Characterization of Salmonella *Typhimurium and monophasic* Salmonella *Typhimurium isolated in Abruzzo and Molise regions, Italy, from 2012 to 2017*

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S. Typhimurium, Monophasic *S.* Typhimurium, Antimicrobial resistance, MLVA.

Summary
Salmonellosis is currently the second most common zoonosis in European Union but in the 6-years periods, between 2012 and 2017, there has been a significant decrease trend in the yearly number of infections caused by *Salmonella*. In Italy, *S.* Typhimurium and monophasic *S.* Typhimurium represent the most prevalent serotypes. In this paper, we investigated these two serovars isolated from 2012 to 2017 in Abruzzo and Molise regions. A set of 345 strains isolated from human sporadic cases, surface water, food and animals were collected and analyzed. Monophasic *S.* Typhimurium strains were found to be resistant to streptomycin, sulfisoxazole, ampicillin, tetracycline and nalidixic acid, while *S.* Typhimurium isolates showed high levels of resistance to tetracycline, sulfisoxazole and ampicillin. The 5-loci Multilocus Variable-Number Tandem Repeat Analysis (MLVA) identified 88 genotypes (GT), six of which were common for the two serovars. Several MLVA profiles were shared by human and non-human isolates. MLVA had sufficient typing resolution for epidemiological studies of *S.* Typhimurium but demonstrated poor discriminatory in trace-back study of monophasic *S.* Typhimurium.

Introduction

Salmonella spp. is one of the most common foodborne pathogens and salmonellosis is the second most frequently reported zoonosis in Europe (EFSA and ECDC 2019, EFSA and ECDC 2018). Monophasic variants of *Salmonella* Typhimurium-like strains, lacking the fljB-encoded second phase H antigen appear to be of increasing importance in many European Union (EU) Member States and a rapid emergence and dissemination of such strains in livestock animals, companion animals and humans has been observed (EFSA J 2010). While the number of *Salmonella*-positive samples collected annually is generally decreasing, the monophasic *S.* Typhimurium is one of the few serovars for which the opposite trend has been described both in humans and in animals and it is currently considered an emerging epidemic serovar. On the basis of genetic similarity, the monophasic strains with formula 4,[5],12:i:- are regarded as variants deriving from *S.* Typhimurium that display similar virulence and antimicrobial resistance characteristics. In 2017 in the EU, a total of 91,857 human salmonellosis cases were reported; *Salmonella* Enteritidis and *Salmonella* Typhimurium were the most common serovars, followed by monophasic *S.* Typhimurium, *S.* Infantis and *S.* Newport (EFSA and ECDC 2018). In 2018, the number of reported confirmed human cases and the EU notification rate were at the same level as in 2017 (EFSA and ECDC 2019, EFSA and ECDC 2018).

In Italy, *S.* Typhimurium and monophasic *S.* Typhimurium are two of the most commonly serovars isolated from humans and animals. A similar situation has also been reported in other European countries (Molbak *et al.* 2014, Mueller-Doblies *et al.* 2018). In Italy, over last few decades, various trends have been observed in the prevalence of different *Salmonella* serotypes. Between 1980 and 1988 *S.* Typhimurium was the serotype most commonly isolated from humans, and in the 90's the highest number of cases were caused by *S.* Enteritidis. Since 2000, and until 2012, *S.* Typhimurium was once again the most prevalent serotype reported. The

monophasic *S.* Typhimurium was isolated for the first time in Italy from a patient in 2003 and the number of cases has increased in the last years (Graziani *et al.* 2013, Dionisi *et al.* 2009).

S. Typhimurium was the predominant serovar isolated from veterinary samples between 2002 and 2011, however in 2012, the numbers of monophasic *S.* Typhimurium-positive samples exceeded the numbers of *S.* Typhimurium and a steady rise in the prevalence of the monophasic variant has been observed in the following years (https://www.izsvenezie.it/temi/malattie-patogeni/salmonella/enter-vet/) (accessed on 22 October 2019). The emergence of a clonal group of serovar *S.* Typhimurium and monophasic *S.* Typhimurium R-type ASSuT (resistante to ampicillin, streptomycin, sulphonamides and tetracycline) strains in Italy, Denmark and the United Kingdom has also been described (Lucarelli *et al.* 2010).

Multilocus variable-number tandem repeat analysis (MLVA) is a highly discriminative typing method (EFSA 2013) that has emerged as a powerful tool for subtyping of food-borne bacterial pathogens (Nadon *et al.* 2013) and it is used for *S.* Typhimurium subtyping, especially in outbreak investigations (Torpdahl *et al.* 2007, Petersen *et al.* 2011, Ross *et al.* 2011).

The main aims of this study were to investigate the epidemiology of *S.* Typhimurium and monophasic *S.* typhimirium (4,[5],12:i:-) in Abruzzo and Molise regions, to elucidate the characteristics of the resistotypes and genotypes circulating in the environment and in animal and human populations between 2012 and 2017.

Materials and methods

Strains collection

A set of 345 strains including 65 isolates of *S.* Typhimurium and 280 isolates of monophasic *S.* Typhimurium were collected in Abruzzo and Molise regions of Italy between January 2012 and December 2017. The strains of *S.* Typhimurium were isolated from fecal samples of chickens (n = 2), pigs (n = 9), wild birds (n = 6), humans (n = 13), pork meat (n = 6), and surface water samples (n = 31). The strains of monophasic *S.* Typhimurium were isolated from fecal samples (from 15 pigs, 2 chickens and from 145 humans), from food (21 from pork, 2 from mixed beef-pork minced meat, 1 from lamb, 1 from turkey, 8 from bivalve molluscs) and from 85 surface water samples. The strains isolated from human feces and surface water were sent to our laboratory by regional hospitals and environmental protection agency, respectively, within the Enternet Italia surveillance network. The Enternet Italia is coordinated by the Istituto Superiore di Sanità and relies on the collaboration of peripheral laboratories. It is incorporated into the European surveillance system for Food- and Waterborne Diseases (FWD) and coordinated by the ECDC. The strains isolated from animals were collected in farms. Isolates from food were obtained from the producers and from retail stores during official veterinary controls.

All the strains were serotyped according to the Kauffmann-White-Le Minor schema by the slide agglutination method (Grimont *et al.* 2007) using commercial antisera (Statens Serum Institute, Copenhagen, Denmark). To confirm the strains serotyped as *S.* Typhimurum or monophasic variant, a PCR assay was also performed, as previously described (Barco *et al.* 2011).

Antimicrobial susceptibility testing

The susceptibility to sixteen antibiotics was performed by the disc diffusion method (Kirby-Bauer) in accordance with the Clinical Laboratory Standards Institute guidelines (Patel *et al.* 2017) using the following BD Sensi-DiscTM (Becton and Dickinson, Berkshire, U.K): ampicillin (A) 10 µg, amoxicillin-clavulanic acid (AmC) 20/10 µg, cefazolin (Cz) 30 µg, gentamycin (G) 10 µg, kanamycin (K) 30 µg, enrofloxacin (En) 5 µg, trimethoprim/sulfamethoxazole (Sxt) 1.25/23.75 µg, tetracycline (T) 30 µg, ceftazidime (Caz) 30 µg, colistine (Cl) 10 µg, sufisoxazole (Su) 300 µg, nalidixic acid (Na) 30 µg, streptomycin (S) 10 µg, chloramphenicol (C) 30 µg, cephalothin (Cf) 30 µg and ciprofloxacin (Cip) 5 µg. *Escherichia coli* ATCC 25922 was used as a control.

Multilocus Variable-Number Tandem Repeat Analysis (MLVA)

The 345 strains were typed using 5 locus MLVA (STTR9-STTR5-STTR6-STTR10-STTR3) according to the protocol previously described by ECDC (Takkinen *et al.* 2011) using multiplex PCR and capillary electrophoresis on an ABI 3500 instrument with POP 7 polymer (Thermo Fisher Scientific, MA, USA). The MLVA profiles were expressed according to the nomenclature published by Larsson and colleagues (Larsson *et al.* 2009).

The genetic diversity of each locus was determined using the Simpson's diversity index (SDI) calculated for a dataset of *S.* Typhimurium and monophasic *S.* Typhimurium, comprising of only one strain per MLVA genotype and using the online tool available at the Comparing Partitions website (http://www.comparingpartitions.info/?link=Tool).

MLVA clustering was performed using the

BioNumerics software package version 7.6. (Applied-Maths, Sint-Martens-Latem, Belgium) with MST (Minimum Spanning Tree) method. Clonal complexes (CCs) were retrieved with the most stringent (conservative) definition (Spratt *et al.* 2004) where all members assigned to the same CC differed only by one locus with the nearest member included in the same CC. Table I shows GTs-MLVA patterns conversion.

Table I. *GTs-MLVA patterns convertion.*

***Salmonella* Typhimurium**		**Monofasic *Salmonella* Typhimurium**			
MLVA pattern	**GTs**	**MLVA pattern**	**GTs**	**MLVA pattern**	**GTs**
2-10-6-6-112	2	1-9-NA-NA-210	1	3-14-9-NA-210	60
2-13-NA-NA-311	3	2-19-NA-NA-211	10	3-15-11-NA-211	61
2-16-6-12-212	4	3-10-10-NA-211	15	3-15-12-NA-210	63
2-16-6-14-112	5	3-10-11-NA-211	16	3-15-12-NA-211	64
2-17-NA-NA-105	6	3-10-12-NA-211	17	3-15-7-NA-211	65
2-17-NA-NA-211	7	3-10-15-NA-211	18	3-15-8-NA-NA	66
2-17-5-7-212	8	3-10-9-NA-112	19	3-15-8-NA-211	67
2-17-7-14-112	9	3-11-10-NA-211	21	3-15-9-NA-211	68
2-20-NA-NA-211	11	3-11-11-NA-211	22	3-17-12-NA-311	70
2-22-9-14-112	12	3-11-15-NA-211	23	3-9-12-NA-211	75
2-6-4-9-212	13	3-11-8-NA-211	24	3-9-5-NA-211	76
2-8-5-9-212	14	3-11-9-NA-211	25	3-10-9-NA-210	81
3-11-9-NA-211	25	3-12-NA-NA-211	26	3-13-NA-NA-211	82
3-12-10-NA-211	27	3-12-10-NA-211	27	3-11-NA-NA-211	83
3-12-12-12-211	31	3-12-11-NA-211	28		
3-13-14-NA-211	43	3-12-11-18-NA	29		
3-13-7-NA-211	44	3-12-12-NA-211	30		
3-14-NA-21-311	49	3-12-13-NA-211	32		
3-14-12-7-211	55	3-12-3-NA-211	33		
3-14-16-17-311	57	3-12-7-NA-211	34		
3-15-11-NA-211	61	3-12-8-NA-211	35		
3-15-11-NA-311	62	3-12-9-NA-NA	36		
3-15-12-NA-211	64	3-12-9-NA-211	37		
3-17-12-NA-211	69	3-13-NA-7-211	38		
3-17-12-NA-311	70	3-13-10-NA-211	39		
3-19-12-NA-311	71	3-13-11-NA-211	40		
3-19-NA-NA-311	72	3-13-12-NA-211	41		
3-8-10-NA-211	73	3-13-13-NA-211	42		
3-9-NA-NA-210	74	3-13-7-7-211	45		
3-9-12-NA-211	75	3-13-8-NA-211	46		
4-12-9-7-211	77	3-13-9-NA-210	47		
4-13-9-22-311	78	3-13-9-NA-211	48		
6-24-15-22-11	79	3-14-10-NA-211	50		
6-9-13-9-211	80	3-14-11-NA-211	51		
6-19-15-18-NA	84	3-14-11-9-211	53		
2-19-6-14-112	85	3-14-12-NA-211	54		
2-17-NA-NA-210	86	3-14-13-NA-211	56		
3-10-NA-NA-210	87	3-14-3-NA-211	58		
2-20-5-13-112	89	3-14-8-NA-211	59		

Results

The antimicrobial resistance traits of the two *Salmonella* serovars are shown in Table II. More than a half of tested isolates were resistant to tetracycline (61.35% for *S.* Typhimurium and 66.39% for monophasic *S.* Typhimurium). Moreover, a large number of monophasic *S.* Typhimurium strains were resistant to streptomycin (81.89%), sulfisoxazole (58.49%), ampicillin (52.08%) and nalidixic acid (48.30%), while *S.* Typhimurium isolates showed high levels of resistance to sulfisoxazole (38.64%) and ampicillin (36.36%). Fewer monophasic *S.* Typhimurium isolates were resistant to cefazolin, kanamycin, ceftazidime, colistin, cephalotin and ciprofloxacin, while *S.* Typhimurium isolates were fully susceptible to these antimicrobials (Table II). More than 30% of monophasic *S.* Typhimurium belonged to the ASSuT R-type (95 strains) and only the 5.6% to the ACSSuT type (16 strains) while for *S.* Typhimurium the ASSuT and ACSSuT profiles accounted for 6.87% (3 strains for each serovars).

The MLVA analysis classified the 345 strains (Figure 1) into 88 GTs. Six of the MLVA profiles were common for both serovars, while 33 were specific for *S.* Typhimurium, and 49 for monophasic *S.* Typhimurium. The six common genotypes between the two serotypes were GTs 25 (3-11-9-NA-211 MLVA profile), 27 (3-12-10-NA-211), 61 (3-15-11-NA-211), 64 (3-15-12-NA-211), 70 (3-17-11-NA-211) and 75 (3-8-10-NA-211).

Table II. *Antibiotic resistance rates (%) of* S. *Typhimurium and* S. *4,[5],12:i:- isolated between 2012 and 2017 in Abruzzo and Molise regions.*

Antibiotic	**% Resistant**	
	***S.* Typhimurium**	***S.* 4,[5],12:i:-**
Ampicillin (A) 10µg	36.36	52.08
Amoxicillin/Clavulanic acid (Amc) 20/10 µg	11.36	20.38
Cefazolin (Cz) 30 µg	0,00	3.02
Gentamicyn (G) 10 µg	4.55	3.02
Kanamycin (K) 30 µg	0,00	5.66
Enrofloxacin (En) 5 µg	6.82	12.08
Trimethoprim/sulfamethoxazole (Sxt) 1.25/23.75 µg	4.55	9.81
Tetracycline (T) 30 µg	61.36	66.79
Ceftazidime (Caz) 30 µg	0,00	0.01
Colistine (Cl) 10 µg	0,00	1.89
Sufisoxazole (Su) 300 µg	38.64	58.49
Nalidixic acid (Na) 30 µg	9,09	48.30
Streptomycin (S) 10 µg	22.73	81.89
Chloramphenicol (C) 30 µg	6.82	6.04
Cephalothin (Cf) 30 µg	0.00	6.79
Ciprofloxacin (Cip) 5 µg	0.00	0.00

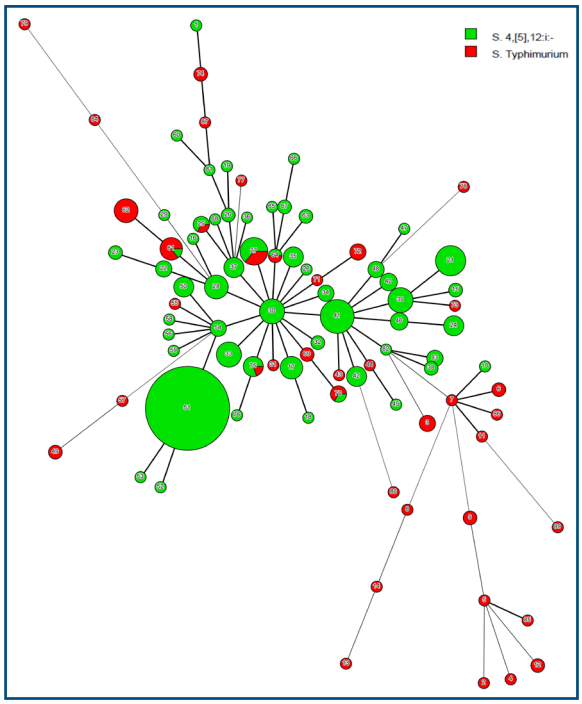

Figure 1. *Minimum Spanning Tree (MST) of 65 S. Typhimurium and 280 S. 4,[5],12:i:- analyzed between 2012 and 2017 in Abruzzo and Molise regions.* The node labels represent the unique genotype (GT). The diameter of each node is proportional to the number of isolates. The branch length is proportional to the loci differences.

The degree of polymorphism of MLVA genotypes associated with the two serovars was quantified by calculating the diversity index of each locus (Table III). For *S.* Typhimurium, STTR9 showed the lowest number of alleles (n = 4) with a SDI of 0.622, STTR5 resulted as the most variable locus (15 allele) with SDI of 0.934. For monophasic *S.* Typhimurium only STTR5 and STTR6 were highly discriminating, other loci showed an SDI below to 0.325 (Table III).

Ten CCs were assigned using MST analysis of *S.* Typhimurium based on MLVA typing results (Figure 2). The major clonal complex (CC1) included 8 genotypes, three of which were associated with human and non-human isolates. GT62 (3-15-11-NA-311) included human and pig samples, GT72 (3-19-NA_NA-311) and GT61 (3-15-11-NA-211) contained strains isolated from humans and the surface water. GT27 (3-12-10-NA-211) included *S.* Typhimurium isolated from humans and surface water and belonged to CC2 that contained isolates from pig. CC5 included only isolates from surface water, while CC3, CC4 and CC7 comprised of strains from pig and surface water. GT85 (2-19-6-14-112) group included only poultry isolates and clustered closely with GT5 (2-16-6-14-112) samples from surface water in CC9. We identified four genotypes that were shared between the human and environmental or animal samples. The genotype that contained the higher number of isolates was G62 (MLVA profile 3-15-11-NA-311) that was identical for seven pigs and 1 human isolate. GT61, with a MLVA pattern 3-15-11-NA-211, was assigned to isolates from four surface water samples and from one human sample. Genotype 27 (3-12-10-NA-211) was found in one water, two wild birds and 1 human isolates, and G72 (3-19-12-NA-311) was shared between one strain collected from surface water and two strains isolated from humans.

For monothasic *S.* Typhimurium, clustering analysis retrieved only one major CC which grouped almost all the MLVA genotypes (45 out of 55) (Figure 3). Several genotypes, *i.e.* GT28 (3-12-11-NA-211) and GT37 (3-12-9-NA-211) included, beside human isolates, samples from more than one source (pig, turkey, poultry, water, etc.). Three MLVA profiles, 3-11-10-NA-211 (GT21), 3-12-3-NA-211 (GT33) and 3-11-15-NA-211 (GT23), were shared between humans and bivalve molluscs; while, 3-11-11-NA-211 (GT22) included monophasic variant strains isolated from humans and sheep. Fifteen genotypes were retrieved from human isolates only.

GT51 (MLVA profile 3-14-11-NA-211) was the most common genotype identified in monophasic *S.* Typhimurium (85 human feces and 25 surface water strains), followed by GT41 (3-11-10-NA-211) assigned to 14 surface water and 3 human isolates. Other frequently identified MLVA pattern included

Table III. *Number of partition, Simpson's diversity index (SDI) and confidence interval (CI 95 %) for the five loci of the MLVA panel for S. Typhimurium and S. 4,[5],12:i:-.* The calculation of confidence intervals for Simpson's index was performed by use of the large sample approximation, thereby improving the objective assessment of the discriminatory power of typing techniques.

Locus	*S.* Typhimurium			*S.* 4,[5],12:i:-		
	#partitions	SDI	CI (95%)*	#partitions	SDI	CI (95%)*
STTR9	4	0.622	(0.536-0.708)	3	0.072	(1.000-0.168)
STTR5	15	0.934	(0.903-0.964)	9	0.868	(0.843-0.893)
STTR6	13	0.899	(0.853-0.944)	11	0.894	(0.868-0.921)
STTR10	11	0.725	(0.580-0.869)	4	0.173	(0.038-0.308)
STTR3	8	0.777	(0.684-0.871)	5	0.325	(0.168-0.481)

#Number of different repeats present at this locus; *Precision of the Diversity Index, expressed as 95% upper and lower boundaries.

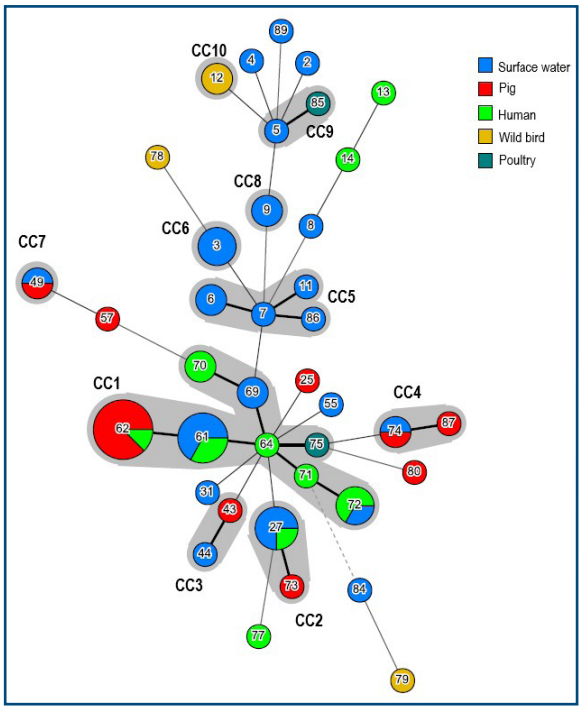

Figure 2. *Minimum spanning tree (MST) of 65 strains of S. Typhimurium isolated in Abruzzo and Molise regions between 2012 and 2017.* The node labels represent the unique genotype (GT) and are colored according to the source of isolation. The diameter of each node is proportional to the number of isolates. The branch length is proportional to number of different loci. Clonal complexes (CCs) are shaded in grey.

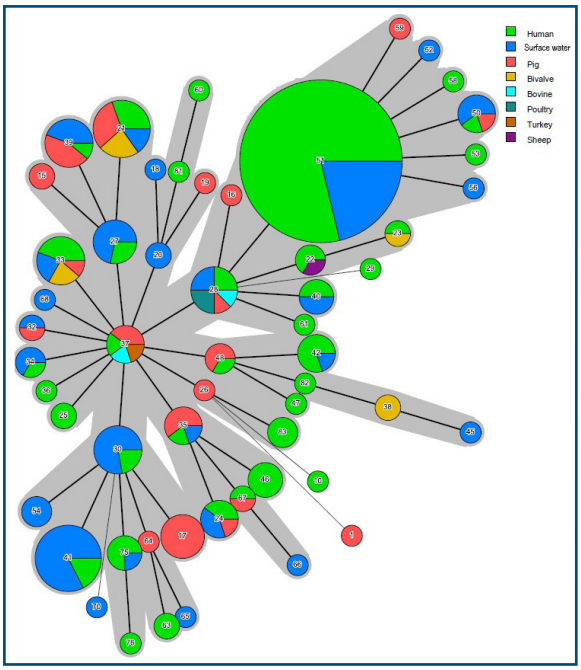

Figure 3. *Minimum spanning tree (MST) of strains of 280 monophasic S. Typhimurium:- isolated in Abruzzo and Molise regions between 2012 and 2017.* The node labels represent the unique genotype (GT) and are colored according to source of isolation. The diameter of each node is proportional to the number of isolates. The branch length is proportional to number of different loci. Clonal complexes (CC) are shaded in grey.

3-11-10-NA-211 (G21) assigned to humans (n = 4), pigs (n = 4), surface water (n = 3) and bivalve molluscs (n = 3). 3-12-11-NA-211 MLVA profile (GT28) found in human (n = 1), surface river (n = 1), pigs (n = 1), cattle (n = 1) and chikens (n = 2) strains and 3-12-3-NA-211 (GT33) assigned to human (n = 4),pigs (n = 3),surface river (n = 2) and strains isolated from bivalve molluscs (n = 2).

Discussion

In Italy, *S.* Typhimurium and monophasic *S.* Typhimurium are two of the most commonly reported serovars isolated from human and veterinary samples. While, in general, the total number of yearly *Salmonella* isolation have been decreasing, the opposite trend has been observed for a few serovars including monophasic *S.* Typhimurium (EFSA and ECDC 2019). Our data confirmed this observation as were reported four times greater number of isolated monophasic variant than *S.* Typhimurium strains. The high dissimilarity of number of sampling between the two serogroups was partly due to an epidemic outbreak (MLVA 3-14-11-NA-211) of monophasic *S.* Typhimurium observed in Abruzzo region during 2013 and 2014 (67.80% of the strains) where a possible implication of environmental sources was suspected (Cito *et al.* 2016).

The MLVA protocol designed for *Salmonella* Typhimurium routine typing (Takkinen *et al.* 2011) is also commonly used for the characterization of monophasic *S.* Typhimurium and it has facilitated the successful detection and investigation of multiple food-borne disease outbreaks (Cito *et al.* 2016, Barco *et al.* 2014, Raguanaud *et al.* 2012). For routine surveillance of *Salmonella*, MLVA seems to have notable advantages over Pulsed field gel electrophoresis (PFGE) method. The protocol is fast and can be completely automated and the generated data are easily analyzed and compared between laboratories. Moreover, MLVA has been shown to have a higher discriminatory power than PFGE (Torpdahl *et al.* 2007, Hopkins *et al.* 2007, Best *et al.* 2009). In our study, the analysis of the MLVA profiles of *S.* Typhimurium and *S.* 4,[5],12:i:- isolates showed that, in spite of the high similarity and close relationship between the two serovars (Switt *et al.* 2009), only six MLVA profiles were in common between the two serovars. Moreover, the genetic resolution power of three MLVA loci was much lower for monophasic *S.* Typhimurium than for *S.* Typhimurium. Cluster analysis performed for monophasic variant retrieved only one major clonal complex that grouped almost all the MLVA profiles,

confirming the lack of discrimination of STTR3, STTR10 and STTR9 loci, as previously demonstrated by Barco and colleagues (Barco *et al.* 2015) On the contrary, for *S.* Typhimurium, the diversity of MLVA profiles was conferred by all the five loci (SDI > 0.6).

Hopkins and colleagues (Hopkins *et al.* 2007) reported that since MLVA is a highly discriminatory method, some minor changes in targeted loci can be found in outbreak-related strains. Therefore, for *Salmonella* the authors proposed a difference of no more than two repeats at one single locus to identify isolates that are part of the same outbreak. As the MLVA protocol for typing of monophasic *S.* Typhimurium includes three out of five highly stable loci, the correct interpretation of MLVA profiles can be critical especially for large scale epidemiological investigations and for source attribution studies. In our work, MLVA analysis of *S.* Typhimurium assigned only four genotypes in common between human and non-human isolates (pig or surface water), while, for monophasic *S.* Typhimurium about half of genotypes were shared by human isolates and the isolates from other sources (pig, turkey, poultry, water, etc.). These findings support the conclusion of Barco and colleagues (Barco *et al.* 2015) that 5-loci MLVA scheme applied to monophasic *S.* Typhimurium may not provide a sufficient resolution for source attribution studies. In the definition of an outbreak caused by monophasic *S.* Typhimurium, Cito and colleagues (Cito *et al.* 2016) reported the PFGE profile and the R-type. Another study confirmed the insufficient resolution of MLVA scheme in the characterization of *S.* 4,[5],12:i:- isolates, given that isolates with considerable diversity in terms of single-nucleotide polymorphisms (SNPs) were indistinguishable by their MLVA profiles (Petrin *et al.* 2019).

The EFSA-ECDC report from 2017 (EFSA and ECDC 2018) describing the prevalence of antimicrobial resistance in EU included data on *Salmonella* isolated from humans, fattening pigs and calves. In 2017 approximately 40% of human *S.* Typhimurium isolates, and 80% of monophasic *S.* Typhimurium were resistant to multiple antimicrobials. In pigs, multi-drug resistance (MDR) was observed in approximately 60% of *S.* Typhimurium and 77% of the monophasic variant isolates (EFSA and ECDC 2019). In recent years, there has been an overall decline in the level of antimicrobial resistance in serovar *S.* Typhimurium in several European countries related to a decrease in the number of isolates of multiresistent DT104 (Meakins *et al.* 2008). To some extent this reduction has been counterbalance by the increase in prevalence of monophasic *S.* Typhimurium that expresses resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (Mueller-Doblies *et al.* 2018, Lucarelli *et al.* 2010). In our study, we found that more than 30% of *S.* Typhimurium were resistant to four antibiotics (ASSuT resistance type) and only 5.6% were additionally resistant to chloramphenicol (ACSSuT resistance type). Overall, the majority of monophasic *S.* Typhimurium isolates appeared to show a multidrug resistance phenotype while the majority of *S.* Typhimurium isolates were resistant to only a few antimicrobial drugs (data not showed).

In conclusion, based on the characterization of human and non-human *S.* Typhimurium and monophasic *S.* Typhimurium strains obtained over a 6-year surveillance at the regional level, we found antimicrobial resistance results comparable to those reported by the last EFSA report from 2017. The MLVA 5-loci scheme resulted suitable for *S.* Typhimurium typing but the discriminatory power observed for monophasic *S.* Typhimurium was insufficient when used as an exclusive typing method in epidemiological studies. Use of powerful discrimination typing method based on Whole Genome Sequencing (WGS) such as SNPs analysis or core genome MLST are recommended to identify epidemiological relationship of monophasic *S.* Typhimurium isolates.

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