Austin Journal of Microbiology

Open Access

Austin Publishing Group

Research Article

Genetic Characterization by Pulsed-Field Gel Electrophoresis (PFGE) of Emerging *Salmonella* Serotypes in Northwest Italy

Tramuta C*, Bianchi DM, Pitti M, Cazzaniga GF and Decastelli L

S.C. Sicurezza e Qualità degli Alimenti, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Italy

***Corresponding author:** Clara Tramuta, Sicurezza e Qualità degli Alimenti, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Via Bologna 148, 10154, Turin, Italy

Received: April 28, 2022; **Accepted:** May 23, 2022; **Published:** May 30, 2022

Abstract

Here we investigated the genetic relatedness of emerging *Salmonella* serotypes of human origin circulating in northwest Italy and created a regional database to facilitate foodborne outbreak investigations and to monitor outbreaks at an earlier stage. A total of 112 strains of S. Derby (n=34), S. Infantis (n=38), and S. Napoli (n=40) analysed in hospital laboratories between 2016 and 2018 were characterized by Pulsed-Field Gel Electrophoresis (PFGE). Cluster analysis indicated high genetic similarity (≥83%) among the three circulating strains of S. Derby (88%), S. Infantis (95%), and S. Napoli (95%). Most of the isolates possessed the same or similar fingerprinting in each cluster, suggesting that foods of animal origin are a source of human infection. PFGE proved a powerful and discriminatory tool for revealing genetic relationships among the emerging serotypes and for monitoring and preventing the spread of *Salmonella*.

Keywords: Emerging *Salmonella* Serotypes; Genetic Characterization; Human Strains; Northwest Italy; PFGE

Background

The annual report of the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) ranks *Salmonella* infection due to *Salmonella enterica* as the second most common foodborne pathogen in humans in the European Union [1]. *Salmonella enterica* is present in nature with more than 2600 different serotypes. Although all *Salmonella* serotypes are potentially pathogenic to humans and animals, few cause infection [2]. *Salmonella* can infect the intestines of animals and humans where it causes salmonellosis. Salmonellosis in humans is generally contracted through the consumption of contaminated water and foods of animal origin (e.g., poultry, fish, eggs, beef, dairy products) and raw or slightly cooked fruit and vegetables [3]. Transmission of *S. enterica* to humans and animals can also occur through contact with surfaces, tools, and food handled by infected people.

There are six subspecies of *S. enterica: S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica*) [2]. The serovars most frequently associated with human illness are: *S. enterica* serotype Enteritidis, Typhimurium and its monophasic variant (4, [5], 12: i:-), which are becoming a major public health problem [4]. A retrospective study by the Regional Center for *Salmonella* typing (Centro di Riferimento Regionale per la tipizzazione delle Salmonelle - CeRTiS) reported that the most frequent serovars of 450 *Salmonella* isolates of human origin in 2018 were: S. 4, [5], 12: i:- (48%), S. Enteritidis (18%), S. Typhimurium (8%), S. Derby (5%), S. Napoli (3%), and S. Infantis (2%). Salmonellosis caused by S. Derby, S. Infantis, and S. Napoli infection in humans have been reported in Piedmont, with an increase (at least of 30%) in the prevalence of these three emerging serotypes between 2015 and 2018 (CeRTiS data not published). The CeRTiS assembled a large collection of *S. enterica* strains of these emerging serotypes isolated from biological samples of patients hospitalized in healthcare facilities in Piedmont (northwest Italy).

Several molecular typing methods to investigate the molecular epidemiology of bacterial pathogens [5] have proved effective in determining clonal and strain distribution in various environments and sources. Pulsed-Field Gel Electrophoresis (PFGE) is the most widely used molecular method to investigate widespread outbreaks of bacterial foodborne illness. It provides one of the most suitable approaches to characterize strains, particularly by laboratories where Next-Generation Sequencing (NGS) techniques are not available. By virtue of its specificity, PFGE can be employed to detect outbreaks and control them at an earlier stage, as well as enhance the detection of geographically dispersed outbreaks [6]. Owing to the stability of generated profiles and the method's discriminatory power and reproducibility, PFGE is the gold standard for genotyping *Salmonella*. PFGE detects genomic differences between isolates by digesting the whole DNA using restriction enzymes to yield strain-specific fragment patterns. It is these differences accumulated by genetic variation that cause slight, detectable differences between DNA fingerprint patterns [7]. With this study we wanted to characterize emerging *S. enterica* subsp. *enterica* serovars, S. Derby, S. Infantis, and S. Napoli of human origin by PFGE and evaluate genetic correlations between the strains circulating in northwest Italy. Also, we wanted to create a regional database that could aid in facilitating foodborne outbreak investigations and monitor them at an earlier stage.

Citation: Tramuta C, Bianchi DM, Pitti M, Cazzaniga GF and Decastelli L. Genetic Characterization by Pulsed-Field Gel Electrophoresis (PFGE) of Emerging Salmonella Serotypes in Northwest Italy. Austin J Microbiol. 2022; 7(1): 1037.

Materials and Methods

Salmonella strains

A total of 112 *Salmonella* strains obtained from the faeces of 112 patients (mean age 50 years, range 1-89) were isolated in hospital laboratories in Piedmont between 2016 and 2018. The isolates, delivered refrigerated to the CeRTiS on sheep blood agar plates and tryptic soy agar plates, were analyzed by Mass Spectrometry MALDI-TOF (VITEK® MS Biomerieux) [8], in order to confirm their species. The method is based on the analysis of positive ions derived from ribosomal proteins and the spectrum of the tested strain is compared with a library of reference spectra, in order to be identified. Strains were then serotyped according to ISO 6579-3 with the Kaufmann-White [9,10] scheme, using O and H antisera (Statens Serum Institut). The identification of serotypes is based on O (somatic/cell wall) antigens that consist of the lipopolysaccharide-protein chains exposed on the cell surface and H (flagellar) antigens. The different antigens are identified by numbers and letters: each serotype is given an antigenic formula and classified into a group.

PFGE analysis

Characterization was performed by PFGE analysis of emerging *Salmonella* strains isolated according to a standardized PulseNet protocol [11]. Briefly, equal volumes of 1% agarose (SeaKem Gold Agarose, Lonza) were mixed with 100µL cell standardized suspension to form plugs. The bacteria within the plugs were lysed with cell lysis buffer and incubated at 55°C for 2h. The plugs were washed with sterile deionised water and TE buffer. Plug slices (1.5mm thick) were then digested with 10 units of restriction enzyme *Xba*I (Thermo Scientific) at 37°C for 2h and loaded onto a 1% agarose gel (SeaKem Gold Agarose). PFGE was performed on a CHEF Mapper system (Bio-Rad) under the following conditions: initial switch time 6s-final switch time 24s, gradient 6V/cm, included angle 120, running time 20h at 14°C. The agarose gel was then stained with GelRed (Biotium) and photographed under ultraviolet transillumination (Gel-Doc Bio-Rad). *Xba*I restricted-*Salmonella* Braenderup, strain H9812 was used as the DNA size marker and positive control. The PFGE patterns obtained from the 112 strains were compared using Bionumerics version 7.6 software (Applied Maths) with the Dice coefficient [12] with 2% band tolerance and 2% optimization and the unweighted pair group method with arithmetic averages (UPGMA) [13]. PFGE types were assigned using an ad hoc created regional nomenclature. PFGE profiles with a coefficient of similarity ≥83% were considered genetically related, while *Salmonella* strains sharing 100% similarity were considered identical and were assigned to the same PFGE type.

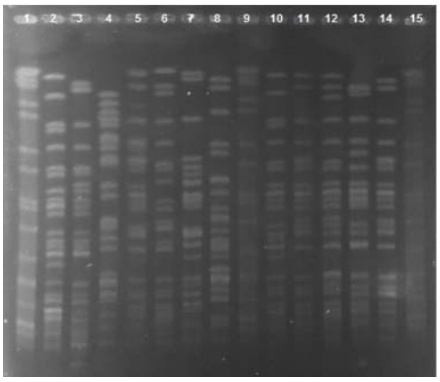

Figure 1: PFGE banding patterns of representative emerging *S. enterica* strains, showing distinct polymorphic bands ranging from 40 to 1100 bp (lanes 2-8, 10-14) and S. Branderup used as the standard ladder (lanes 1, 9, 15).

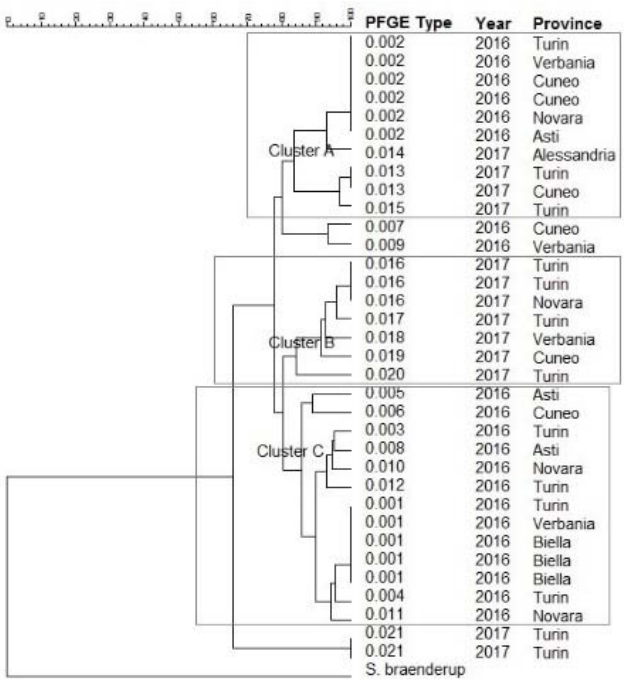

Figure 2: Dendrogram of 34 pulsed-field gel electrophoresis-based profiles of *Salmonella enterica* serovar Derby strains isolated from humans in Piedmont between 2016 and 2017.

Results

A total of 112 *Salmonella* human strains were identified as S. Derby (n=34), S. Infantis (n=38), and S. Napoli (n=40). PFGE analysis with *XBa*I restriction enzyme was performed to determine the genetic relatedness of emerging *S. enterica* subsp. *enterica* serovars, S. Derby, S. Infantis, and S. Napoli of human origin and to evaluate genetic correlations between the strains circulating in Piedmont. All isolates had appreciable restricted digestion patterns ranging from approximately 40 to 1100 kb (Figure1). Analysis of the dendrogram highlighted a similarity coefficient of 66% for the 34 *S. enterica* subsp. *enterica* serovar Derby strains (Figure 2). A total of 21 PFGE types were identified, the most frequent of which were 0.002 (17.6%), 0.001 (14.7%), 0.016 (8.8%), 0.013 (5.9%), and 0.021 (5.9%). All remaining PFGE types were a single strain. The strains with genetic homologies ≥83% were grouped into three clusters (A, B, C). Cluster A consisted of ten strains that shared four different PFGE profiles, eight of which with 100% genetic similarity (five strains with PFGE profile 0.002 and two strains with PFGE profile 0.013). Cluster B consisted of seven strains, three of which with 100% pattern homology (PFGE type 0.016); cluster C included 13 strains, five of which had an identical profile (PFGE type 0.001). The strains in clusters A and C were isolated from samples taken in nearly all eight provinces of the region, whereas cluster B strains were isolated from samples taken in four provinces (Figure 2). The distribution of the three clusters over time was mapped by year of isolation: cluster A strains were isolated in

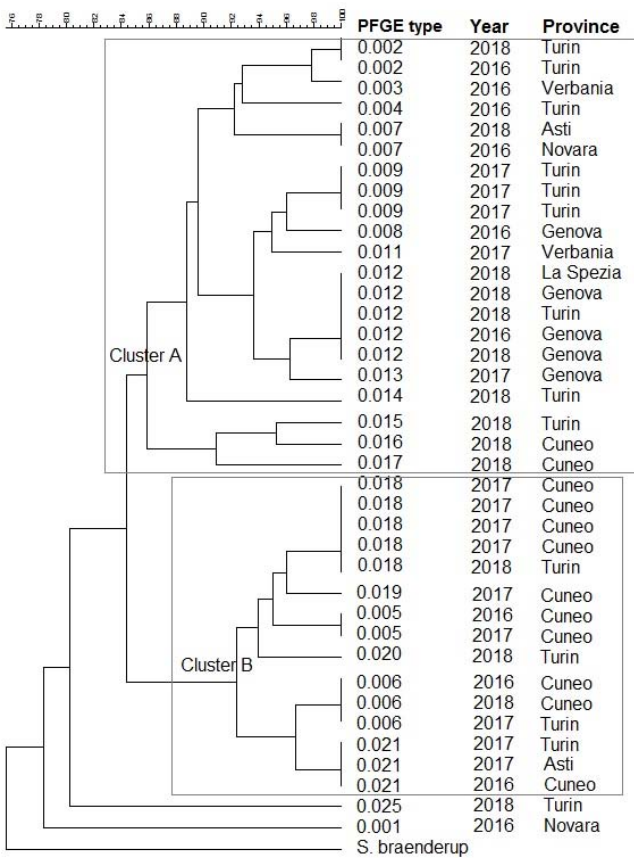

Figure 3: Dendrogram of 38 pulsed-field gel electrophoresis-based profiles of *Salmonella enterica* serovar Infantis strains isolated from humans in Piedmont between 2016 and 2018.

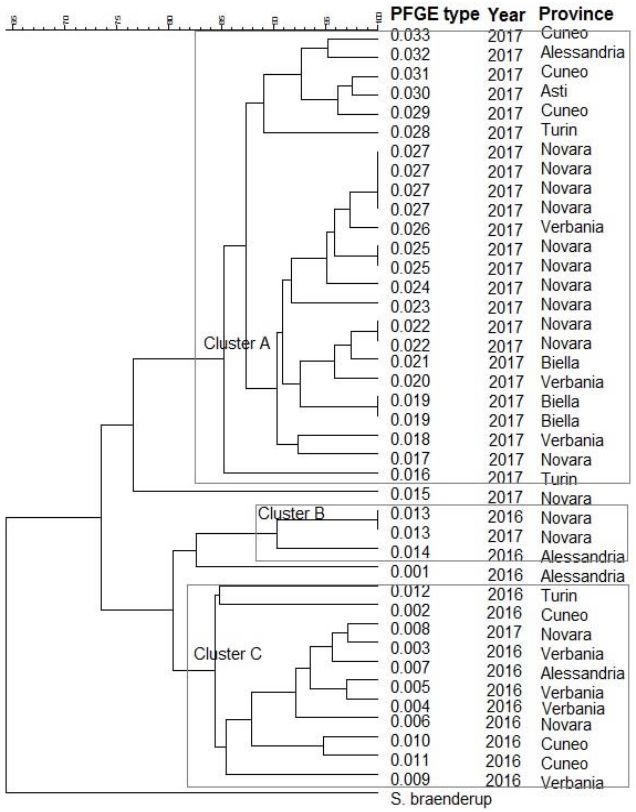

Figure 4: Dendrogram of 40 pulsed-field gel electrophoresis-based profiles of *Salmonella enterica* serovar Napoli strains isolated from humans in Piedmont between 2016 and 2018.

2016 (60%) and 2017 (40%); cluster B strains in 2016 (100%), cluster C strains in 2017 (100%) (Figure 2).

Analysis of the dendrogram showed a coefficient of similarity of 78% between isolates of 38 *S. enterica* subsp. *enterica* serovar Infantis (Figure 3). A total of 21 PFGE types were identified, the most frequent of which were: 0.012 (13.2%), 0.018 (13.2%), 0.006 (7.9%), 0.009 (7.9%), 0.021 (7.9%), 0.002 (5.3%), 0.005 (5.3%), and 0.007 (5.3%). All the remaining PFGE types were a single strain. We set the cut-off at 83% similarity and identified two main clusters: cluster A (86% similarity) and cluster B (92% similarity), which accounted for 55.3% and 39.5%, respectively, of the isolates. Cluster A included 21 strains, for a total of 13 PFGE types: PFGE type 0.012 was observed in five strains, PFGE type 0.009 in three strains, PFGE types 0.007 and 0.002 in two strains. Cluster B included 15 strains, for a total of six PFGE types: PFGE type 0.018 in six strains, PFGE type 0.006 and 0.021 in three strains, and PFGE type 0.005 in two strains. Cluster A consisted predominantly of strains isolated from samples from one province (Turin) (43%). Cluster B included strains isolated predominantly from two provinces (Cuneo, 66.7%, and Turin, 26.7%) (Figure 3). The cluster A and the cluster B strains were isolated in all three years: 42.9% of the cluster A strains were isolated in 2018, 33.3% in 2016, and 23.8% in 2017. Cluster B strains were isolated predominantly in 2017 (66.7%) and 2016 (26.7%) (Figure 3).

There were 33 different PFGE patterns that had 73.5% homology among the 40 *S. enterica* subsp. *enterica* serovar Napoli, (Figure 4). The dendrogram showed the presence of three main clusters consisting of 38 strains that shared 83% genetic homology: cluster A (85% similarity), cluster B (89.5 % similarity), and cluster C (83% similarity). Cluster A included 24 strains (60%) with a total of 18 PFGE types: four strains with PFGE profile 0.027 showed 100% genetic similarity, as did the six strains with PFGE type 0.025 (n=2), 0.022 (n=2), and 0.019 (n=2). Cluster B included three strains with two PFGE types: two strains appeared genetically identical (PFGE type 0.013) and 89.5% similar to the strain with a PFGE profile 0.014. Finally, cluster C included 11 genetically highly related strains, each with a different PFGE type. Cluster A included strains from all eight provinces of Piedmont, especially from Novara (46%). Cluster B included strains isolated from two provinces (Alessandria and Novara), while cluster C included strains from four provinces (Alessandria, Cuneo, Novara, Vercelli) (Figure 4). Cluster A strains were isolated in 2016 and clusters B and C in 2017 (Figure 4).

Discussion

As found for the rest of Europe S. Infantis accounted for 2.5% of all *Salmonella* serotypes [1], while S. Napoli and S. Derby, which are not included among the EU target serovars, are among the most frequent serovars isolated from humans in Italy [14]. Based on the distribution of serovars collected by the ENTER-NET network from humans in Italy, the most common serovars detected between 2016 and 2018 were: S. 4, [5],12:i:- (35.0%), S. Enteritidis (14.7%),

S. Typhimurium (10.2%), S. Napoli (4.9%), S. Derby (3.0%), and S. Infantis (2.7%) [14]. The CeRTiS reported an increase in the prevalence of S. Derby, S. Infantis, and S. Napoli isolated from human samples in Piedmont since 2015. Prevention, control, and monitoring programs to reduce the presence of *Salmonella* strains in the food production chain, coupled with better knowledge of the regional features of circulating *Salmonella* serotypes and contamination sources, can help to inform food safety intervention priorities and implement appropriate control measures [15]. Furthermore, molecular typing is a useful infection control tool for establishing the clonal relationship between strains of the same serotype in epidemiological investigations of *S. enterica*. While sophisticated analytical techniques such as Whole Genome Sequencing (WGS) can be used in studying the epidemiology of salmonellosis, PFGE is the gold standard recognized by PulseNet International [16]. PFGE has been successfully employed for molecular typing of *Salmonella* serotypes isolated from different sources worldwide. Recent studies in Italy showed high similarity among S. infantis, S. Derby, and S. Napoli strains isolated from different sources characterized by PFGE [17-19]. With this study we wanted to characterise by PFGE human isolates of *S. enterica* subsp. *enterica* of three emerging serotypes (S. Derby, S. Infantis, S. Napoli) in Piedmont and to evaluate the genetic correlations between strains circulating in the area. For each serotype, only bacterial strains with a correlation coefficient ≥83% were considered to be genetically closely related. Our study results are shared by epidemiological evidence that supports the clonal distribution of these emerging *Salmonella* serotypes. Analysis of restriction profiles of the 34 S. Derby strains identified a total of 20 pulsotypes, grouped in three main clusters with a similarity of 78%. The broad genetic similarity observed in this serotype is in line with the literature and shared by a previous study that demonstrated the relationship between porcine and human cases of salmonellosis caused by S. Derby [17]. A total of 19 pulsotypes identified by PFGE for the 38 strains of S. Infantis in the two main clusters showed similarities of 84.5%. Our data are consistent with previous studies reporting that S. Infantis isolated from humans, animals, and the environment had a homology >90% [18]. Wide genetic similarity was also shown by examination of the dendrogram obtained by analysis of the 40 strains of S. Napoli: the 33 pulsotypes were grouped into three main clusters (similarity coefficient of 73.5%). Overlapping data were reported in an Italian study on numerous strains of this serotype isolated in Italy between 2011 and 2015. Human and environmental strains of S. Napoli belonging mainly to cluster A in northern Italy and cluster B in central Italy suggest direct infection [19]. The geographical distribution of the clusters was mapped for each serotype, taking into account the geographical province where the bacterial strain was isolated. In general, we observed a fairly heterogeneous distribution of pulsotypes across the provinces.

The clusters were then compared for year of strain isolation. We noted that all cluster C S. Derby strains were isolated in 2016, while the cluster B strains were isolated in 2017. Similar observations were made for S. Napoli; the cluster B and C strains were isolated prevalently in 2016 and the cluster A strains in 2017. These data suggest a possible correlation between year of isolation of the S. Derby and S. Napoli strains in Piedmont and their cluster formation. PFGE proved a powerful discriminatory tool for revealing genetic relationships among emerging *Salmonella* serotypes other than for monitoring the incidence and dissemination of S. Derby, S. Infantis, and S. Napoli throughout the region. As the spread of these *Salmonella* clones has been observed in Piedmont over the last three years, we will continue to characterize emerging *Salmonella* serotype isolates from humans and animals. The uncontrolled spread of these emerging *Salmonella* clones in the environment and via associated foodborne illnesses poses a serious public health concern in the areas where they circulate.

Conclusions

The present study revealed the high similarity shared by three emerging *Salmonella* strains (S. Derby, S. Napoli, S. Infantis) isolated from humans in northwest Italy. The study data were used to create a database for identifying outbreaks at an early stage. Two or three similar or identical PFGE profiles (coefficient of similarity ≥83%) were found for each *Salmonella* serotype in several areas of the region, highlighting the presence of genetically related *Salmonella* strains. The data obtained by PFGE strongly suggest that closely related clones are circulating and that foods of animal origin (e.g., poultry meat, eggs, pork) are the probable source of human infection.

Funding

This study was funded by Regione Piemonte D.D. 354 28/05/2018.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

1. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union one health 2020 zoonoses report. EFSA J. 2021; 19: 6971.
2. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, et al. Host Adapted Serotypes of Salmonella enterica. Epidemiol Infect. 2000; 125: 229-255.
3. Bintsis T. Foodborne pathogens. AIMS Microbiol. 2017; 3: 529-563.
4. Graziani C, Mughini-Gras L, Owczarek S, Dionisi AM, Luzzi I, Busani L. Distribution of Salmonella enterica isolates from human cases in Italy, 1980 to 2011. Euro Surveill. 2013; 18: 20519.
5. Ranjbar R, Karami A, Farshad S, Giammanco GM, Mammina C. Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. New Microbiol. 2014; 37: 1-15.
6. Mughini-Gras L, Smid J, Enserink R, Franz E, Schouls L, Heck M, et al. Tracing the sources of human salmonellosis: a multi-model comparison of phenotyping and genotyping methods. Infect Genet Evol. 2014; 28: 251-260.
7. Zakaria Z, Hassan L, Sharif Z, Ahmad N, Ali RM, Husin SA, et al. Analysis of Salmonella enterica serovar Enteritidis isolates from chickens and chicken meat products in Malaysia using PFGE, and MLST. BMC Vet Res. 2020; 16: 393.
8. Krishnamurthy T, Ross PL. Rapid identification of bacteria by direct matrix-assisted laser desorption ionization mass spectrometric analysis of whole cells. Rapid Commun Mass Spectrom. 1996; 10: 1992-1996.
9. ISO 6579-3. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella Guidelines for serotyping of Salmonella spp. Geneva, Switzerland. 2014.
10. Kauffmann-White and Le Minor. Antigenic formulae of the salmonella serovars. Bergey's Manual of Systematic Bacteriology. 1984; 1: 429-445.
11. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for

PulseNet. Foodborne Pathog Dis. 2006; 3: 59-67.

12. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA. 1979; 76: 5269-5273.

13. Sneath PHA, Sokal RR. Numerical Taxonomy. San Francisco: W.H. Freeman. 1973; 230-234.

14. Leati M, Zaccherini A, Ruocco L, D'Amato S, Busani L, Villa L, et al. The challenging task to select Salmonella target serovars in poultry: the Italian point of view. Epidemiol Infect. 2021; 149: e160.

15. Machado SCA, Pereira VLA, Aquino MHC, Santos AFM, Rodrigues DP, Giombelli A, et al. Serotyping and Genotyping of Salmonella Strains Isolated from Broilers, Chicken Carcasses Before and After Chilling, and Frozen Chicken Breasts Produced in the States of Mato Grosso do Sul and Santa Catarina, Brazil. Braz. J Poultry Sci. 2017; 19: 135-142.

16. Tang S, Orsi RH, Luo H, Ge C, Zhang G, Baker RC, et al. Assessment and Comparison of Molecular Subtyping and Characterization Methods for Salmonella. Front Microbiol. 2019; 10: 1591.

17. Bonardi S, Alpigiani I, Bruini I, Barilli E, Brindani F, Morganti M, et al. Detection of Salmonella enterica in pigs at slaughter and comparison with human isolates in Italy. Int. J. of Food Microbiol. 2016; 218: 44-50.

18. Dionisi AM, Lucarelli C, Benedetti I, Owczarek S, Luzzi I. Molecular characterization of multidrug-resistant Salmonella enterica serotype Infantis from humans, animals and the environment in Italy. Int J Antimicrob Agents. 2011; 38: 384-399.

19. Sabbatucci M, Dionisi AM, Pezzotti P, Lucarelli C, Barco L, Mancin M, et al. Molecular and Epidemiologic Analysis of Reemergent Salmonella enterica Serovar Napoli, Italy, 2011-2015. Emerg Infect Dis. 2018; 24: 562-565.