular, the study of antimicrobial resistance focused on the five serotypes mainly involved in human infections and isolated from food and animal sources, that are: *S. Infantis*, *S. Enteritidis*, monophasic variant of *S. Typhimurium* (MVST), *S. Typhimurium* and *S. Derby* (1). In addition, *S. Kentucky* has been included in the study due to its growing relevance in laying hens in last years (2).

2. Materials and Methods

2.1. Samples collection.

The food and animal samples collection was obtained according to the monitoring plans based on the European regulation about the control of: foods of animal origin, poultry population and animal feed (3-5) and also from research activity or outbreak investigation.

Among the human origin strains collected in hospitals from symptomatic or asymptomatic patients and sent voluntary to the ENTER-NET local point, only those belonged to the six serotypes of interest were selected and sent to IZSPB.

2.2. Salmonella Isolation and Typing.

The isolation of *Salmonella* strains from food, feed and animal samples was made according to ISO 6579-1:2017 and ISO/TS 6579-2:2012 (Microbiology of food and animal feeding stuffs: horizontal method for the detection of Salmonella spp.). The presence of *Salmonella* was confirmed using appropriate biochemical miniaturized tests (API 20E®, Biomerieux, Lyon, France) and serological tests. Serotype identification of the isolated strains was carried out according to ISO/TR 6579-3:2014 (Microbiology of the food chain—Horizontal method for the detection, enumeration and serotyping of Salmonella—Part 3: Guidelines for serotyping of Salmonella spp.) using Statens Serum antisera (SSI Diagnostic, Denmark). One strain of *Salmonella* from each sample was preserved in Microbank ™ vial (Pro-Lab Diagnostics, Canada) at -20°C.

2.3. Antimicrobial Susceptibility Testing

Salmonella strains typed like: *Enteritidis, Infantis, Typhimurium, MVST, Kentucky* and *Derby* were characterized phenotypically by broth microdilution using Sensititre® EUVSEC3® and EUVSEC2® plates (Termofisher scientific,UK). The plates contained the antimicrobials specified in Commission Implementing Decision (EU) 2020/1729 (6). The definition of sensibility or resistance was based on the Epidemiological Cut-Off value (ECOFF) shown in previously indicated Decision, except for azithromycin, for which the sensibility or resistance values reported in CLSI 2021 document M100 were used, due to the lack of specific indications in the aforementioned legislation (7). The quality control of batch was performed with *E. coli* ATCC® 25922. The antimicrobial test was carried out on colonies grown on non-selective agar medium, incubated at 36±1 °C overnight, touched with a sterile swab and transferred to sterile saline solution, until reaching 0,5McFarland turbidity. Then, 0,1 ml of each suspension was inoculated in 9,9 ml of Cation Adjusted Muller Hinton Broth and 50 µl of this suspension was added into the wells of the EUVSEC3® or EUVSEC2® plates. The plates were incubated at 36±1 °C for 24 hours before the reading.

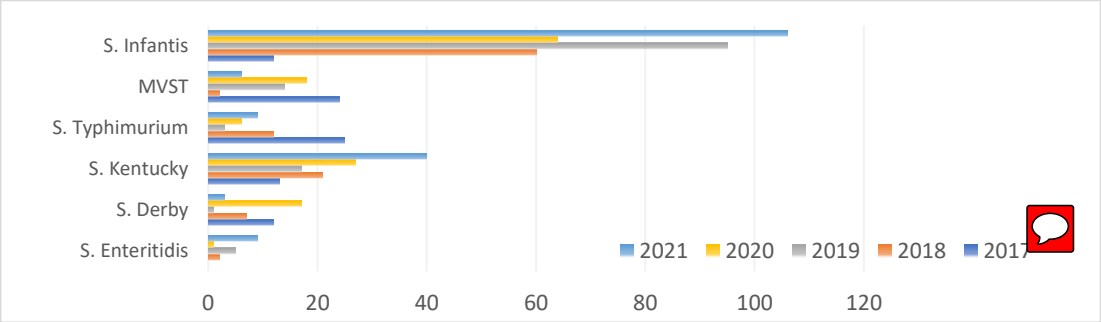
3. Results

3.1. Salmonella strains collected and typed.

Among the 1050 strains collected in IZSPB between 2017 and 2021 from food and animal (indicated below as FA) matrices there were: 337 *S. Infantis*, 118 *S. Kentucky*, 64 *monophasic variant of Salmonella Typhimurium* (MVST), 55 *S. Typhimurium*, 40 *S. Derby*, 30 *S. London*, 29 *S. Bredeney*, 26 *S. Abony*, 25 *S. Kaseny*, 22 *S. Muenster*, 19 *S. Agona*, 18 *S. Anatum*, 17 *S. Enteritidis*, 16 *S. Rissen*, 13 S. *Newport*, 10 *S. Branderup*; the remaining 275 strains belonged to other 45 serotypes having less than 10 strains per serotype.

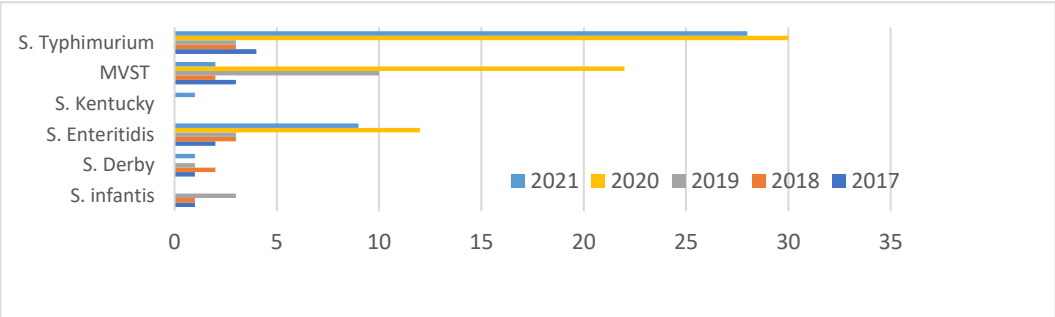
Salmonella Enteritidis, S. Derby, S. Kentucky, S. infantis, S. Typhimurium and its monophasic variant (MVST) represented 60% of the typed strains. The distribution per year of this kind of strains is shown in Figure 1.

Figure 1. Distribution of S. Infantis, MVST, S. Typhimurium, S. Kentucky, S. Derby and Enteritidis per year. FA strains.



Among the 146 *Salmonella* strains of human origin (indicated below as H) collected between 2017 and 2021 by ENTER-NET local network (Department of Basic Medical Sciences, Neurosciences and Sense Organs of Bari University) and sent to IZSPB there were: 5 *S. Infantis*, 5 *S. Derby*, 29 *S. Enteritidis*, 1 *S. Kentucky*, 39 *S. monophasic variant of Salmonella Typhimurium* (MVST) and 68 *S. Typhimurium*. Figure 2 shows the distribution per year of this kind of strains.

Figure 2. Distribution of *S. Infantis*, MVST, S. *Typhimurium*, *S. Kentucky*, *S. Derby* and *Enteritidis* per year. H strains.



3.2. Distribution on territory and sampling.

The food, feed and animal matrices positive for *Salmonella* spp. were sampled within the scope of: the National Control Plan for foods of animal origin 49,62% (Reg. (EU) 2017/625); the *Salmonella* National Control Program in poultry population 27,40% (Reg. (EC) 2160/2003); screening, research cooperation and other unspecified reasons 14,09%;

outbreak investigation 3,43%; the National Control Plan for feedingstuffs 3.24% (Reg. (EU) 2017/625) (3, 4). The *Salmonella* strains were detected principally in food and animal samples (56,1% and 41,33% respectively) and to a small extent in feed (2,57%). The main source of *Salmonella* in food was chicken meat whit about 27% of the total isolates (1050 strains), followed by pork meat (10,3%), mixed meat preparations (5,6%), clams (3,71%) and bovine meat (3,24%). As regards animal carcasses and swabs, *Salmonella* was isolated primarily from laying hens (13,43% on 1050 strains), followed by broilers (10,76%), bovine (4,48%), turtles (3,9%), etc. Food and animal strains originated from the following provinces of Apulia and Basilicata regions: Bari (22,32%), Foggia (21,11%), Matera (15,86%), Brindisi (9,19%), Potenza (8,79%), Barletta-Andria-Trani (8,18%), Lecce (8,18%), Taranto (6,36%).

On the human strains 67 out of 146 were collected from children 0-6 years old (45,89%), 16 from kids 7-18 years old (10,95%), 11 from adults 18-60 years old (7,53%), 11 from adults 60-90 years old (7,53%), while for the remaining 39 samples the age is unknown. The human samples originated from the following geographical areas: Bari (63,01%), Foggia (8,22%), Matera (1,37%), Brindisi (26,02%), Barletta-Andria-Trani (1,37%), territories outside Apulia and Basilicata regions (1,37%).

3.3. Antimicrobial susceptibility testing.

As regards *Salmonella* strains isolated from food and animal matrices, the antimicrobial susceptibility test was performed only on those belonged to the six serovars of interest (206), in particular: 100 *Salmonella Infantis*, 35 *Salmonella Kentucky*, 27 MVST, 26 *Salmonella Typhimurium*, 13 *Salmonella Derby*, 5 *Salmonella Enteritidis*.

All 146 human strains sent to IZSPB were examined.

Among the FA strains, the most resistant serotype versus all evaluated antimicrobial classes was *S. Infantis*, while for H strains it was *S. Typhimurium*.

All *Salmonella* strains were resistant to sulfamethoxazole. None was resistant to meropenem.

Salmonella FA strains showed antimicrobial resistance mainly to: nalidixic acid (63,59%), ciprofloxacin and tetracycline (60,68%), trimethoprim (42,72%), ampicillin (41,26%), cefotaxime (17,48%), ceftazidime (14,08%), followed by other antimicrobial classes with resistance percentages of less than 10%.

As regards the *Salmonella* H strains resistance was detected principally versus: ampicillin (57,53%), tetracycline (50.68%), azithromycin (36.30%), chloramphenicol (9.59%), ciprofloxacin (8.90%), nalidixic acid (8.22%) followed by others antimicrobial classes with resistance percentages of less than 8%.

Table 1 shows the resistance percentage of the total strains, distinguished with respect to H and FA.

Table 1. Resistance percentage of the total strains versus single antimicrobial agent. In bold were reported the Highest Priority Critically Important Antimicrobials.

Antimicrobial agents	**Human resistant strains/TOT**	**Food-animal resistant strains/TOT**
Sulfamethoxazole (SUL)	100,00%	100,00%
Nalidixic Acid (NAL)	**8,22%**	**63,59%**
Ciprofloxacin (CIP)	**8,90%**	**60,68%**
Tetracycline (TET)	50,68%	60,68%
Trimethoprim (TRI)	6,16%	42,72%
Ampicillin (AMP)	57,53%	41,26%

Cefotaxime (FOT)	**4,11%**	**17,48%**
Ceftazidime (TAZ)	**3,42%**	**14,08%**
Tigecycline (TGC)	2,74%	7,77%
Azithromycin (AZM)	**36,30%**	**7,28%**
Chloramphenicol (CLO)	9,59%	6,31%
Amikacin (AMI)	3,42%	4,37%
Colistin (COL)	**2,74%**	**2,91%**
Gentamicin (GEN)	0,00%	1,46%
Meropenem (MERO)	0,00%	0,00%

Among the FA strains, *S. Infantis* displayed resistance mainly to tetracycline (73,00%), followed by nalidixic acid and ciprofloxacin (70,00% and 69,00% respectively), trimethoprim and ampicillin (55,00% and 44,00% respectively), cephems (19,00% - 23,00%). Ciprofloxacin and nalidixic acid resistance were predominant in *S. Derby* strains (76,92% and 69,23% respectively), followed by trimethoprim (61,54%), ampicillin (46,15%), ceftazidime and cefotaxime (30,77% each). The MVST strains were mainly resistant to tetracycline (70,37%) and ampicillin (62,96%). *Salmonella Typhimurium* strains were resistant to nalidixic acid (50%), tetracyclines and ampicillin (42,31% each) and ciprofloxacin (38,46%). Table 2 shows all resistance percentages of FA strains.

Table 2. Resistance versus single antimicrobial agent showed for serotype of FA strains. In bold were reported the Highest Priority Critically Important Antimicrobials.

Antimicrobial agents	S. Infantis	S. Derby	S. Enteritidis	S. Kentucky	VMST	S. Typhimurium
Ampicillin	44,00%	46,15%	40,00%	14,29%	62,96%	42,31%
Meropenem	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
Ciprofloxacin	69,00%	76,92%	80,00%	77,14%	18,52%	38,46%
Azithromycin	9,00%	0,00%	0,00%	5,71%	3,70%	11,54%
Amikacin	5,00%	15,38%	0,00%	2,86%	3,70%	0,00%
Gentamicin	0,00%	7,69%	0,00%	0,00%	7,41%	0,00%
Tigecycline	11,00%	7,69%	0,00%	2,86%	11,11%	0,00%
Ceftazidime	19,00%	30,77%	20,00%	5,71%	3,70%	7,69%
Cefotaxime	23,00%	30,77%	40,00%	11,43%	0,00%	11,54%
Chloramphenicol	5,00%	0,00%	0,00%	2,86%	11,11%	15,38%
Colistin	2,00%	7,69%	0,00%	2,86%	0,00%	7,69%
Nalidixic Acid	70,00%	69,23%	80,00%	85,71%	18,52%	50,00%
Tetracycline	73,00%	53,85%	60,00%	34,29%	70,37%	42,31%
Trimethoprim	55,00%	61,54%	60,00%	25,71%	29,63%	19,23%
Sulfamethoxazole	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%

As regards the H strains: *S. Infantis* showed simultaneous resistance to ampicillin, ciprofloxacin, trimethoprim and azithromycin (85,71%), it was also resistant both to nalidixic acid and tetracycline (71,43%), to cephems (57,14%) and tigecycline (28,57%). The highest level of resistance observed on *S. Derby* and *S. Enteritidis* was to azithromycin (66,67% and 14,81% respectively). The MVST strains revealed a resistant phenotype mainly to ampicillin (63,64%), followed by tetracycline and azithromycin (57,58% and

42,42% respectively), likewise *S. Typhimurium* whose resistance percentages were respectively 75,00%, 65,28% and 34,72%. Table 3 shows all the resistances.

Table 3. Resistance versus single antimicrobial agent in serotypes of H strains. In bold were reported the Highest Priority Critically Important Antimicrobials.

Antimicrobial agents	***S. Infantis***	***S. Derby***	***S. Enteritidis***	***S. Kentucky***	***MVST***	***S. Typhimurium***
Ampicillin	85,71%	33,33%	3,70%	0,00%	63,64%	75,00%
Meropenem	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
Ciprofloxacin	85,71%	16,67%	0,00%	0,00%	6,06%	5,56%
Azithromycin	85,71%	66,67%	14,81%	0,00%	42,42%	34,72%
Amikacin	14,29%	16,67%	3,70%	0,00%	3,03%	1,39%
Gentamicin	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
Tigecycline	28,57%	0,00%	3,70%	0,00%	0,00%	1,39%
Ceftazidime	57,14%	16,67%	0,00%	0,00%	0,00%	0,00%
Cefotaxime	57,14%	16,67%	0,00%	0,00%	0,00%	1,39%
Chloramphenicol	14,29%	16,67%	7,41%	0,00%	6,06%	11,11%
Colistin	14,29%	0,00%	7,41%	0,00%	0,00%	1,39%
Nalidixic Acid	71,43%	0,00%	7,41%	0,00%	9,09%	2,78%
Tetracycline	71,43%	33,33%	3,70%	0,00%	57,58%	65,28%
Trimethoprim	85,71%	0,00%	0,00%	0,00%	3,03%	2,78%
Sulfamethoxazole	100,00%	100,00%	100,00%	100,00%	100,00%	100,00%

Resistance to two or more antimicrobials was detected in 175 out of the 206 FA strains (84,95%) and in 113 out of the 146 H strains (77,40%).

The most common MDR pattern found in all FA *Salmonella* strains was CIP-NAL-TET-TRI-SUL associated to other antimicrobial, with the exception of MVST which didn't show so much resistance to quinolones.

Salmonella Infantis showed the two main MDRs detected in FA strains: CIP-NAL-TET-TRI-SUL-AMP-FOT- TAZ in 14 strains and CIP-NAL-TET-TRI-SUL in 9 strains. The most common MDR in H strains was AMP-TET-SUL associated to other antimicrobials. The main MDR in H strains was AMP-TET-SUL-AZM, detected in 11 *S. Typhimurium* and in 8 MVST. In *Salmonella Infantis* this kind of MDR was included in a wider multidrug resistance pattern comprising up to 11 resistances (AMP-CIP-AZM-TIG-FOT-TAZ-COL-NAL-TET-TRI-SUL) and it was detected also in *S. Derby* and *Enteritidis* associated to other antimicrobials.

4. Discussion

The extreme diffusion of *Salmonella* spp. and its ability to infect human from animal, food and environmental sources made this bacterium very important in health surveillance systems. Several European regulations provide rules regarding the management of the *Salmonella* disease and the surveillance of antibiotic resistance; thus, they have contributed to produce a large amount of data related to these topics. In Europe, the trend in human salmonellosis has been stable over the last five years, while *Salmonella* positivity increased significantly in laying hens and breeding flocks (8). The top five *Salmonella* serovars for human infections from food-animal sources were *S. Infantis*, *S. Enteritidis*, *monophasic variant of Salmonella Typhimurium, S. Typhimurium and S. Derby*. The vast majority

of the salmonellosis foodborne outbreaks was caused by S. Enteritidis and the most implicated food vehicles were eggs and egg products (1, 8). The relevance of *Salmonella Kentucky* is growing (2) and in our opinion, even if it has little impact on human cases, it could be a source of antibiotic resistance to be considered in relation to European data (1).

In last five years *S. Infantis* from FA samples accounted for more than 30% of the total strains isolated in Apulia and Basilicata regions. The prevalence value increased over the time: it was detected only 12 times in 2017 while 106 samples were positive in 2021, as shown in Figure 1. In particular, *S. Infantis* was detected in 94% of broiler samples and only in 6% of the other sources. This result is in agreement with the European data, where *S. Infantis* was the most frequently isolated serovar in broiler flocks and the fourth one in breeding flocks and laying hens in the Member States (1). The significant increase in the prevalence of *Salmonella Infantis* seems to be linked to the presence of an ESBL-producing multidrug resistant clone harboring a conjugative pESI-like meg, already detected since 2014 (2, 9, 10). The characteristic resistance pattern of pESI-like meg *S. Infantis* is towards tetracyclines, trimethoprim, sulphonamides, aminoglycosides associated to fluoroquinolone and macrolide resistance (10). A stable population of this kind of *S. Infantis* has been recorded in Italy (2, 9). Our data seem to confirm it since a lot of *S. Infantis* strains isolated from both human and veterinary samples displayed simultaneous resistance to sulphonamides, tetracyclines and trimethoprim, sometimes associated with fluoroquinolones or aminoglycosides or beta-lactams. The MDR versus the HpCIAs is particularly interesting, a signal that there is still much work to be done on the use of these antibiotics, as well as about EsBL resistance. In 23 FA and 3 H strains there was resistance versus one of tested cephems, showing a worrying incidence of this kind of resistance, as reported by many authors (10, 11). Colistin resistance was very low, detected only in 2 FA strains and 1 H strain; this result is in agreement with the European data (8). Both EsBL and colistin resistance were always associated with MDR complexes which reached up to 11 antimicrobial resistances at the same time.

Salmonella Enteritidis emerged as a global epidemic in the 1980s and persists in most countries as the leading cause of human salmonellosis due to its ability to colonize reproductive systems in poultry and contaminate the eggs (2). *Salmonella Enteritidis* is the second most common serovar in laying hens, but European data shows that is very infrequent in broilers (2, 8). The distribution of *S. Enteritidis* detected in our FA samples was slightly different, but the low number of strains does not allow any consideration. The strains detected in human samples were 27 out of 146 and there were 2 colistin resistant strains, consistent with a previous study on ENTER-NET data (12). Resistance to azithromycin was the most frequent one in *S. Enteritidis* strains of human origin, followed by resistance to quinolones and fluoroquinolones. Conversely, this resistance was inexistent in strains of veterinary origin, which showed resistance mostly to quinolone and fluoroquinolone. Some H and FA strains were MDR, showing resistance to colistin and cefotaxime/ceftazidime respectively always associated with other antimicrobial agents. In these MDR strains there were many HpCIAs and up to 9 resistances at the same time, making them particularly dangerous for human health. It should be noted that there were a strain of animal origin and a human one with the same MDR pattern (AMP-CIP-TAZ-FOT-NAL-TET-TRI-SUL) except for azithromycin, present only in the human strain. this kind of MDR could be of great concern for people, because they can become infected with the strain from food or animals and provide it an additional chance to acquire antimicrobial resistance, making the picture worst. Furthermore, given the great invasion capacity of *Salmonella Enteritidis*, the fact that 3 out of 5 strains of veterinary origin were MDR can be of great concern for public health.

Salmonella Typhimurium is the second most common serovar in humans with epidemic potential in animal and human populations. This kind of *Salmonella* often evolved towards multiple antimicrobial resistance like DT104 strain that has enhanced virulence in humans and animals (2, 13, 14). *Monophasic variant of S. Typhimurium* (MVST) strains emerged in pig populations since 1980 and rapidly spread, reaching the prevalence value

of 8,3% in samples of animal origin and 14% in samples of human origin in 2008 (9). MVST and *S. Typhimurium* were the most frequent serovars isolated from humans in Italy, representing, respectively, 35% and 10,2% of all reported isolates between 2016 and 2018 (2, 15). Nowadays they belong to the top five serovars in humans and animals (8). The presence of integrons, gene of invasion (*invA*) and virulence (*spv*, *Salmonella* plasmid virulence) played a fundamental role in the spread of this kind of *Salmonella*, whose main source is today pig (13, 14). Furthermore, some studies report the presence of *S. Typhimurium* and MVST in wildlife mammals in southern and northern Italy (16, 17). One of the latest big outbreak of MVST detected in Italy occurred in Abruzzo region between 2013 and 2014 and involved 30 people; it was caused by a particular MDR profile (15), to which it should be paid more attention because of its ability to persist in the environment and its infectious capacity. Overall, in our study 107 H and 119 FA strains of *S. typhimurium* and its monophasic variant were analysed. The most common resistance and MDR in human strains were versus AMP, TET, AZM and SUL. Chloramphenicol resistance seems to occur frequently; streptomycin was not tested in our panel, therefore it is not possible to include these strains in the ASSuT or ACSSuT patterns as appropriate. The assessment of the ASSuT or ACSSuT patterns will be detected by molecular biology methods in our future studies (18, 19).

S. Kentucky, accounting for about 40% of all identified isolates each year in laying hens in Italy, was the most frequently detected serovar by far. Conversely, in humans it was detected at low frequency (0,24%) (2). Our study reveals that the presence of *S. Kentucky* has increased from 2017 to 2021. In particular, 64% of *S. Kentucky* originated from laying hens, 23% from broilers but, in our opinion, it is interesting to note that the 12,71% of strains were collected from other sources (swine, bovine, turtles, wild birds) as well as the detection of a human strain in 2021. *Salmonella Kentucky* is an emergent strain and the sequence type ST198 has rapidly spread in broiler populations in many EU and non-EU countries (8, 20-22). The study of the STs of our populations to assess their belonging to the clones circulating in Europe will soon be in-depth. The H strain of *Salmonella Kentucky* was sensible to all antimicrobial agents. The occurrence of MDR in FA *S. Kentucky* strains was not so abundant, but among MDR strains there were 4 strains resistant to cephems and 1 strain resistant to colistin. The MDR strains were the 28,57% of the total. These data contrast with those shown by the EFSA where MDR was reported in 73,7% of *S. Kentucky* isolated from human cases (23). The resistance to quinolones and fluoroquinolones is confirmed as the most frequent one, in agreement with the EFSA report: in fact, in our study 77,14% of *S. Kentucky* strains were resistant to ciprofloxacin and the EFSA reports 82,1%, associated predominantly with poultry (23).

According to the ENTER-NET network data, *S. Derby* represented 3,0% of all reported isolates between 2016-2018 (2); in 2019 it has been highlighted the EsBL production (23). Our study confirms this finding, making *S. Derby* one of the special supervised ones, even if it does not have a prevalence comparable to the other *Salmonella* serovars of the present study. *Salmonella Derby* was detected in 40 FA and 6 H samples. 11 out of 13 FA strains were MDR, 4 of these were resistant to CIP-NAL-TET-TRI-SUL. Among the H strains 2 were MDR and one of these had cephems resistance in MDR profile.

In general, in our study the resistance to ampicillin, sulfamethoxazole and tetracyclines was the most observed within the human strains. The level of resistance to cefotaxime and ceftazidime was low (0,03%) and none of the strains were resistant to meropenem. In FA strains the resistance percentage value versus ampicillin was lower than ciprofloxacin, nalidixic acid, tetracyclines and sulfamethoxazole, but it still remained quite high. The resistance to cefotaxime and ceftazidime was at the a medium level of 17.48%, higher than that found in 2019 according to monitoring plans in the EU Member States (8, 23). Sulfamethoxazole and tetracyclines are categorized as "Highly Important antimicrobials" by the World Health Organization and despite the invitation to moderate or exclude their use, the resistance remains high, as shown by our survey; the same must be said for the

use of quinolones and fluoroquinolones. Resistance to azithromycin, tigecycline, colistin was low, as shown in the European data (1).

In conclusion it is possible to argue that our data, limited to Apulia and Basilicata regions, agrees with the European ones, although they differ from other Italian works (16, 17).

The resistance to sulfamethoxazole shown by 100% of the strains is certainly noteworthy, especially since our data are more similar to those registered in Russia and China rather than in Europe (23-25). We suspect that this high prevalence of resistance to sulfamethoxazole is linked to the ease of spread of type 1 integron associated with the presence of the aadA2 gene. This topic will certainly be a starting point for further insights into the molecular biology in future studies.

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