TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2022) 46: 826-829

doi:10.55730/1300-0128.4259

Typing of rarely isolated *Salmonella* serotypes with MLST

Seyyide SARIÇAM İNCE*, İnci Başak MÜŞTAK, Hamit Kaan MÜŞTAK
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Received: 30.05.2022 • **Accepted/Published Online:** 16.10.2022 • **Final Version:** 08.12.2022

Abstract: Salmonellosis caused by nontyphoidal *Salmonella* is the second most common zoonotic infection worldwide. The major foodborne route of this infection is the consumption of contaminated poultry meat and egg. Therefore, monitoring and control of *Salmonella* agents in poultry populations is critical to reduce the risks for public health. In the poultry industry, the most common serotypes in *Salmonella* infections are *Salmonella enterica* subsp. *enterica* serotype Enteritidis and Typhimurium. In recent years, other frequently isolated *Salmonella* serotypes have also increased in Turkey because of the *Salmonella* control programs regulated by many countries. Hence, other *Salmonella* serotypes (except for the most frequently isolated serotypes, *S.* Enteritidis, *S.* Typhimurium) may become dominant in the future, so their epidemiology needs to be investigated. In this study, Multilocus Sequence Typing (MLST) was used to type rarely found *Salmonella* serotypes by sequencing seven housekeeping genes for each strain. The broiler chicken originated 54 *Salmonella* strains were used as *Salmonella* Bazenheid (n = 4), *Salmonella* Burgas (n = 5), *Salmonella* Gombe (n = 8), *Salmonella* Hayindogo (n = 9), *Salmonella* Jamaica (n = 4), *Salmonella* Kamoru (n = 10), *Salmonella* Paris (n = 2), and *Salmonella* Stuttgart (n = 12). This study is the first study to use MLST to serotype *S.* Bazenheid (sequence type, ST544), *S.* Burgas (ST1814), *S.* Gombe (ST413), *S.* Hayindogo (ST1959), *S.* Jamaica (ST32), *S.* Kamoru (ST1995), *S.* Paris (ST82), and *S.* Stuttgart (ST19) in our country. The presence of a single sequence type for each serotype suggests that these serotypes may be the dominant sequence types in broiler chicken flocks in Turkey.

Key words: Broiler chicken, poultry, *Salmonella*, Multilocus sequence typing

1. Introduction

Salmonella genus belongs to *Enterobacteriaceae* family, which consists of two species: *Salmonella enterica (S. enterica)* and *Salmonella bongori.* Of the six subspecies of *S. enterica*, most of the zoonotic *Salmonella* agents are found in *S. enterica* subsp. *enterica*. There are approximately 2700 different serotypes within the *Salmonella* genus [1].

Salmonellosis infection caused by nontyphoidal *Salmonella* members is the second most common zoonotic infection worldwide with 94 million cases and approximately 155,000 human deaths annually [2,3]. Salmonellosis due to foodborne outbreaks most commonly causes complications such as enteric fever, stomach cramps, diarrhea, sickness, and vomiting. Mortality is less than 1%. In poultry, there are different clinical manifestations, including weakness, loss of appetite, arthritis, lameness, septicemic diarrhea and death [4].

Contact with infected animals and humans directly spreads the agent while contact with carrier parameters, such as animal products and environmental factors indirectly spread the agent. The intestines are the reservoir in both domestic and wild animals, with nontyphoidal *Salmonella* serotypes being colonized in the intestine and being scattered through feces. The bacteria are transmitted to the food chain by food (eat, milk, eggs, vegetables, fruits, etc.) and drinking water contamination [4]. The major foodborne route of salmonellosis infection is contaminated poultry meat and egg consumption. Therefore, monitoring and control of *Salmonella* agents in poultry populations is very important to reduce the risks for public health [5]. *Salmonella* control programs in poultry populations aim to reduce the rate of salmonellosis cases. According to the EFSA 2011 zoonotic agents report, *Salmonella* control programs in European countries reduced the incidence of salmonellosis in humans from 37.9% (2007) to 5.4% (2010). This shows the success of *Salmonella* control programs implemented in poultry populations between 2008–2011 [1]. According to the EFSA 2017 zoonotic agents report, the two most commonly reported *Salmonella* serovars in human cases were *S.* Enteritidis (48.5%) and *S.* Typhimurium (21.8%), followed by *S.* Infantis, *S.* Newport, and *S.* Kentucky [6]. Between 2011 and 2016, the proportion of salmonellosis cases caused by *S.* Infantis increased from 2.2% to 2.4%,

* Correspondence: s.saricam-92@hotmail.com

S. Newport increased from 1.0% to 1.1%, and *S*. Kentucky from 0.7% to 0.8%. According to the EFSA 2017 report, the most common serotypes in broiler chicken flocks were *S*. Enteritidis and *S*. Typhimurium while the proportion of salmonellosis cases caused by *S*. Infantis increased from 2.17% (2011) to 2.4% (2016) in poultry. The proportion of *Salmonella* serotypes other than these three serotypes was 23.7% in 2011 and 27.3% in 2016. The 15.2% increase in the prevalence of other rare *Salmonella* serotypes between 2011 and 2016 suggests that serotypes with low isolation rates may become dominant in the future. However, almost no genotypic typing studies have examined rarely observed *Salmonella* serotypes [1,6].

Salmonella agents have been serotyped using conventional serotyping analysis based on agglutination of surface (Vi), somatic (O) and flagellin (H) antigens with specific antisera according to the Kauffmann-White-Le Minor scheme. However, this method is time-consuming, costly and labor-intensive. Therefore, molecular methods are frequently used for typing *Salmonella* agents [7]. Genotypic analysis methods such as Ribotyping, RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), PFGE (Pulsed-Field Gel Electrophoresis) and MLST (Multilocus Sequence Typing) can identify, correlate and evaluate *Salmonella* isolates based on their origin. MLST is the most commonly used genotyping method in epidemiological and phylogenetic association studies of *Salmonella* species. The seven housekeeping gene fragments (*aro*C, *dna*N, *hem*D, *his*D, *pur*E, *suc*A and *thr*A) have been characterized in *Salmonella enterica* subsp. *enterica* by MLST analysis. The nucleotide sequences of these genes can then be compared to show the diversity of serotypes or strains in genetic clusters and to determine the transmission routes of each strain [8]. A major advantage of MLST analysis is that the results can be easily stored in an electronic database so that the results of any typed strain can be compared with existing types to indicate phylographic and host specificity. These databases also allow new sequence types to be recorded and record of the current situations according to global information changes [9,10].

This study characterized *S*. Bazenheid, *S*. Burgas, *S*. Gombe, *S*. Hayindogo, *S*. Jamaica, *S*. Kamoru, *S*. Paris and *S*. Stuttgart serotypes isolated from broiler chicken. These have rarely been identified by MLST analysis.

2. Materials and methods

2.1. *Salmonella* strains and conventional serotyping

Salmonella strains were derived from Ankara University, Faculty of Veterinary Medicine, Microbiology Department culture collection, between 2017–2018. Totally, the broiler chicken originated 54 *Salmonella* strains were used as follows: *S*. Bazenheid (n = 4), *S*. Burgas (n = 5), *S*. Gombe (n = 8), *S*. Hayindogo (n = 9), *S*. Jamaica (n = 4), *S*. Kamoru (n = 10), *S*. Paris (n = 2), and *S*. Stuttgart (n = 12). The strains were revived overnight at 37 °C on Nutrient agar plates. All isolates were confirmed by conventional serotyping with commercial *Salmonella* polyvalent and monovalent O and H antisera (SSI, Denmark) according to the Kauffmann White Le Minor Scheme [11].

2.2. DNA extraction

DNA extraction was performed with GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. DNA samples were stored at –20 °C until PCR analysis.

2.3. Multilocus sequence typing analysis

The housekeeping genes *aro*C (826 bp), *dna*N (833 bp), *hem*D (666 bp), *his*D (894 bp), *pur*E (510 bp), *suc*A (643 bp), and *thr*A (852 bp) were amplified for each strain using primer sequences obtained from *Salmonella enterica* MLST at PubMLST database [12]. BigDye Terminator v3.1 Cycle Sequencing Kit was used for sequence analysis. The PCR products were purified by Sephadex (Oxoid, UK) gel filtration and sequenced on an ABI 3500 genetic analyzer system (Applied Biosystems, USA). CLC Main Workbench version 8.1.0 software was used for editing the sequence data. For each isolate, the seven housekeeping gene sequences were loaded into the PubMLST database to compare and determine the sequence type (ST).

3. Results

The MLST analysis identified allelic sequences and sequence types for 54 *Salmonella* strains. Similar alleles and sequence type were found for each serotype with combinations of these alleles. Table shows the sequence types of the strains (*S*. Bazenheid strains ST544, *S*. Burgas strains ST1814, *S*. Gombe strains ST413, *S*. Hayindogo strains ST1959, *S*. Jamaica strains ST32, *S*. Kamoru strains ST1995, *S*. Paris strains ST82, and *S*. Stuttgart strains ST19).

4. Discussion

As one of the most common zoonotic infections worldwide, Salmonellosis poses important public health risks. The major foodborne route of infection is contaminated poultry meat and egg consumption. Therefore, monitoring and control of *Salmonella* agents in poultry populations is critical to reduce public health risks. According to the EFSA 2017 zoonotic agents report, the most common serotypes in broiler chicken flocks are *S*. Enteritidis and *S*. Typhimurium. In addition, the proportion of other nontyphoidal *Salmonella* serotypes have recently increased, especially *S*. Infantis [5,6]. This requires investigation of the epidemiology of rare *Salmonella* serotypes.

Molecular typing methods such as MLST can be used for phylogenetic investigation of *Salmonella* agents and

Table. MLST results of *Salmonella* strains.

Number of strain	Serotype	*aro*C	*dna*N	*hem*D	*his*D	*pur*E	*suc*A	*thr*A	Sequence type (ST)
4	*S.* Bazenheid	4	164	134	195	132	46	158	**544**
5	*S.* Burgas	2	13	10	540	459	416	34	**1814**
8	*S.* Gombe	15	70	93	78	113	6	68	**413**
9	*S.* Hayindogo	313	445	3	507	480	388	333	**1959**
4	*S.* Jamaica	17	18	22	17	5	21	19	**32**
10	*S.* Kamoru	5	101	18	561	36	12	12	**1995**
2	*S.* Paris	41	42	43	12	9	12	2	**82**
12	*S.* Stuttgart	10	7	12	9	5	9	2	**19**

determination of transmission routes. In many studies, *Salmonella* serotypes commonly observed in poultry have been examined by MLST analysis. These include *S.* Enteritidis, *S.* Typhimurium, and *S.* Infantis [2,13,14]. The present study, however, used MLST analysis to genotype rarely isolated *Salmonella* serotypes from broiler chicken flocks. A total of 54 strains were used including *S.* Bazenheid (n = 4), *S.* Burgas (n = 5), *S.* Gombe (n = 8), *S.* Hayindogo (n = 9), *S.* Jamaica (n = 4), *S.* Kamoru (n = 10), *S.* Paris (n = 2), and *S.* Stuttgart (n = 12).

To our knowledge, this study is the first MLST analysis for these *Salmonella* serotypes. We therefore used the PubMLST database to compare the obtained genotypes [12]. The database had no genotype record for the *S.* Bazenheid, *S.* Hayindogo and *S.* Jamaica serotypes identified in this study. In addition, it is the first study to serotype *S.* Bazenheid, *S.* Burgas, *S.* Gombe, *S.* Hayindogo, *S.* Jamaica, *S.* Kamoru, *S.* Paris, and *S.* Stuttgart by MLST analysis.

All the *S.* Bazenheid and *S.* Hayindogo strains were ST544 and ST32 genotypes, respectively. Previously, strains recorded as ST544 genotype were known to be *S.* Molade and *S.* Duisburg strains isolated from human or environmental sources. All poultry strains recorded as ST1959 genotype were *S.* Liverpool strains while *Salmonella* strains recorded as ST32 in the PubMLST database included different serotypes, such as *S.* Derby, *S.* Enteritidis, and *S.* Infantis. These originate particularly from human, poultry, and environmental sources [12]. This finding suggests that MLST analysis may not be suitable for distinguishing between these serotypes, which are frequently isolated in Turkey.

Previously, one *S.* Burgas of unknown origin and S. Gombe and *S.* Kamoru strains of human origin have been recorded in the PubMLST database as ST6400, ST6473 and ST487, respectively. In our study, however, all the *S.* Burgas strains were ST1814 genotype whereas strains recorded as ST1814 genotype in the database are known to be *S.* Salford, *S.* Nchanga, and *S.* Brooklyn serotypes. Among these strains, five foodborne *S.* Salford strains were isolated in Turkey between 2002 and 2012 and recorded in the database. All the *S.* Gombe and *S.* Kamoru strains isolated in the present study were ST413 and ST1995 genotype, respectively, whereas the ST413 genotype strains recorded in the database are known to be serotypes like *S.* Mbandaka, *S.* Weltevreden, *S.* Menden, *S.* Enteritidis, and *S.* Berta [12]. Finally, the strains of ST1995 genotype recorded in the database are known to be *S.* Coeln.

Previously, one *S.* Paris strain of human origin as ST6396 genotype and one *S.* Stuttgart strain of human origin as ST4792 genotype have been recorded in the PubMLST database [12]. In our study, however, all *S.* Paris strains were ST82 genotype. The database records 15 different serotypes, including *S.* Kottbus, S. Jericho, *S.* Derby and *S.* Muenchen, and *S.* Kottbus and *S.* Derby from poultry origin, as ST582 genotype whereas all *S.* Stuttgart strains isolated in our study were ST19 genotype. In the database, the major serotype recorded as ST19 genotype is *S.* Typhimurium strains. Because different serotypes have the same sequence type, MLST analysis should be supported by conventional or molecular serotyping analysis.

This is the first study to use MLST analysis to identify serotypes of *S.* Bazenheid, *S.* Burgas, *S.* Gombe, *S.* Hayindogo, *S.* Jamaica, *S.* Kamoru, *S.* Paris and *S.* Stuttgart with new sequence types. The presence of a single sequence type for each serotype suggests that these serotypes may be the dominant sequence types in broiler chicken flocks in Turkey.

Acknowledgments
This research received no grant from any funding agency/ sector.

Conflict of interest
The authors declare no competing interests.

References

1. EFSA. The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA Journal 2013; 3129.
2. Almeida F, Pitondo-Silva A, Oliveira MA, Falcao JP. Molecular epidemiology and virulence markers of Salmonella Infantis isolated over 25 years in Sao Paulo State, Brazil. Infection, Genetics and Evolution 2013; 19: 145-151. doi: 10.1016/j.meegid.2013.07.004
3. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bulletin World Health Organization 2004; 82 (5): 346-353.
4. Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG et al. Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science 2015; 8 (3): 284-293. doi: 10.1080/21553769.2015
5. Mezal EH, Sabol A, Khan MA, Ali N, Stefanova R et al. Isolation and molecular characterization of Salmonella enterica serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiology 2014; 38: 67-74. doi: 10.1016/j.fm.2013.08.003
6. EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal 2017; 5077.
7. Ho YN, Chou MY, Tsai HC, Huang TY, Fan CW et al. Empirical testing of modified Salmonella MLST in aquatic environmental samples by in silico analysis. Science of the Total Environment 2017; 581: 378-385. doi: 10.1016/j.scitotenv.2016.12.144
8. Kidgell C, Reichard U, Wain J, Linz B, Torpdahl M, et al. Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old. Infection, Genetics and Evolution 2002; 2 (1): 39-45. doi: 10.1016/s1567-1348(02)00089-8
9. Achtman M, Wain J, Weill FX, Nair S, Zhou ZM et al. Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. Plos Pathogens 2012; 8 (6): 1-10. doi: 10.1371/journal.ppat.1002776
10. Aanensen DM, Spratt BG. The multilocus sequence typing network: mlst.net. Nucleic Acids Research 2005; 33: 728-733. doi: 10.1093/nar/gki415
11. Grimont PAD, Weill FX. Antigenic formulae of the Salmonella serovars. 9th ed: Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella; 2007.
12. Jolley KA, Bray JE, Maiden CJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications [version 1; peer review: 2 approved]. Wellcome Open Research 2018; 3: 124.
13. Cai YQ, Tao J, Jiao Y, Fei X, Zhou L et al. Phenotypic characteristics and genotypic correlation between Salmonella isolates from a slaughterhouse and retail markets in Yangzhou, China. International Journal of Food Microbiology 2016; 222: 56-64. doi: 10.1016/j.ijfoodmicro.2016.01.020
14. Ghaderi R, Tadayon K, Khaki P, Mosavari N. Iranian clonal population of Salmonella enterica serovar Enteritidis, characterized by multi-locus sequence typing (MLST) method. Iranian Journal of Microbiology 2015; 7 (5) : 251-259.