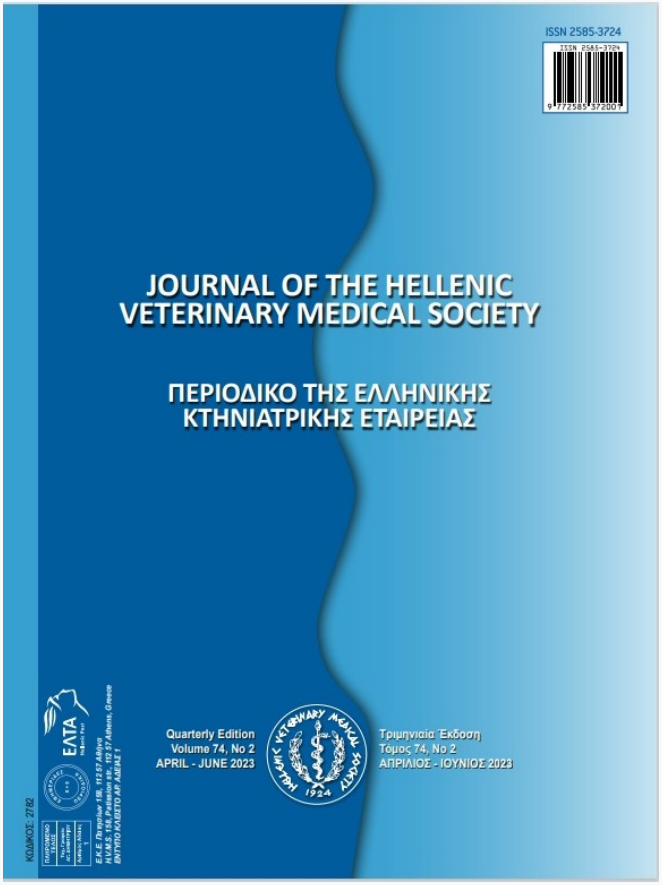
Journal of the Hellenic Veterinary Medical Society

Vol 74, No 3 (2023)



Occurrence and molecular characterization of Salmonella Typhimurium and Salmonella Enteritidis isolates from contaminated food samples from Palestine

G Adwan, S Abuseir, O Khreishi , A Hussein, N Khraim, M Abed Al-Daym

doi: 10.12681/jhvms.31088

To cite this article:

Adwan, G., Abuseir, S., Khreishi , O., Hussein, A., Khraim, N., & Abed Al-Daym , M. (2023). Occurrence and molecular characterization of Salmonella Typhimurium and Salmonella Enteritidis isolates from contaminated food samples from Palestine. *Journal of the Hellenic Veterinary Medical Society*, *74*(3), 6201–6211. https://doi.org/10.12681/jhvms.31088

J HELLENIC VET MED SOC 2023, 74 (3): 6201-6211
ΠΕΚΕ 2023, 74 (3): 6201-6211

Research article
Ερευνητικό άρθρο

Occurrence and molecular characterization of *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates from contaminated food samples from Palestine

G. Adwan[1*], S. Abuseir[2*], O. Khreishi[3], A. Hussein[4], N. Khraim[2], M. Abed Al-Daym[2]

[1]*Department of Biology and Biotechnology, Molecular Microbiology/Virology, An-Najah National University, Nablus, Palestine*

[2]*Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, Palestine*

[3]*Qalqilya Veterinary Services and Animal Health Directorate, Ministry of Agriculture, Nablus Street, Al-Sabri Neighborhood, Qalqilya, Palestine*

[4]*Medical Laboratory Sciences Department, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine*

ABSTRACT: *Salmonella* is one of the most frequently isolated foodborne pathogens. It is of major public health concern worldwide. Poultry meat and eggs represent important sources of Salmonellae organisms with an impact on consumers' health. This study aims to evaluate the occurrence of *Salmonella* Typhimurium and *Salmonella* Enteritidis using multiplex PCR (mPCR) among isolates collected from samples of the local food market and to assess genetic relationships between isolates of *S.* Typhimurium, which was the only serotype isolated from the tested food samples. This was achieved using *virulence factors* profiling and *fingerprint* profiling, which were assessed by random amplified polymorphic DNA (RAPD-PCR) and repetitive sequence PCR (REP-PCR). Both enterobacterial repetitive intergenic consensus (ERIC-PCR) and interspersed repetitive DNA sequence BOXAIR-PCR were used for this purpose.
The overall occurrence percentage of *S.* Typhimurium and *S.* Enteritidis out of 51 isolates was 54.9% and 0.0%, respectively. Only 13 out of 17 virulence genes were detected in these isolates. The occurrence of the detected virulence genes among these isolates was 100%, 50.0%,46.4%, 39.3%, 35.7%, 35.7%, 32.1%, 25.0%, 25.0%, 17.6%, 14.3%, 14.3%, 3.6% for *invA*, *sopB*, *prgH*, *sitC*, *pefA*, *tolC*, *cdtB*, *msgA*, *sifA*, *iroN*, *spiA*, *ipfC* and *pagC*, respectively. The remaining virulence genes were absent in all of the isolates. Based on the combination of the presence and absence of virulence genes, eight profiles were detected among these isolates, the most common genetic profile was V5 (each 32.1%). Based on this genetic profile at cut-off point 96.0%, both ERIC and BOX primers allowed for discrimination into 4 and 6 clusters or clones of 16 *S.* Typhimurium isolates, respectively. Results of PCR typing methods showed that, three strains clustered together using both ERIC-PCR and BOX-PCR typing methods and they had the same virulotype (V1), while other four strains also clustered together by both typing methods and had the same virulotype (V4).
Contamination of food with Salmonellae, especially with *S.* Typhimurium, was high and affected the microbiological quality of food. This emphasizes the need for rigorous public health and food safety control methods to lower the human health hazard and risk associated with Salmonellae infection.

Keywords: foodborne pathogens, *Salmonella* Typhimurium, *Salmonella* Enteritidis, molecular characterization, Palestine.

Corresponding Authors:
Ghaleb Adwan, Department of Biology and Biotechnology, Molecular Microbiology/Virology, An-Najah National University P. O. Box 7 Nablus, West Bank, Palestine.
E-mail address: adwang@najah.edu,
Sameh Abuseir, Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, An-Najah National University P. O. Box 7 Nablus, West Bank, Palestine.
E-mail address: sameh.abuseir@najah.edu

Date of initial submission: 15-08-2022
Date of acceptance: 26-01-2023

INTRODUCTION

Foodborne microorganisms are major pathogens affecting food safety and causing human illness worldwide. These foodborne infections and intoxications result from the consumption of various foodstuffs, mainly contaminated animal products. Most of these food-borne pathogens have zoonotic nature, resulting in a significant impact on both human public health and the economic sector (Abebe et al., 2020). In the developed counties, the annual incidence of foodborne illnesses is estimated to be around 30.0% of the population (De Giusti et al., 2007).

According to the WHO, *Salmonella* spp. are among the 31 pathogenic agents showing the highest ability of provoking intestinal or systemic disease in humans among diarrheal and/or invasive pathogens, and the third causative agent of death among food-borne diseases (Ferrari et al., 2019). Salmonellosis is an important zoonotic infection seen in all animal species (Seifi et al., 2019). It is considered the second major cause of food-borne diseases worldwide (Jeníková et al., 2000; Scallan et al., 2011; EFSA 2019; Abuseir et al., 2020). Non-typhoid *Salmonella* accounts for 93.8 million foodborne infections and 155,000 deaths per year (Eng et al., 2015).

Salmonella enterica serotype Typhimurium (*S.* Typhimurium), *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport are epidemiologically important non-typhoidal *Salmonella* serotypes, which have been responsible for the majority of human *Salmonella* disease-burden worldwide (Jajere, 2019). In the European Union, the second most frequently involved bacterial genus in gastrointestinal outbreaks in humans is *Salmonella* and more particularly the subspecies *S.* Enteritidis and *S.* Typhimurium (Paniel and Noguer, 2019). Contaminated poultry products such as meat and eggs continue to play a central role in spreading the infection of the *S*. Enteritidis and *S*. Typhimurium serovars to humans (Tarabees et al., 2017; Ferrari et al., 2019; Paniel and Noguer, 2019; Wang et al., 2019).

Among more than 2,500 serovars of *Salmonella enterica*, *S.* Typhimurium was also one of the most frequently isolated serovars worldwide (Medeiros et al., 2015), and it is one of the leading serovars that cause salmonellosis globally (Medeiros et al., 2015).

In 2015, *S.* Enteritidis was representing 45.7% of all reported serovars in confirmed human cases (EFSA 2016) and accounted for 57.9% of all *Salmonella* outbreaks and 48.7% of human cases in all *Salmonella* outbreaks in the EU countries (EFSA 2021).

Salmonella spp. can establish an infection and cause illness through the expression of several virulence genes that interact with host cells (Elemfareji and Thong, 2013; Borges et al., 2013; Mezal et al., 2014; Rowlands et al., 2014; Gharieb et al., 2015; Tarabees et al., 2017; Srisanga et al., 2017; Thung et al., 2018; Liaquat et al., 2018; Elkenany et al., 2019). Virulence genes play a very important role in the broad spectrum of pathogenic mechanisms. These mechanisms include invasion, adhesion, toxin production, systemic infection, antibiotic resistance, fimbrial expression, intracellular survival, and iron and Mg^{2+} uptake (Hensel, 2004). The genes *prgH*, *invA*, *spaN* (*invJ*), *spiA*, *tolC*, *orgA*, *sipB*, *pagC*, *pefA*, *msgA*, *sopB, spvB*, *lpfC* and *sifA* are expressed to produce certain proteins associated with invasiveness traits, such as cellular invasion/survival and adhesion or pili production. Other genes encode certain proteins thought to be very important virulence factors. These factors include *iroN* and *sitC*, which are involved in iron acquisition, and *cdtB* gene, a putative toxin-encoding gene (Skyberg et al., 2006). The function of these virulence factors are presented in table 1.

The main aim of this study was to estimate the occurrence of the main *Salmonella* strains found in food samples, namely *S.* Typhimurium and *S*. Enteritidis. In addition, the fingerprinting of the main strain,

Table 1. Function of virulence factors of *Salmonellae* used in this study in virulence genotyping (Skyberg et al., 2006).

Virulence factor	Virulence-related function
invA, orgA, prgH, tolC, sopB, lpfC, cdtB, pefA	Host recognition/invasion
spaN	Entry into non-phagocytic cells, killing of macrophages
sipB	Entry into non-phagocytic cells, killing of macrophages
iroN, sitC	Iron acquisition
pagC, msgA, spiA	Survival within macrophage
sifA	Filamentous structure formation
spvB	Growth within-host

recovered by random amplified polymorphic DNA (RAPD-PCR) and repetitive sequence PCR (REP-PCR) using enterobacterial repetitive intergenic consensus (ERIC-PCR) and interspersed repetitive DNA sequence BOXAIR-PCR primers was accomplished. The genetic relationships between the strains, and the pathogenic potential of the recovered isolates were assessed by using virulotyping PCR assay, targeting 17 virulence gene sequences.

MATERIALS AND METHODS

Samples collection

A total of 51 *Salmonella* isolates were recovered from different types of food samples, which were collected from the local retail market in different governorates and areas in the West Bank, Palestine during 2019. These samples were fresh chicken meat (24), Kebab (1), fresh turkey meat (3), cheese (1), beef burger (11), Hummus (1), Parsley (1), Tahini (4), restaurant's salad (1), Halawa (1), fish-Fillet (1) and beef meat (2). All *Salmonella* isolates were collected and identified by the Analysis, Poison Control and Calibration Center, An-Najah National University, and the Central Laboratories, Ministry of Health, Palestine**.** Identification of these isolates was done according to ISO 6579-1: 2017, by conventional methods using enrichment, selective and differential media, Gram staining, and biochemical tests. All cultures that were negative for Lactose/Sucrose, positive for Glucose, produce H_2S, and motile were kept for serological confirmation. The serological confirmation used is genus-specific, to confirm the *Salmonella* isolates. The positive isolates were stored at -70ºC.

DNA extraction

DNA of *Salmonella* isolates was prepared for PCR according to the method described previously (Adwan et al., 2013). The concentration of the DNA sample was determined using a nanodrop spectrophotometer (Genova Nano, Jenway), and the DNA samples were stored at -20ºC for further analysis.

Salmonella spp. confirmation and *S.* Typhimurium and *S.* Enteritidis identification by multiplex PCR (mPCR)

For mPCR detection, three primer pairs were used to identify specific target genes, including *invA* for *Salmonella* spp. identification, *STMO159* for *S.* Typhimurium identification, and *SEN1383* for *S.* Enteritidis identification. Target gene, primer sequence, and amplicon size for these primer pairs are presented in table 2.

The mPCR reaction mix was carried according to the method described previously (Ranjbar et al., 2017). A final volume of 25 µl PCR reaction mix was performed as follows: 12.5 µl of PCR premix (Ready-MixTM Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.3 µM of each primer and 3 µl (50-70 ng) of target DNA template. DNA amplification was carried out using a thermal cycler (Mastercycler Personal, Eppendorf, Germany) according to the following conditions: initial denaturation at 94ºC for 3 min; followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 57ºC for 1 min and extension at 72ºC for 1 min. Then, these cycles were followed by a single final extension step at 72ºC for 5 min. The PCR amplicons were then detected by electrophoresis through 1.5% agarose gels to determine the size of amplicons after staining with a final concentration of 0.5 µg/ml of ethidium bromide dye. The sizes of the PCR products were determined by comparing them with a 100-bp DNA ladder. Live attenuated vaccines for *S.* Typhimurium and *S.* Enteritidis (Biovac Company), were used as positive controls and *E. coli* ATCC 25922 strain was used as a negative control.

Molecular typing by ERIC-PCR and BOX-AIR-PCR

S. Typhimurium isolates that were the only *Salmonella* strain recovered from different food samples in

Table 2. Oligonucleotide primers used for *Salmonella* spp. confirmation, *S.* Typhimurium, and *S.* Enteritidis detection.

Target gene	Primer Sequence 5'→3'	Amplicon size (bp)	Reference
invA- secretory protein (*Salmonella* spp.)	invA F: GTATTGTTGATTAATGAGATCCG invA R: ATATTACGCACGGAAACACGTT	404	Ranjbar et al., 2017
SEN1383-a hypothetical protein (*S.* Enteritidis)	SEN1383 F: TGTGTTTTATCTGATGCAAGAGG SEN1383 R: TGAACTACGTTCGTTCTTCTGG′	304	Ranjbar et al., 2017
STM0159-a putative restriction endonuclease (*S.* Typhimurium)	STM0159 F: ATGATGCCTTTTGCTGCTTT' STM0159 R: TCCCAGCTCATCCAAAAA	224	Ranjbar et al., 2017

this study were fingerprinted to assess genetic relationships between the strains of *S.* Typhimurium from different sources, using ERIC-PCR primers and interspersed repetitive DNA sequence (BOX) primers as shown in table 3.

Each PCR reaction mix (25 µl) was composed of 10 mM PCR buffer pH 8.3, 3 mM $MgCl_2$ 0.4 mM of each dNTP, 0.8 µM of each primer, 1.5 U of Taq DNA polymerase and 3 µl of DNA template. The DNA amplification for ERIC-PCR was carried out using a thermal cycler (Master-cycler Personal, Eppendorf, Germany) according to the following conditions: initial denaturation for 2 min at 94ºC, followed by 40 cycles of denaturation at 94ºC for 50 s, annealing at 50ºC for 40 s and extension at 72ºC for 1 min. Then, these cycles were followed with a final extension step at 72ºC for 5 min. For BOXAIR-PCR, the thermal conditions were: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 40 s and extension at 72°C for 2 min. After that, these cycles were followed with a final extension step at 72°C for 5 min.

The PCR products were analyzed by electrophoresis on 1.5% agarose gels. The bands in gel images were analyzed using a binary scoring system, which recorded the absence and presence of bands as 0 and 1, respectively. The binary matrix was analyzed by the unweight pair group method for arithmetic averages (UPGMA), using SPSS statistical software version 20 (IBM). The clusters of the fingerprints in the constructed dendrogram were described at a 96% similarity level. The number of different bands in each fingerprint was considered for comparison between *S.* Typhimurium strains as previously described (Adwan et al., 2016; Adwan et al., 2016; Adwan and Rabaya, 2016; Adwan and Haya, 2018), based on the following criteria: identical clones (no different bands), "closely related clones" (have 1 different band),"possibility different clones" (have two different bands), "different clones" (have three or more different bands).

Molecular typing by RAPD-PCR

Recovered *S.* Typhimurium isolates were fingerprinted by RAPD-PCR using the RAPD primer OPP-16 (Table 3) to assess genetic relationships between the strains of *S.* Typhimurium from these sources (Albufera et al. 2009).

RAPD-PCR was carried as described previously with some modification (Hashemi and Baghbani-Arani, 2015). PCR reaction mix was carried out as in ERIC- PCR mix and BOXAIR-PCR mix. DNA amplification for RAPD-PCR was carried out using a thermal cycler (Mastercycler Personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94ºC, followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 35ºC for 1 min and extension at 72ºC for 2 min. Then, these cycles were followed with a final extension step at 72ºC for 5 min. The PCR products were analyzed as well as the ERIC- PCR, and BOXAIR-PCR.

Virulotyping of *S.* Typhimurium isolates by mPCR

Three mPCR reactions were used to amplify the seventeen virulence genes. Pools of reaction, target gene, primer sequence, amplicon size for these primers are presented in table 4.

The mPCR was carried as described previously with some modification (Skyberg et al., 2006). Each PCR reaction mix (25 µl) was composed of 10 mM PCR buffer pH 8.3,6 mM $MgCl_2$, 0.3 mM of each dNTP, 0.3 µM of each primer, 1.5 U of Taq DNA polymerase, 3% DMSO, and 3 µl of target DNA template. DNA amplification for mPCR was carried out using a thermal cycler (Master-cycler Personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94ºC, followed by 25 cycles of denaturation at 94ºC for 40 s, annealing at 62ºC for 40 s and extension at 72ºC for 2 min. After that, these cycles were followed with a

Table 3. The primers for ERIC-PCR, BOXAIR-PCR and RAPD-PCR used in this study.

Target gene	Primer Sequence 5'→3'	Reference
Enterobacterial repetitive intergenic consensus (ERIC)	ERIC1: ATGTAAGCTCCTGGGGATTCAC ERIC2: AAGTAAGTGACTGGGGTGAGC G	Fendri et al., 2013
Interspersed repetitive DNA sequence (BOX)	BOXAIR: CTACGGCAAGGCGACGCTGACG	Dombek et al., 2000 Hashemi and Baghbani-Arani, 2015
Random amplified polymorphic DNA (RAPD-PCR)	OPP-16: CCAAGCTGC C	Albufera et al., 2009

Table 4. Virulence gene primers used in this study (Skyberg et al., 2006).

Target gene	Primer sequence (5'→3')	Amplicon size (bp)	pool
spvB	spvB F: CTA TCA GCC CCG CAC GGA GAG CAG TTT TTA spvB R: GGA GGA GGC GGT GGC GGT GGC ATC ATA	717	1
spiA	spiA F: CCA GGG GTC GTT AGT GTA TTG CGT GAG ATG spiA R: CGC GTA ACA AAG AAC CCG TAG TGA TGG ATT	550	1
pagC	pagC F: CGC CTT TTC CGT GGG GTA TGC pagC R: GAA GCC GTT TAT TTT TGT AGA GGA GAT GTT	454	1
cdtB	cdtB F: ACA ACT GTC GCA TCT CGC CCC GTC ATT cdtB R: CAA TTT GCG TGG GTT CTG TAG GTG CGA GT	268	1
msgA	msgA F: GCC AGG CGC ACG CGA AAT CAT CC msgA R: GCG ACC AGC CAC ATA TCA GCC TCT TCA AAC	189	1
invA	invA F: CTG GCG GTG GGT TTT GTT GTC TTC TCT ATT invA R: GTT TCT CCC CCT CTT CAT GCG TTA CCC	1070	2
sipB	sipB F: GGA CGC CGC CCG GGA AAA ACT CTC sipB R: ACA CTC CCG TCG CCG CCT TCA CAA	875	2
prgH	prgH F: GCC CGA GCA GCC TGA GAA GTT AGA AA prgH R: TGA AAT GAG CGC CCC TTG AGC CAG TC	756	2
spaN	Span F: AAA AGC CGT GGA ATC CGT TAG TGA AGT span R: CAG CGC TGG GGA TTA CCG TTT TG	504	2
orgA	orgA F: TTT TTG GCA ATG CAT CAG GGA ACA orgA R: GGC GAA AGC GGG GAC GGT ATT	255	2
tolC	tolC F: TAC CCA GGC GCA AAA AGA GGC TAT C tolC R: CCG CGT TAT CCA GGT TGT TGC	161	2
iroN	Iron F: ACT GGC ACG GCT CGC TGT CGC TCT AT iron R: CGC TTT ACC GCC GTT CTG CCA CTG C	1205	3
sitC	sitC F: CAG TAT ATG CTC AAC GCG ATG TGG GTC TCC sitC R: CGG GGC GAA AAT AAA GGC TGT GAT GAA C	768	3
lpfC	lpfC F: GCC CCG CCT GAA GCC TGT GTT GC lpfC R: AGG TCG CCG CTG TTT GAG GTT GGA TA	641	3
sifA	sifA F: TTT GCC GAA CGC GCC CCC ACA CG sifA R: GTT GCC TTT TCT TGC GCT TTC CAC CCA TC	449	3
sopB	sopB F: CGG ACC GGC CAG CAA CAA AAC AAG AAG AAG sopB R: TAG TGA TGC CCG TTA TGC GTG AGT GTA TT	220	3
pefA	pefA F: GCG CCG CTC AGC CGA ACC AG pefA R: GCA GCA GAA GCC CAG GAA ACA GTG	157	3

final extension step at 72ºC for 5 min. The PCR products were then detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragments after staining with a final concentration of 0.5 μg/ml of ethidium bromide dye. The sizes of the amplicons of these genes were determined by comparing them with a 100-bp DNA ladder.

RESULTS

Salmonella spp. confirmation and *S.* Typhimurium and *S.* Enteritidis detection

All *Salmonella* isolates were subjected to mPCR using specific primers to confirm that these isolates belonged to a *Salmonella* genus and to determine the occurrence of *S.* Typhimurium and *S.* Enteritidis serotypes among these isolates. PCR confirmation of conventional and serological methods positive strains was documented by the appearance of the amplified DNA fragment of 404-bp for the *invA* gene, a target for *Salmonella* genus, in all 51 (100%) *Salmonella* isolates examined and confirmed. In addition, 28 (54.9%) isolates were *S.* Typhimurium serotype and produced amplified DNA fragment of 224-bp for *STMO159* gene (a putative restriction endonuclease), while amplified DNA fragment of 304-bp for *SEN1383* gene (a hypothetical protein) for *S.* Enteritidis serotype was not detected among the *Salmonella* isolates. Multiplex PCR profile specific for genes responsible for detection of *Salmonella* genus (*invA* gene; 404-bp),*S.* Typhimurium serotype (STMO159 gene; 224-bp), and *S.* Enteritidis serotype (SEN1383 gene; 304-bp) is shown in figure 1.

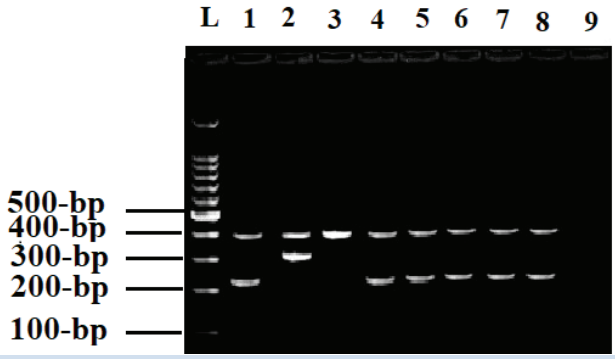


Fig. 1: Multiplex PCR profile specific for genes responsible for detection isolates of *Salmonella* genus, (*invA* gene; 404-bp) *S.* Typhimurium serotype (STMO159; 224-bp) and *S.* Enteritidis (SEN1383; 304-bp). Lanes: L represents 100 bp ladder, lanes 4, 5, 6, 7 and 8 represent *S.* Typhimurium serotype, lane 3 represents *Salmonella* genus, lane 9 represents *E. coli* as a negative control; Lanes 1 and 2 represent live attenuated vaccine strains for *S.* Typhimurium and *S.* Enteritidis serotypes (Biovac Company) as a positive control, respectively.

Virulotyping of *S.* Typhimurium serotype isolates by mPCR

As shown in table 5, only 13 genes among the targeted 17 genes were detected in the 28 *S.* Typhimurium isolates. The occurrence of the detected genes among these isolates was 100%, 50.0%, 46.4%, 39.3%, 35.7%, 35.7%, 32.1%, 25.0%, 25.0%, 17.6%, 14.3%, 14.3%, 3.6% for *invA*, *sopB*, *prgH*, *sitC*, *pefA*, *tolC*, *cdtB*, *msgA*, *sifA*, *iroN*, *spiA*, *ipfC* and *pagC*, respectively. The remaining virulence genes (spvB, sipB, spaN and orgA) were absent from all *S.* Typhimurium isolates.

Table 5. Presence and occurrence percentage of the virulence genes in the 28 S. Typhimurium isolates.

Present genes	Occurrence %	Absent genes
spiA	14.3%	***spvB***
pagC	3.6%	***spaN***
cdtB	32.1%,	**orgA**
msgA	25.0%	***sipB***
invA	100%	
prgH	46.4%,	
tolC	35.7%,	
iroN	17.6%,	
sitC	39.3%,	
lpfC	14.3%	
sifA	25.0%	
sopB	50.0%,	
pefA	35.7%,	

Based on the combination of presence and absence of virulence genes, 7 profiles were detected among these isolates, the most common genetic profile wasV5 (each 32.1%). Figure 2 and table 6 showed the data about virulence gene profiles detected in this study.

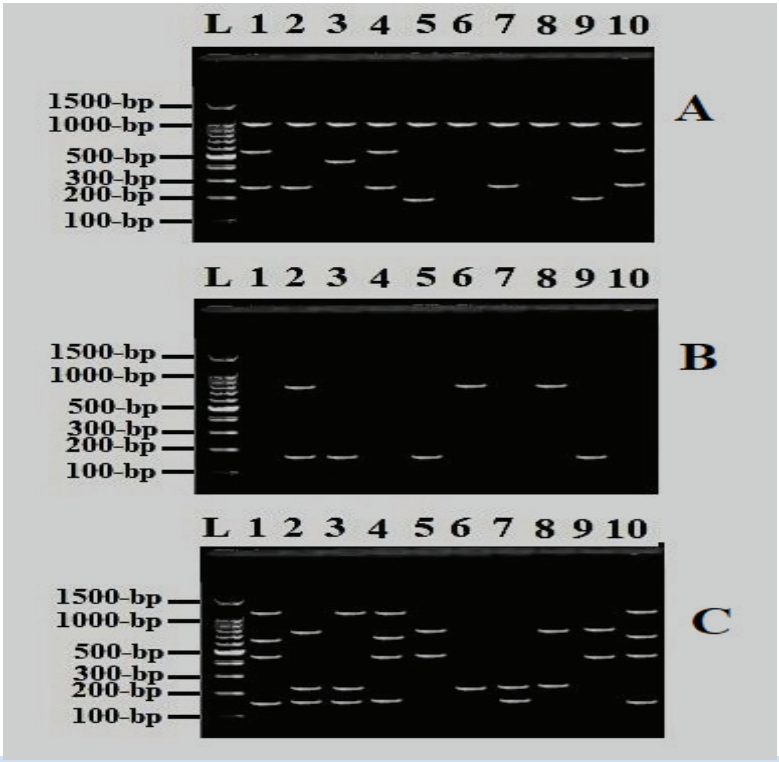


Fig. 2: Multiplex PCR profiles specific for *S.* Typhimurium virulence factors. Figure A: *spiA* gene (550-bp), *pagC* gene (454-bp), *cdtB* gene (268-bp) and *msgA* gene (189-bp). Figure B: *prgH* gene (756-bp) and *tolC* gene (161-bp). Figure C: *iroN* gene (1205-bp), *sitC* gene (768-bp), *ipfC* gene (641-bp), *sifA* gene (449-bp), *sopB* gene (220-bp) and *pefA* gene (157-bp).

Table 6. Virulence gene profile of the 28 *S.* Typhimurium isolated from different types of food samples

Virulotypes (V)	Gene combinations	No. of strains (%)
V1	*invA, spiA, cdtB, iroN, ipfC, sifA, pefA*	4 (14.3)
V2	*invA, cdtB, prgH, tolC, sitC, sopB, pefA*	2 (7.1)
V3	*invA, pagC, tolC, iroN, sopB, pefA*	1 (3.6)
V4	*invA, msgA, tolC, sitC, sifA*	7 (25.0)
V5	*invA, prgH, sopB*	9 (32.1)
V6	*invA, cdtB, sopB, pefA*	3 (10.7)
V7	*invA, prgH, sitC, sopB*	2 (7.1)

Genotyping of *S.* Typhimurium serotype by PCR-based methods

In the present study, ERIC and BOX primers allowed for discrimination into 4 and 6 clusters or clones of 16 *S.* Typhimurium serotype isolates respectively, based on their genetic profile at a cut-off point of 96.0%.

RAPD-PCR using the RAPD primer OPP-16 did not allow for discrimination between *S.* Typhimurium isolates, as it did not produce any amplified fragments during PCR amplification.

According to the ERIC-PCR profile, strains of cluster C1 and C2, C3 and C4, and C3 and C2 are closely related clones. Strains of C4 and C1, and C3 and C1 are different clones, while strains of C4 and C2 are possibly different clones. ERIC-PCR DNA fingerprint pattern, dendrogram, and the relationship between the clones of 16 *S.* Typhimurium strains recovered from different food samples are presented in figure 3 and table 7.

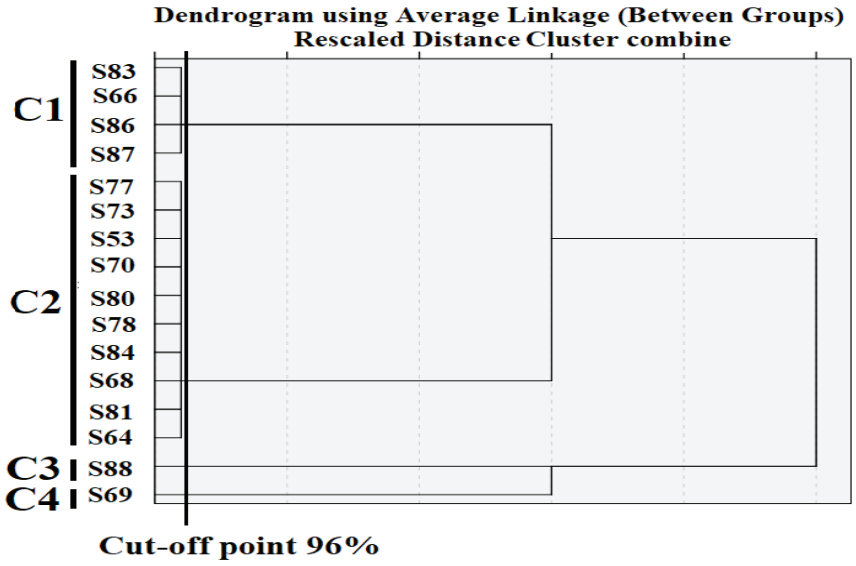


Fig. 3: Dendrogram of 16 *S.* Typhimurium serotype isolates based on the UPGMA method using the average group linkage/squared Euclidean distance by SPSS software version 20 derived from analysis of the ERIC-PCR-profiles at a 96.0% similarity level. C: Cluster.

Table 7. Relationship between the clones or the clusters depending on the number of different bands based on ERIC-PCR profile of 16 *S.* Typhimurium serotype isolates.

Cluster or clone	Cluster relationship			
	C1	**C2**	**C3**	**C4**
C1	1	2	4	4
C2		1	2	3
C3			1	2
C4				1

1: Identical clones. 2: Closely related clones. 3: Possibility different clones. 4: Different clones.
C: cluster or clone.

According to the BOX-PCR profile, strains of cluster C1 and C2, C3 and C4, C4 and C5, and C4 and C6 are closely related clones. Strains of cluster C2 and C3, C5 and C2, C5 and C3, C6 and C2, C6 and C3, and C6 and C5 are possibility different clones, while strains of C3 and C1, C4 and C1, C4 and C2, C5 and C1, and C6 and C1 are different clones. BOX-PCR DNA fingerprint pattern, dendrogram, and the relationship between the clusters or clones of 16 *S.* Typhimurium serotype isolates recovered from different food samples are presented in figure 4 and table 8.

Results of PCR typing methods showed that strain S83 (isolated from chicken wings) and strains S86 and S87 (isolated from chicken) are clustered together using both ERIC-PCR and BOX-PCR typing methods and they had the same virulotype pattern (V1). However, strains S78 and S80 (isolated from beef burgher) and strains S53 and S73 (isolated from chicken) also clustered together by both typing methods and had the same virulotype pattern (V4).

DISCUSSION

Salmonellosis remains a significant public health problem causing food poisoning in humans. Contamination with *Salmonella* in food products can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, handling, and preparation (Tarabees et al., 2017), and monitoring of *Salmonella* spp. along all these steps in food chain should be conducted (EFSA 2018).

The current study supported the ability of specific primer sets to detect *invA* gene which confirms the

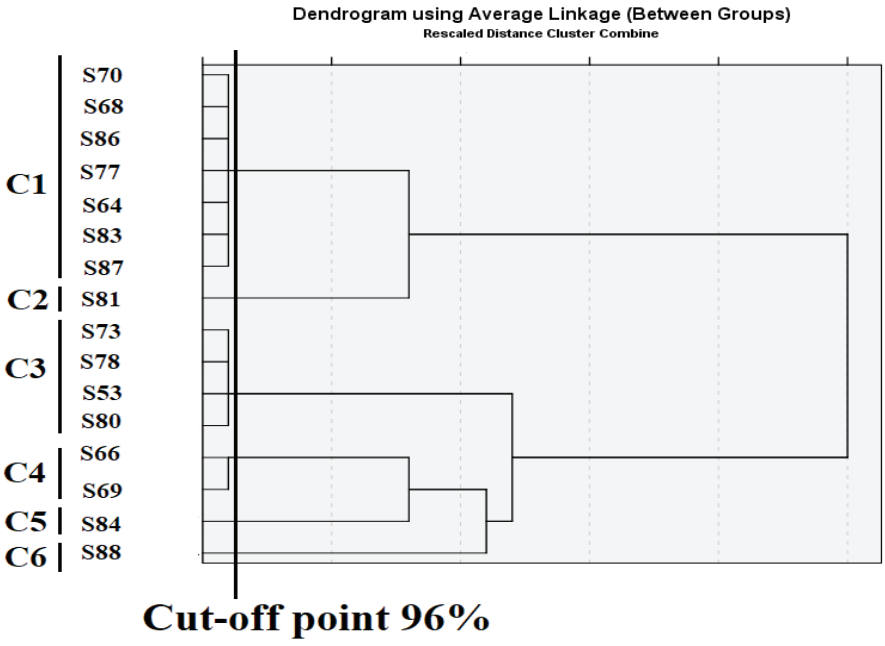


Fig. 4: Dendrogram of 16 *S.* Typhimurium serotype isolates based on the UPGMA method using average group linkage/squared Euclidean distance by SPSS software version 20 derived from analysis of the BOX-PCR-profiles at a 96% similarity level. C: Cluster.

Table 8. Relationship between the clones or the clusters depending on the number of different bands based on BOX-PCR profile of 16 *S.* Typhimurium serotype isolates.

	Cluster relationship					
Cluster or clone	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**
C1	1	2	4	4	4	4
C2		1	3	4	3	3
C3			1	2	3	3
C4				1	2	2
C5					1	3
C6						1

1: Identical clones. 2: Closely related clones. 3: Possibility different clones. 4: Different clones.
C: cluster or clone.

isolates as *Salmonella* spp. The protein encoded by the *invA* gene is essential for the invasion of host epithelial cells (Darwin and Miller, 1999). Several studies had also proven the effective detection of all *Salmonella* isolates using specific primers for the *invA* gene (Helmy et al., 2009; Moussa et al., 2010; Shanmugasamy et al., 2011; Borges et al., 2013; Ammar et al., 2016; Ranjbar et al., 2017; Srisanga et al., 2017; Proroga et al., 2018), which was used as a target gene in PCR assays and a confirmatory test for *Salmonella* detection.

The use of PCR protocols for the recognition and the identification of *S.* Typhimurium and *S*. Enteritidis can be used as an alternative method to the conventional serotyping method according to ISO 6579-3: 2014 that is highly expensive and time consuming.

Despite the results of many research work showing that *S*. Enteritidis was the main *Salmonella* serotypes isolated from food samples (El-Baz et al., 2017; Moussa et al., 2010; EFSA 2018; Magwedere et al., 2015), results of this research study showed that *S.* Typhimurium was the prevalent among the isolates, and no *S*. Enteritidis samples were detected. These results are similar to the results of other research work conducted on the prevalence of the main *Salmonella* serotypes in food samples (Abuseir et al., 2020; Ammar et al., 2016).

The previous studies and others may also indicate that *Salmonella* serovars vary geographically. *S.* Enteritidis and *S*. Typhimurium were considered the most common serovars recorded and clinically significant (EFSA 2015; Ammar et al., 2016). The differences in occurrence rates of *Salmonella* serotypes may be affected by different factors such as differences in the sampling method, sample types, *Salmonella* detection protocol, geographic region, and the housing and husbandry conditions (Busani et al., 2005; Nouichi et al., 2018).

Results of this study showed that using more than one molecular method is useful in epidemiological studies. Both ERIC and BOX primers allowed for discrimination into 4 and 6 clusters or clones of 16 *S.* Typhimurium isolates, respectively, based on their genetic profile at cut-off point 96.0%. RAPD-PCR using the RAPD primer OPP-16 did not allow for discrimination between *S.* Typhimurium isolates. On the other hand, a study conducted in Colombia (Lozano-Villegas et al., 2019) revealed that genotyping of *Salmonella* spp. using RAPD primers allowed the typing of 34 of 49 strains of *Salmonella* spp. The best discriminatory index was observed when GTG 5 (0.92) and OPP 16 (0.85) primers were used alone or combined with RAPD-PCR and BOX-PCR (0.99).

PCR-based fingerprinting methods are considered as a simple and easily applicable typing technique and potentially available to any molecular laboratory. It was suggested that RAPD, ERIC-PCR, REP-PCR, BOXAIR-PCR are good discriminatory techniques to type the different clinical *Salmonella* isolates and these methods are sufficient to determine genetic relationships among *Salmonella* strains for epidemiological purposes when different techniques were combined (Hashemi and Baghbani-Arani, 2015). BOX-A1R-based repetitive extragenic palindromic-PCR (BOX-PCR) is considered to be the best method comparing to other repetitive element-based PCR typing methods, specifically, ERIC-, poly-trinucleotide (GTG) and REP-PCR. BOX-PCR provides a convenient molecular typing method to distinguish *Salmonella* spp. of the same and different serotypes according to genetic relatedness and should be proper for application in typing and tracking route of transmission in outbreaks. Similar results were reported by Pooncharoen et al. (2019) and Lozano-Villegas et al. (2019), who showed that BOX-PCR can differentiate the genetic relationship between *Salmonella* isolates as well as grouping them into different clusters according to their origin.

The virulence of *Salmonella* is linked to a combination of chromosomal and plasmid factors (Yehia et al., 2020). Pathogenicity of *Salmonella* strains is controlled by a set of factors encoded by specific virulence genes that assist these types of pathogens to express the virulence in the host cells. This led to the appearance of typical symptoms of infection in an infected individual (Gharieb et al., 2015). In addition, virulotyping techniques are useful approaches to study *Salmonella* epidemiology. *S.* Typhimurium isolates have a range of virulence factors that play a role in *Salmonella* infection, diseases and interact with their host cells (Tarabees et al., 2017).

Results of the current study are in contrast to a previous study from Egypt, which reported that only 9 genes *sitC*, *iroN*, *sopB*, *sifA*, *lpfC*, *span*, *sipB*, *invA*, and *tolC* were successfully amplified in cases of *S.* Typhimurium isolated from chicken meat (Tarabees et al., 2017). The results of this research agree with many recent studies in Egypt (Awadallah and Abd-Elall, 2015) and Nigeria (Smith et al., 2015) conduct-

ed on *Salmonella* isolated from humans, animals, food, and water samples in which *invA* gene (284 bp) was prevalent at 96.0%.A wide prevalence of this gene (100%) had also been recorded earlier among *Salmonella* isolates, irrespective of their serovars or sample source by previously published works (Ammar et al., 2016; Ranjbar et al., 2017; Prorogaet al., 2018; Thung et al., 2018; Elkenany et al., 2019).

The *Salmonella* outer protein encoded by *sopB* gene was found in 50.0% of *S.* Typhimurium isolates. A study from Malaysia reported that 50.0% of the *S.* Typhimurium isolates harbored *sopB* virulence gene (Thung et al., 2018). The obtained percentage was approximately similar to that reported from *S.* Typhimurium (44.4%) isolated from broilers in Egypt (Ammar et al., 2016). The occurrence of *sopB* factor in this study was less than that reported from *S.* Typhimurium isolated in India, which showed that all tested *S.* Typhimurium isolates carried *sopB* gene (Rahman, 2006).

Fimbriae in *Salmonella* spp. play a significant role in the pathogenicity, because they contribute to the attachment of these pathogens to the host epithelial cells. The plasmid-encoded fimbriae are encoded by the *pef* operon (Murugkaret al., 2003). Among the *S.* Typhimurium isolates tested, the *pefA* gene was detected in 35.7% of the isolates. These results were in similar to some studies and different from others (Ammar et al., 2016; Thunget al., 2018; Elkenanyet al., 2019). A study conducted in Italy showed that the occurrence of *pefA* gene among *S.* Typhimurium isolates of human origin was 8.2% (Proroga et al., 2018). According to relevant studies, in Brazil, the occurrence of the virulence gene *pefA* was higher than that in Palestine; it was 66.7% among *S.* Typhimurium isolates, associated or not with foodborne Salmonellosis (Rowlands et al., 2014).

Virulotyping of *S.* Typhimurium serotype in this study showed that the occurrence of *prgH* gene was 46.4%. This result was, in contrast, to a study conducted by Srisanga et al., (2017), which showed that the occurrence of this gene among different *S. enterica* including *S.* Typhimurium serotype recovered from dogs and cats was 91.8% (Srisanga et al., 2017).

CONCLUSION

The preliminary data from this study have considerable epidemiological implications. Molecular assays using PCR-based methods for identification, virulotyping, and genotyping of *S.* Typhimurium is a useful approach for drawing up a group of genes to use in the epidemiological characterization of *S.* Typhimurium isolates. Early detection of the virulence gene provides many benefits for public health, especially for rapid diagnosis and control of contamination and infection.

The present study emphasizes the need for rigorous public health and hygienic measures throughout the food chain to minimize the human health hazard risk associated with *Salmonella* diseases. Moreover, the recovered *S.* Typhimurium isolates exhibit multiple virulence genes, which constitute a possible risk to humans from consumption of contaminated food products.

REFERENCES

Abebe E, Gugsa G, Ahmed M (2020) Review on Major Food-Borne Zoonotic Bacterial Pathogens. Journal of Tropical Medicine 3: 1-19.

Abuseir S, Abed Al-Daym M, Adwan G, Khraim N (2020) Prevalence of *Salmonella* spp. in layer and broiler farms in Palestine in 2018, with special emphasis on *Salmonella enterica* serovar Enteritidis. J HELLENIC VET MED SOC 72 (1): 2723-2732.

Adwan G, Adwan K, Jarrar N, Salama Y and Barakat A (2013) Prevalence of *seg*, *seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. British Microbiology Research journal 3(2): 139-149.

Adwan G, Haya I (2018) Prevalence and Characterization of *Staphylococcus aureus* Isolated from Bulk Tank Milk Dairy Cow Farms in West Bank-Palestine. Microbiology Research Journal International 23(5): 1-13.

Adwan G, Rabaya' D, Adwan K, Al-Sheboul S (2016) Prevalence of β-lactamases in clinical isolates of *Enterobacter cloacae* in the West Bank-Palestine. International Journal of Medical Research and Health Sciences 5(7): 49-59

Adwan G, Rabaya D (2016) Prevalence and molecular characterization of β-lactamases in clinical isolates of *Klebsiella pneumoniae* from North of Palestine. International Journal of Current Research 8(3): 28058-28067.

Adwan G, Shtayah A, Adwan K, Al-Sheboul S, Othman S (2016) Prevalence and molecular characterization of *P. aeruginosa* isolates in the West Bank-Palestine for ESBLs, MBLs, and integrons. Journal of Applied Life Sciences International 8(2): 1-11.

Albufera U, Bhugaloo-Vial P, Issack M, Jaufeerally-Fakim Y (2009) Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. Infection, Genetics and Evolution 9: 322-327.

Ammar AM, Mohamed AA, Abd El-Hamid MI, El-Azzouny MM (2016) Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. Journal of infection in developing countries 10(4): 337-346.

Awadallah MAI, Abd-Elall AMM (2015) Diversity and virulence-associated genes of *Salmonella enterica* serovars isolated from wastewater agricultural drains, leafy green producing farms, cattle, and human along their courses. Revue de Médecine Vétérinaire 166(3): 96-106.

Borges KA, Furian TQ, Borsoi A, Moraes HLS, Salle CTP, NascimentoVP (2013) Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in south of Brazil. Brazilian Journal of Veterinary Research (Pesquisa Veterinária Brasileira) 33:1416-1422.

Busani L, Cigliano A, Taioli E, Caligiuri V, Chiavacci L, Di Bella C, Battisti A, Duranti A, Gianfranceschi M, Nardella MC, Ricci A, Rolesu S, Tamba M, Marabelli R, Caprioli A (2005) Prevalence of *Salmonella enterica* and *Listeria monocytogenes* contamination in foods of animal origin in Italy. Journal of Food Protection 68(8):1729-1733.

Darwin KH, Miller VL (1999) Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clinical microbiology reviews 12: 405-428.

De Giusti M, De Medici D, Tufi D, Marzuillo C, Boccia A (2007) Epidemiology of emerging foodborne pathogens. Italian Journal of Public Health 4(1): 24-31.

Dombek PE, Johnson LK, Zimmerley ST, Sadowsky MJ (2000) Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Applied and Environmental Microbiology 66: 2572-2577.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) 2017. Multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections. EFSA Supporting publication 2016: EN-1110. doi:10.2903/sp.efsa.2016.EN-1110.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2015. EFSA Journal 2016 14(12): 4634, p231, doi:10.2903/j.efsa.2016.4634.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) 2019.The European Union One Health 2018 Zoonoses Report. EFSA Journal 2019 17(12): 5926, doi: 10.2903/j.efsa.2019.5926.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) 2015. EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals, and food in 2013. EFSA Journal 2015 13(2): 4036, p178, doi:10.2903/j.efsa.2015.4036

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) 2021. The European Union One Health 2020 Zoonoses Report. EFSA Journal 2021 19(12): 6971, doi: 10.2903/j.efsa.2021.6971.

El-Baz AH, El-Sherbini M, Abdelkhalek A, Al-Ashmawy MA (2017) Prevalence and molecular characterization of *Salmonella* serovars in milk and cheese in Mansoura city, Egypt. Journal of Advanced Veterinary and Animal Research 4(1): 45-51.

Elemfareji OI, Thong KL (2013) Comparative Virulotyping of *Salmonella* Typhi and *Salmonella* Enteritidis. Indian Journal of Microbiology 53(4): 410-417.

Elkenany R, Elsayed MM, Zakaria AI, El-Sayed SA, Rizk MA (2019) Antimicrobial resistance profiles and virulence genotyping of *Salmonella enterica* serovars recovered from broiler chickens and chicken carcasses in Egypt. BioMed Central Veterinary Research 15(1):124.

Eng SK , Pusparajah P, Mutalib Ab, Syakimaet N, Ser HL ,Chan KG, Lee LH (2015) *S almonella*: A review on pathogenesis, epidemiology, and antibiotic resistance. Frontiers in Life Science 8(3): 1-10.

Fendri I, Hassena AB, Grosset N, Barkallah M, Khannous L, Chuat V, Gautier M, Gdoura R (2013) Genetic diversity of food-isolated *Salmonella* strains through Pulsed Field Gel Electrophoresis (PFGE) and Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR). PLoS One 8(12): e81315.

Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA (2019) Worldwide Epidemiology of *Salmonella* Serovars in Animal-Based Foods: a Meta-analysis. Applied and Environmental Microbiology 85(14): e00591-19.

Gharieb RM, Tartor YH, Khedr MH (2015) Non-Typhoidal *Salmonella* in poultry meat and diarrhoeic patients: prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug-resistant strains. Gut pathogens 7:34.

Hashemi A, Baghbani-Arani F (2015) The effective differentiation of *Salmonella* isolates using four PCR-based typing methods. Journal of Applied Microbiology 118(6):1530-1540.

Helmy MN, Mahmoud AH, Adawy SS (2009) Application of Multiplex Polymerase Chain Reaction (MPCR) for Identification and Characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium. Journal of Applied Sciences Research 5(12): 2343-2348.

ISO 6579-3 (International Organization for Standardization) (2014) Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*-Part 3: Guidelines for serotyping of *Salmonella* spp. https://www.iso.org/standard/56714.html.

ISO 6579-1 (International Organization for Standardization) (2017) Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*-Part 1: Detection of *Salmonella* spp. https://www.iso.org/obp/ui/#iso:std:iso:6579:-1:ed-1:v1:en.

Jajere SM (2019) A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Veterinary world 12(4): 504-521.

Jeníková G, Pazlarová J, Demnerová K (2000) Detection of *Salmonella* in food samples by the combination of immunomagnetic separation and PCR assay. International microbiology 3(4): 225-229.

Liaquat S, Sarwar Y, Ali A, Haque A, Farooq M, Martinez-Ballesteros I, Laorden L, Garaizar J, Bikandi J (2018) Virulotyping of *Salmonella enterica* serovar Typhi isolates from Pakistan: Absence of complete SPI-10 in Vi negative isolates. PLoS Neglected Tropical Diseases

12(11): e0006839.
Lozano-Villegas K, Rodríguez HR, Rondón BI (2019) Effectiveness of six molecular typing methods as epidemiological tools for the study of *Salmonella* isolates in two Colombian regions. Veterinary World 12(12): 1998-2006.
Magwedere K, Rauff D, De Klerk G, Keddy KH, Dziva F (2015) Incidence of Nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012-2014. Clinical Infectious Diseases 61(4):283-289.
Medeiros C, Almeida F, Ine M, Rodrigues P, Falca JP (2015) Genotypic diversity, pathogenic potential and the resistance profile of *Salmonella* Typhimurium strains isolated from humans and food from 1983 to 2013 in Brazil. Journal of Medical Microbiology 64(11): 1395-1407.
Mezal EH, Sabol A, Khan MA, Ali N, Stefanova R, Khan AA (2014) Isolation and molecular characterization of *Salmonella enterica* serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiology 38: 67-74.
Moussa IM, Gassem MA, Al-Doss AA, Mahmoud WAS, Abdel Mawgood AL (2010) Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. African Journal of Biotechnology 9: 612-619.
Murugkar HV, Rahman H, Dutta PK (2003) Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. The Indian Journal of Medical Research 117: 66-70.
Nouichi S, Ouatouat R, Can H, Mezali L, Belkader C, Ouar- korichi M, Bertrand S, Cantekin Z, Hamdi T (2018) Prevalence and antimicrobial resistance of *Salmonella* isolated from bovine and ovine samples in slaughterhouses of Algiers, Algeria. J HELLENIC VET MED SOC 69(1): 863-872.
Paniel N, Noguer T (2019) Detection of *Salmonella* in food matrices, from conventional methods to recent aptamer-sensing technologies. Foods 8(9): 371.
Pooncharoen K, Pulsrikarn C, Nuanmuang N, Khamai P (2019) Effectiveness of BOX-PCR in Differentiating Genetic Relatedness among *Salmonella enterica* Serotype 4,[5],12:i:- Isolates from Hospitalized Patients and Minced Pork Samples in Northern Thailand. International Journal of Microbiology 5086240. doi: 10.1155/2019/5086240.
Proroga YTR, Capuano F, Capparelli R, Bilei S, Bernardo M, Cocco MP, Campagnuolo R, Pasquale V (2018) Characterization of non-typhoidal *Salmonella enterica* strains of human origin in central and southern Italy. Italian journal of food safety 7(1): 6888.
Rahman H (2006) Prevalence and phenotypic expression of *sop*B gene among clinical isolates of *Salmonella enterica*. The Indian Journal of Medical Research123(1): 83-88.
Ranjbar R, Mortazavi SM, Tavana AM, Sarshar M, Najafi A, Zanjani RS (2017) Simultaneous Molecular Detection of *Salmonella enterica* Serovars Typhi, Enteritidis, Infantis, and Typhimurium. Iranian Journal of Public Health 46(1): 103-111.
Rowlands RE, Ristori CA, Ikuno AA, Barbosa ML, Jakabi M, Franco BD (2014) Prevalence of drug resistance and virulence features in *Salmonella* spp. isolated from foods associated or not with salmonellosis in Brazil. Revista do Instituto de Medicina Tropical de São Paulo 56(6): 461-467.
Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL, Jones JL, Griffin PM (2011) Foodborne Illness Acquired in the United States-Major Pathogens Elaine. Emerging Infectious Diseases 17(1): 7-15.
Seifi S, Khoshbakht R, Azizpour A (2019) Occurrence of *Campylobacter*, *Salmonella*, and *Arcobacter* in pet birds of northern Iran**.** J HELLENIC VET MED SOC 70: 3-11.
Shanmugasamy M, Velayutham T, Rajeswar J (2011) *InvA* gene-specific PCR for detection of *Salmonella* from broilers. Veterinary World 4(12): 562-564.
Skyberg J, Logue C, Nolan L (2006) Virulence genotyping of *Salmonella* spp. with multiplex PCR. Avian Diseases 50: 77-81.
Smith SI, Fowora MA, Atiba A, Anejo-Okopi J, Fingesi T, Adamu ME, Omonigbehin EA, Ugo-Ijeh MI, Bamidele M, Odeigah P (2015) Molecular detection of some virulence genes in *Salmonella* spp. isolated from food samples in Lagos, Nigeria. Animal and Veterinary Sciences 3(1): 22-27.
Srisanga S, Angkititrakul S, Sringam P, Le Ho PT, T Vo AT, Chuanchuen R (2017) Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella enterica* isolated from pet dogs and cats. Journal of veterinary science 18(3): 273-281.
Tarabees R, Elsayed MSA, Shawish R, Basiouni S, Shehata AA (2017) Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. Journal of Infection in Developing Countries11(4): 314-319.
Thung TY, Radu S, Mahyudin NA, Rukayadi Y, Zakaria Z, Mazlan N, Tan BH, Lee E, Yeoh SL, Chin YZ, Tan CW, Kuan CH, Basri DF, Wan Mohamed Radzi CWJ (2018) Prevalence, Virulence Genes and Antimicrobial Resistance Profiles of *Salmonella* Serovars from Retail Beef in Selangor, Malaysia. Frontiers in Microbiology 8: 2697.
Wang X, Biswas S, Paudyal N, Pan H, Li X, Fang W, Yue M (2019) Antibiotic resistance in *Salmonella* Typhimurium isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. Frontiers in microbiology 10: 985.
Yehia HM, Elkhadragy MF, Al-masoud AA, Al-Dagal MM (2020) *InvA* Gene to Detect *Salmonella Enterica* Serovar Typhimurium Supported by Serum Anti-*Salmonella* Antibodies and Protein Profiles for Chicken Carcass Isolates**.** Research Square, doi:10.21203/rs.3.rs-87131/v1.