International Journal of Food Microbiology 337 (2021) 108956

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Whole-genome sequence analysis of *Salmonella* Infantis isolated from raw chicken meat samples and insights into pESI-like megaplasmid

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ARTICLE INFO

Keywords:
S. Infantis
Chicken meat
Whole genome sequencing
Clonal spread
Megaplasmid

ABSTRACT

There has been an increase in the number of reports on *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis) isolated from animals and humans. Recent studies using whole genome sequencing (WGS) have provided evidence on the likely contribution of a unique conjugative megaplasmid (pESI; ~280 kb) to the dissemination of this serovar worldwide. In the present study, twenty-two unrelated *Salmonella* strains [*S.* Infantis (n = 20) and *Salmonella* 6,7:r:- (n = 2)] and their plasmids were investigated using next generation sequencing technologies (MiSeq and MinION) to unravel the significant expansion of this bacteria in Turkey. Multi-locus sequence typing, plasmid replicons, resistance gene contents as well as phylogenetic relations between strains were determined. According to the WGS data, all *S.* Infantis possessed the relevant megaplasmid backbone genes and belonged to sequence type 32 (ST32) with the exception of a single novel ST7091. Tetracycline and trimethoprim/sulfamethoxazole resistance were found to be widespread in *S.* Infantis strains and the resistant strains exclusively carried the *tet*A, *sul1*, *sul2* and *dfr*A14 genes. One *S.* Infantis isolate was also a carrier of the plasmid-mediated ampC via bla_{CMY-2}, gene. Moreover, full genomes of four *S.* Infantis isolates were reconstructed based on hybrid assembly. All four strains contained large plasmids (240–290 kb) similar to previously published megaplasmid (pESI) and accompanied by several small plasmids. The megaplasmid backbone contained a toxin-antitoxin system, two virulence cassettes and segments associated with heavy metals resistance, while variable regions possessed several antibiotic resistance genes flanked by mobile elements. This study indicated that pESI-like megaplasmid is widely disseminated within the tested *S.* Infantis strains of chicken meat, warranting further genomic studies on clinical strains from humans and animals to uncover the overall emergence and spread of this serovar.

1. Introduction

Non-typhoidal *Salmonella* (NTS) is one of the most common causes of foodborne bacterial infections in humans, with about 94 million human gastroenteritis cases and 155.000 deaths annually throughout the world (Majowicz et al., 2010). In 2018, there were 91.857 confirmed cases reported in 28 EU countries, with 119 deaths (EFSA, 2019). *Salmonella* caused a total of 1.580 foodborne outbreaks, resulting in 11.581 cases, and the majority of cases were associated with consumption of poultry meat and eggs (EFSA, 2019). In fact, NTS serovars cause acute illnesses with gastrointestinal symptoms characterized with diarrhoea, fever and malaise, which are mostly self-limiting episodes that do not require treatment (Erdem et al., 2005). On the other hand, NTS infections could result in severe clinical manifestations and complication in affected individuals who might need antimicrobial therapy (Sanz-Puig et al., 2015).

There have been more than 2.600 NTS serotypes described, among which *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis) has been recognized as one of the most frequent causes of bacterial food borne illness worldwide (Hendriksen et al., 2011; Issenhuth-Jeanjean et al., 2014). For instance, there were 1.868 reported *S.* Infantis related human infection cases in 2018, placing this organism among the top four

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https://doi.org/10.1016/j.ijfoodmicro.2020.108956
Received 5 July 2020; Received in revised form 19 October 2020; Accepted 29 October 2020
Available online 6 November 2020
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infectious serovars causing human salmonellosis in EU/EEA countries (EFSA, 2020). Unfortunately, multidrug resistant (MDR) *S.* Infantis was reported with a worrying frequency from human and animal sources, representing a major public health concern due to the treatment challenges (Carfora et al., 2018; Dionisi et al., 2011; Nogrady et al., 2012). Consumption of broiler meat was a major source of *S.* Infantis infection in humans (Antunes et al., 2016; EFSA, 2020). It is directly linked with a high frequency of *S.* Infantis carriage reported in broiler flocks (Nogrady et al., 2012; Report, 2018) and further high prevalence in broiler meat observed in EU/EEA countries (EFSA, 2020), Turkey (Acar et al., 2017) and Israel (Aviv et al., 2014). The emergence and dissemination of MDR *S.* Infantis were favored by a unique conjugative megaplasmid (pESI; ~280 kb) without any burden on the host bacteria (Aviv et al., 2014; Franco et al., 2015). Moreover, beneficial accessory genes coding resistance to heavy metals and disinfectant as well as virulence traits were found to be located on the same plasmid. In addition, *S.* Infantis possessing the pESI-like plasmid and resistant to important class of antibiotics including cephalosporins and colistin has been reported from numerous countries including Italy (Carfora et al., 2018; Franco et al., 2015), Switzerland (Hindermann et al., 2017), USA (Tate et al., 2017), Russia (Bogomazova et al., 2020) and Japan (Yokoyama et al., 2015), suggesting global distribution of the serovar.

According to the Turkish Ministry of Health, *S.* Infantis was the third most frequent serovar observed during 2012–2016 accounting for 4.0–6.7% of human *Salmonella* infections after *S.* Enteritidis and *S.* Typhimurium (Report, 2018). Recent national surveillance program has revealed that 24.5% of broiler flocks were being colonized with *Salmonella* spp., among which the most frequent serotype was *S.* Infantis (72.2%), followed by *S.* Kentucky (10.6%) and *S.* Enteritidis (6.1%) (Report, 2018). In our recent investigation, 85.1% of *Salmonella* isolates obtained from chicken meat samples were serotyped as *S.* Infantis ($n = 40$), followed by *Salmonella* 6,7:r:- ($n = 2$), *S.* Enteritidis (n = 2), and single strains of *S.* Orion, *S.* Potsdam, and *Salmonella* 8,20:z4z23:- (Kurekci et al., 2019). This indicates that the situation has changed over the past two decades since *S.* Infantis was not found in the ceacal samples of broilers and layers about 20 years ago in Turkey (Carli et al., 2001). Up to date, there has been no investigation to explore this significant change in the serotype distribution of *Salmonella* spp. in poultry and poultry products in Turkey. Therefore, it is important to obtain information on the characteristics of *S.* Infantis in order to understand its emergence and significant expansion in both human and poultry in recent years. In order to provide new insights into this question, we used two next generation sequencing platforms for genomic characterization of *S.* Infantis ($n = 20$) and *Salmonella* 6,7:r:- (n = 2) strains.

Table 1
AMR phenotypes with AMR genes identified by ResFinder in *S.* Infantis isolates.

Isolate no	City	Company	MLST Type	Antimicrobial resistance profile*	Resistance genes*	Predicted antigenic profile
S18/0149	Sivas	A	32	AmAmcCazCFfcSmxTe	ant(3″)-Ia, bla_{CMY-2}, floR, mdf(A), Sul1, TetA	7:r:1,5
S18/0153	Hatay	A	32	CFfcTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0188	Sivas	B	32	SmxTe	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0141	Hatay	C	32	SmxTe	ant(3″)-Ia, aph(3′)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0143	Sivas	D	32	CFfcSmxTe	ant(3″)-Ia, aph(3″)-Ib, aph(6)-Id, dfrA14, floR, mdf(A), Sul1, TetA	7:r:1,5
S18/0145	Hatay	E	7091	nd	aph(3′)-Ia, dfrA14, mdf(A)	7:r:1,5
S18/0147	Sivas	D	32	SmxTe	ant(3″)-Ia, aph(3″)-Ib, aph(3′)-Ia, aph(6)-Id, dfrA14, floR, mdf(A), Sul1, Sul2, TetA	7:r:1,5
S18/0148	Hatay	B	32	CSmxTe	ant(3″)-Ia, aph(3″)-Ib, aph(3′)-Ia, aph(6)-Id, dfrA14, floR, mdf(A), Sul1, Sul2, TetA	7:r:1,5
S18/0154	Hatay	F	32	SmxTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0157	Sivas	D	32	SmxTe	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0158	Hatay	G	32	nd	mdf(A)	7:r:1,5
S18/0165	Sivas	B	32	CFfcSmxTe	ant(3″)-Ia, dfrA14, floR, mdf(A), Sul1, TetA	7:r:1,5
S18/0169	Sivas	A	32	SmxTe	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0172	Sivas	H	32	SmxTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0174	Hatay	B	32	SmxTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0179	Sivas	D	32	SmxTe	ant(3″)-Ia, aph(3′)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0180	Sivas	A	32	nd	ant(3″)-Ia, aph(3′)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0156	Sivas	A	32	SmxTe	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0163	Sivas	B	32	Te	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0185	Hatay	G	32	SxTe	ant(3″)-Ia, mdf(A), Sul1, TetA	7:r:1,5
S18/0187	Sivas	D	32	CipSmxTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5
S18/0177	Hatay	K	32	SmxTe	ant(3″)-Ia, dfrA14, mdf(A), Sul1, TetA	7:r:1,5

nd: not detected. *The aac(6′)-Iaa gene encoding aminoglycoside modifying enzymes are present in all isolates.

2. Material and methods

2.1. Background of S. Infantis strains

A total of twenty-two *Salmonella* strains serotyped by using White-Kauffmann–Le Minor scheme were included in this study. These strains comprised of 20 *S.* Infantis and two *Salmonella* 6,7:r:- and were epidemiologically unrelated (on the basis of producer of poultry, city and date of isolation and antimicrobial resistance profile; Table 1). All strains were recovered from raw chicken meat samples sold in the retail market of Hatay and Sivas provinces in Turkey over a period of 9 months (January to September 2017). Antimicrobial susceptibility testing was performed using disc diffusion method, according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2015). A panel of antimicrobials included: ampicillin, Am; amoxicillin/clavulanic acid, Amc; cefotaxime, Ctx; ceftazidim, Caz; chloramphenicol, C; florfenicol, Ffc; ciprofloxacin, Cip; gentamycin, Gn; imipenem, Imp; tetracycline, Te and trimethoprim/sulfamethoxazol, Smx.

2.2. Whole genome sequencing

Genomic material was extracted with commercial kit (Genomic Mini, A&A Biotechnology) according to manufacturer's instructions. Quantity and quality of DNA were evaluated by spectrophotometric (NanodropOne, Thermo Fisher Scientific) and fluorometric (Qubit 3.0, Thermo Fisher Scientific) methods. Additionally, integrity of isolated DNA was confirmed by capillary electrophoresis (DNF–488 High Sensitivity Genomic DNA Analysis Kit, Fragment Analyzer, Agilent). Library preparation for short-read sequencing was performed with the use of the NexteraXT kit (Illumina) and dual-indexing system (Illumina) according to manufacturer's recommendation. Quality and quantity of libraries were confirmed by capillary gel electrophoresis (DNF-473 Standard Sensitivity NGS Fragment Analysis Kit, Fragment Analyzer, Agilent) and fluorimeter (BR assay kit, Qubit 3.0, Thermo Fisher Scientific). The paired-end (2 × 300 bp) sequencing was performed using the Reagent kits V3 on the MiSeq platform (Illumina, San Diego, CA, USA).

Additionally, based on the initial analyses of short-read sequences, four strains were selected for long-read WGS for the further characterization of plasmid's structure and assessment of antimicrobial resistance (AMR) genes. These four strains were selected on the basis of featured IncX1 replicon and significant number of multiple resistance genes (MDR profile). Additionally, one of them had AmpC marker (bla_{CMY-2}). Libraries for long-read sequencing were prepared with the use of 1D Genomic DNA sequencing kit SQK-LSK108 (Oxford Nanopore). DNA sheering and repair steps were excluded from the procedure to prevent fragmentation. Size selection and clean-up step were done by AMPure XP (Beckman Coulter). Quantification of libraries was done by fluorimeter (BR assay kit, Qubit 3.0, Thermo Fisher Scientific). Sequencing of long reads was performed on MinION (Oxford Nanopore) and basecalling was done in real-time by MinKNOW.

2.3. Analysis of sequencing data

Short reads from Illumina sequencing were trimmed by using Trimmomatic 0.36 (Bolger et al., 2014) and de novo assembled by SPAdes genome assembler software 3.13.0 (Nurk et al., 2013). Sets of data were searched for the antimicrobial resistance and virulence genes using Abricate 0.9.8 (Seemann T., https://github.com/tseemann/abricate) with ResFinder 3.2 (Zankari et al., 2012) and virulence factor (Chen et al., 2016) databases, respectively. Threshold levels were ≥75% for gene identity without minimum coverage. Serotype identification was done using SeqSero version 1.0.0 software (Zhang et al., 2019). Multilocus sequence typing (MLST) and estimation of plasmid replication genes were done by MLST 2.0 of the PubMLST website (https://pubmlst.org/) developed by Jolley and Maiden (2010) and PlasmidFinder 2.0.1 (Carattoli et al., 2014), respectively.

Hybrid assembly of both short- and long-read reads was done by Unicycler 0.4.8 (Wick et al., 2017) to reconstruct plasmid and chromosome structure. Then, analysis of newly assembled files was performed by CGE web tools. Presence of Insertion Sequence (IS) was confirmed using ISFinder (Siguier et al., 2006). Plasmids were annotated and visualized by CGView Server ^{BETA} (Grant and Stothard, 2008). Comparison of surveyed plasmids composition with the pESI reference from Aviv et al. (2014) was done by Blast software. The assembled short length genome sequences were uploaded to the European Nucleotide Archive under accession number (PRJEB35626).

2.4. Phylogenetic analysis

The core genome MLST (cgMLST) phylogeny analysis was used to identify WGS of *S.* Infantis strains. Fastq files were loaded into Enterobase (https://enterobase.warwick.ac.uk/), following quality check and assembly, and alignment was done using V2 scheme containing 3002 core genes. The tree was built using MStree V2 algorithm. All available *S.* Infantis genomes with the same HierCC50 values as tested sequences of this study were also included for generation of the tree. The software grapetree (Zhou et al., 2018) was used for visualization of the minimum spanning tree.

3. Results

3.1. Serotype, sequence type and phylogenetics

Two *Salmonella* 6,7:r:- strains that could not serotyped completely by White-Kauffmann–Le Minor scheme were further identified as *S.* Infantis by WGS (Table 1). Moreover, WGS data indicated that all *S.* Infantis ($n =$ 22) of the current study were assigned to ST32, except for one strain, which was identified as novel ST7091 – single variant different (hemD-930) from ST32 (Table 1). Moreover, cgMLST – based phylogenetic analysis was used to understand the clonal relationship of *S.* Infantis strains tested in the current study. This analysis also included a wider panel of publicly available *S.* Infantis sequences from Turkey and other countries. The analysis revealed significant genetic similarities among *S.* Infantis strains sequenced in this study, with allele differences between 0 and 84 (Fig. 1). The majority of Turkish strains created a large cluster, together with strains from the United Kingdom and Denmark.

3.2. Plasmid reconstructions

Short-read sequencing indicated the presence of IncFIB replicon gene in all *S.* Infantis strains. Four strains harbored IncX1 replicon, and one of them also had IncI1 (Supplementary Table S1). Hybrid assembly indicated the presence of megaplasmid and small plasmid in four IncX1 positive strains. According to the WGS analysis of hybrid assembly, all megaplasmids had the IncFIB (pN55391) replication gene, while one isolate had additional IncX1. Their size ranged from 239 kb to approximately 289 kb. Small plasmids ranged from 44 kb to 96 kb and were classified as IncX1 ($n = 3$) or IncI1 ($n = 1$) (Fig. 2 and Supplementary Table S2). Comparison of the megaplasmid sequences obtained in the current study to previously published pESI-like megaplasmids (CP016407.1, CP0164097.1, CP016411.1, CP016413.1; Tate et al., 2017) indicated high similarity (99,97% identity on 82% of sequence length). Four isolates had similar megaplasmid backbone containing mainly: toxin/anti-toxin gene clusters, genes responsible for mercury resistance, nickel and iron transporters cassette and two large virulence cassettes (Supplementary Table S2). In addition, pESI-like megaplasmid of *S.* Infantis strains from Turkey harbored variable regions, codding mostly antibiotic resistance (Supplementary Table S3).

In addition, complete plasmid sequences from hybrid assembly were used as a reference for multi-alignment of the remaining *S.* Infantis. All of them were mapped to Turkish pESI-like megaplasmid structure on at

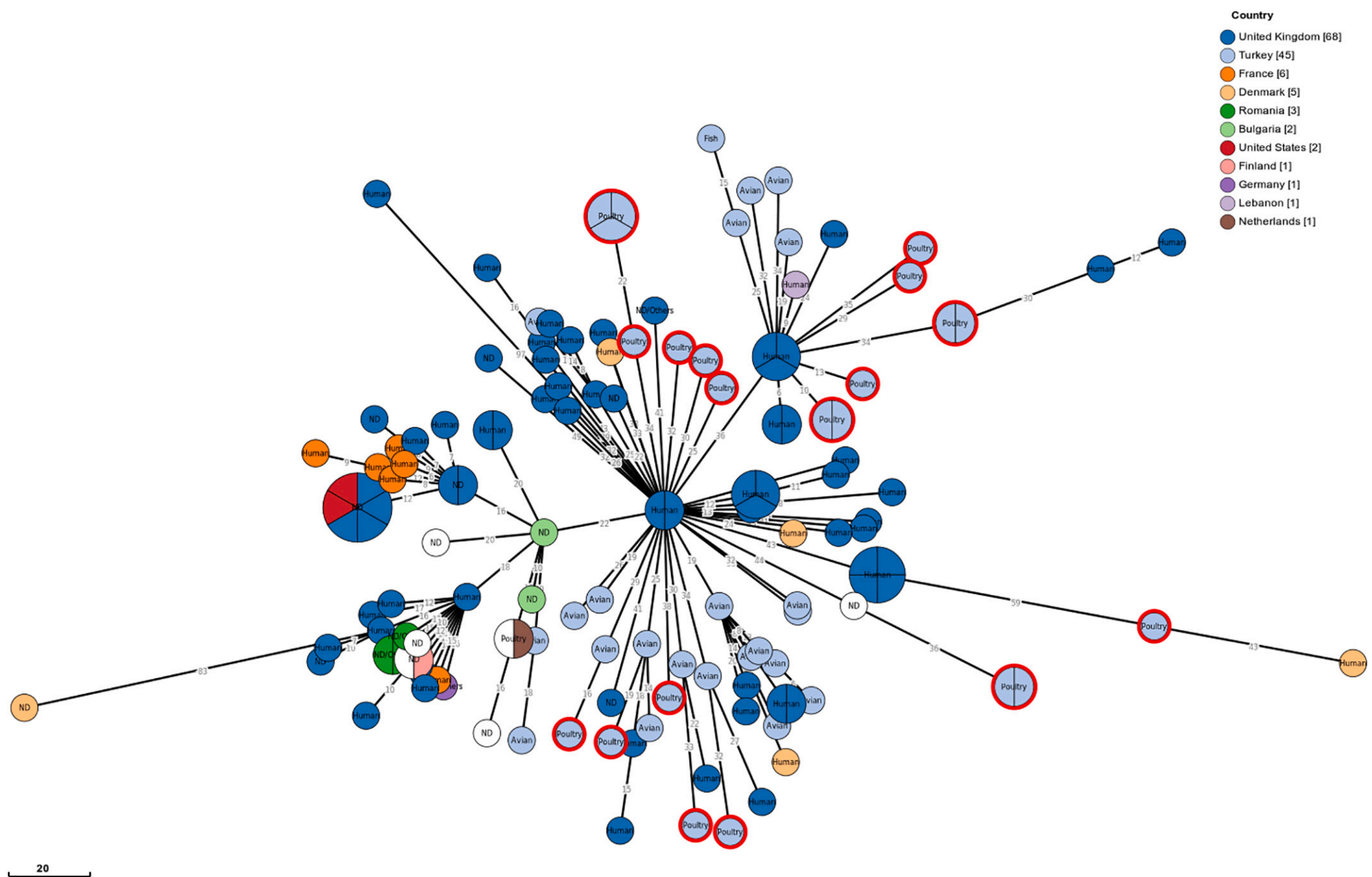

Fig. 1. cgMLST-based phylogenetic tree of *Salmonella* Infantis constructed using HC50(36) sequences available in the Enterobase. Branches were collapsed on distance of 5 allel differences. The country of origin were represented by coloured circles that showed the source of the isolates. Turkish strains characterized in the presented study were additionally circled in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

least 66% of its length (Table S4).

3.3. Resistance genes and phenotypic resistance

A total of twelve genes encoding resistance to six different antibiotic classes were detected in the WGS data (Table 1) and their sequence coverages were given in Supplementary Table S5. Among these, the *mdfA* gene encoding MdfA efflux pumps was identified in the sequences of all *S*. Infantis. Twenty strains possessed *tetA* and *sul* genes in combinations (*sul1*, $n = 18$ and *sul2*, $n = 2$). In addition, 14 strains had the *dfrA14* gene. The *dfrA14*, *sul1* and *tetA* gene combination was present in 13 strains. In most of the cases *tetA*, *sul1* and *ant*(*3*″)*-Ia* genes were co-located on the same contig, in three cases these genes were found to be associated with IncFIB plasmid replicons (Supplementary Table S3). The presence of these genes corresponded well with phenotypic resistance, in which 19 and 17 of *S*. Infantis were resistant to tetracycline and trimethoprim/sulfamethoxazole, respectively (Table 1).

Interestingly, the genes *aac*(*6*′)*-laa* and *ant*(*3*″)*-la* encoding aminoglycoside modifying enzymes were also present, respectively, in 22 and 20 strains. The other aminoglycoside-resistance genes found were *aph* (*3*′)*-la* ($n = 6$), *aph*(*3*″)*-Ib* ($n = 3$), and *aph*(*6*)*-Id* (n = 3). However, all strains were sensitive to gentamycin that was the only aminoglycoside tested in the current study.

Only one strain displayed phenotypical resistance to β-lactams including ceftazidime, amoxicillin/clavulanic acid and ampicillin. That single strain was AmpC-type cephalosporinase producer due to the presence of bla_{CMY-2} that was found to be localized in a vicinity of IncI1 replicon (Supplementary Tables S2 and S3). Reconstructed IncI1 (96 kb) plasmid harbored bla_{CMY-2} gene mobilized by insertion sequence IS1380 (ISEcp1) (Fig. 3). Several other mobile elements and nickel transporter gene were also carried by this plasmid (Supplementary Table S2).

WGS results displayed that five out of 22 *S*. Infantis harbored the *floR* gene for which phenotypical resistance to chloramphenicol ($n = 4$) and florfenicol (n = 3) was observed. One isolate (S18/0147) carrying the *floR* gene was identified as chloramphenicol and florfenicol susceptible, whereas another *S*. Infantis (S18/0153) was identified as resistant to chloramphenicol/florfenicol, but not possessing the *floR* gene (Table 1).

In hybrid assembled strains, aminoglycoside resistance gene (*aac*(*6*′)*-laa*) and MdfA efflux pumps gene (*mdfA*) were localized on chromosome, while all the remaining genes were allocated on plasmids. Variable regions of megaplasmids and small plasmids harbored different combinations of resistance genes (*ant*(3″)-Ia, *aph*(3″)-Ib, *aph*(6)-Id, *floR*, *sul1*, *tet*(A), *dfrA14*) flanked by multiple mobile elements (IS, transposons, integrase, DNA topoisomerase III, recombinase, resolvase, etc.). Those various AMR determinants were located on both large and small plasmids (Supplementary Table S3). Moreover, in three out of four strains, arsenic resistance and type IV secretion complex (T4S - coupling protein VirD4, inner membrane protein and ATPase required for IV system assembly) were associated with small plasmids (Supplementary Table S3).

3.4. Virulence genes

S. Infantis has displayed the presence of numerous virulence genes mostly related to fimbrial adherence determinants and secretion system components. Three fimbrial operons, Agf/Csg (*csg*BAC and *csg*DEFG), *fim*ICDHF and *lpf*ABCDE were the most frequently identified operons in

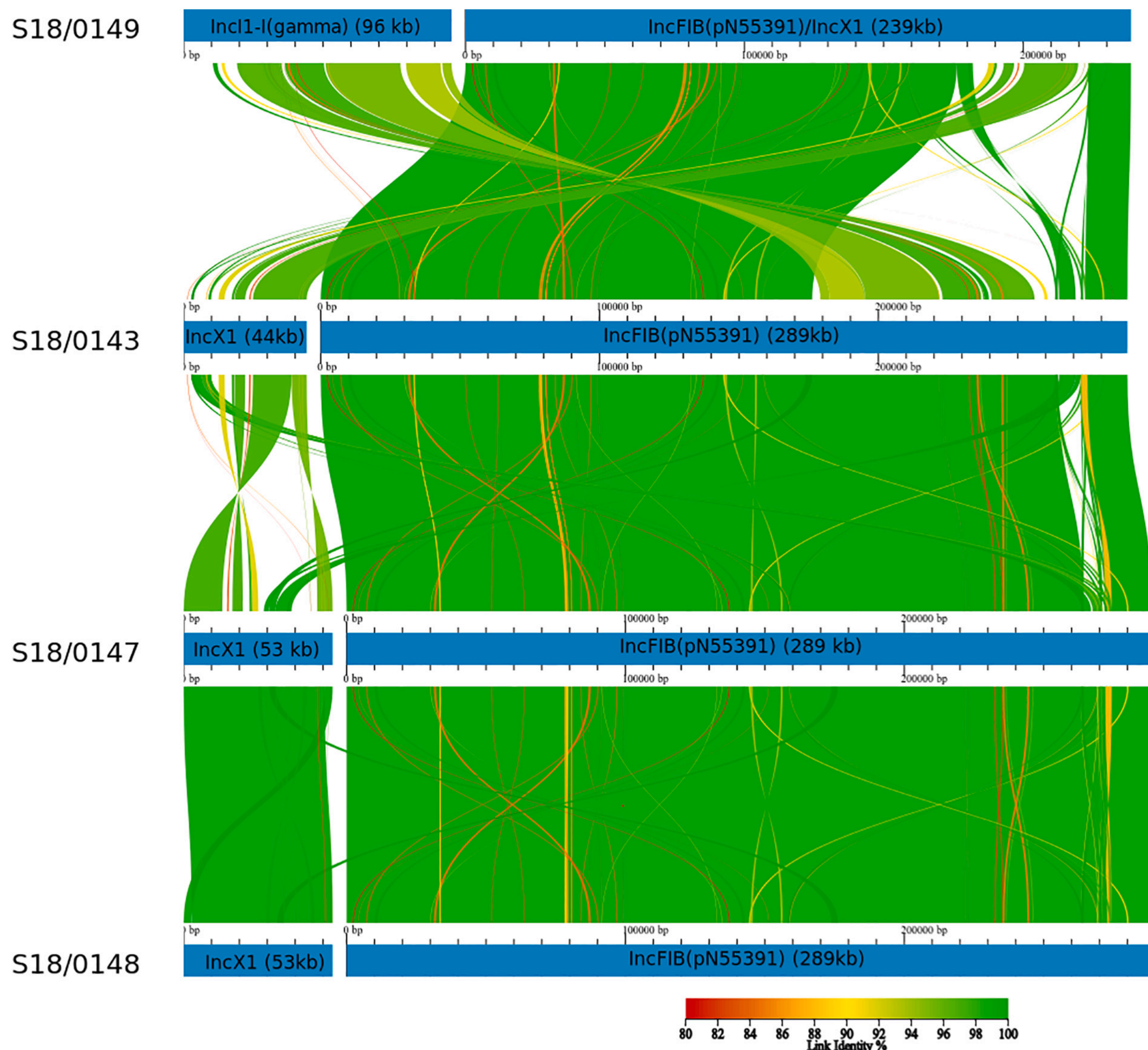

Fig. 2. Comparison of complete plasmid sequences of four Turkish *S.* Infantis strains, generated via hybrid assembly. Green colour visualises high identity of sequences shared. Colour shift to the red spectrum shows decreasing identities between the sequences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

all the isolates. In addition, type III secretion systems (T3SS) genes encoding *Salmonella* pathogenicity island-1/2 (SPI1/SPI2) were identified as the most common virulence traits among *S.* Infantis. Additionally, five variable SPI1 were common to all *S.* Infantis isolates: *inv*, *org*, *prg*, sic, *spa* (Supplementary Table S6).

It was shown that two main virulence cassettes were located on pESI-like megaplasmid. The first contained several fimbria coding genes and the second had Yersiniabactin biosynthetic protein (YbtT, YbtU, Irp1, and Irp2), regulatory and transport operons of *Yersinia pestis*.

4. Discussion

Currently adopted "gold" *Salmonella* serotyping based on the White-Kauffmann–Le Minor scheme utilizes phenotypic variation of antigenic properties [somatic "O" and flagellar "H" antigens] (Grimont and Weill, 2007). Nevertheless, the utility of the method is limited owing to being expensive, laborious, the necessity of technical expertise and the inability to differentiate serotypes, when the isolates do not fully express their antigens (Nair et al., 2014). Several studies have documented WGS as effective tool for genome-based identification of serovars. It gets around the above limitations and in addition to serovar identification, it provides essential data for *Salmonella* characterization (Bale et al., 2016). The current study also showed WGS as reliable, rapid and accurate means of *Salmonella* serovar identification. This includes also sequence type identification where all but one (ST7091) strain belonged to ST32, which is the most often observed in other European countries (Alba et al., 2020; Carfora et al., 2018; Franco et al., 2015) and Japan (Sakano et al., 2013). WGS also enabled us to understand the phylogenetic relationship of *S.* Infantis and the sequence analysis of strains in this study displayed a wide homogeneity of Turkish strains using the means of SNP analysis. However, some Turkish strains were grouped together with those reported from the United Kingdom and Denmark, confirming the clonal spread to distant geographic regions as highlighted by Alba et al. (2020).

As previously reported (Abbasoglu and Akcelik, 2011; Acar et al., 2017; Alba et al., 2020; Siriken et al., 2015), WGS analysis revealed that the majority of *S.* Infantis strains harbored tetracycline and trimethoprim/sulfamethoxazol resistance genes. According to the long-read sequence analysis, most of the antimicrobial resistance genes [except *aac(6′)-Iaa* and *mdf(A)*] seem to be associated with either a megaplasmid or small plasmids. For example, the *sul1* gene was located on the megaplasmid, whereas the *sul2* gene was on the small plasmid (53 kb, IncX1)

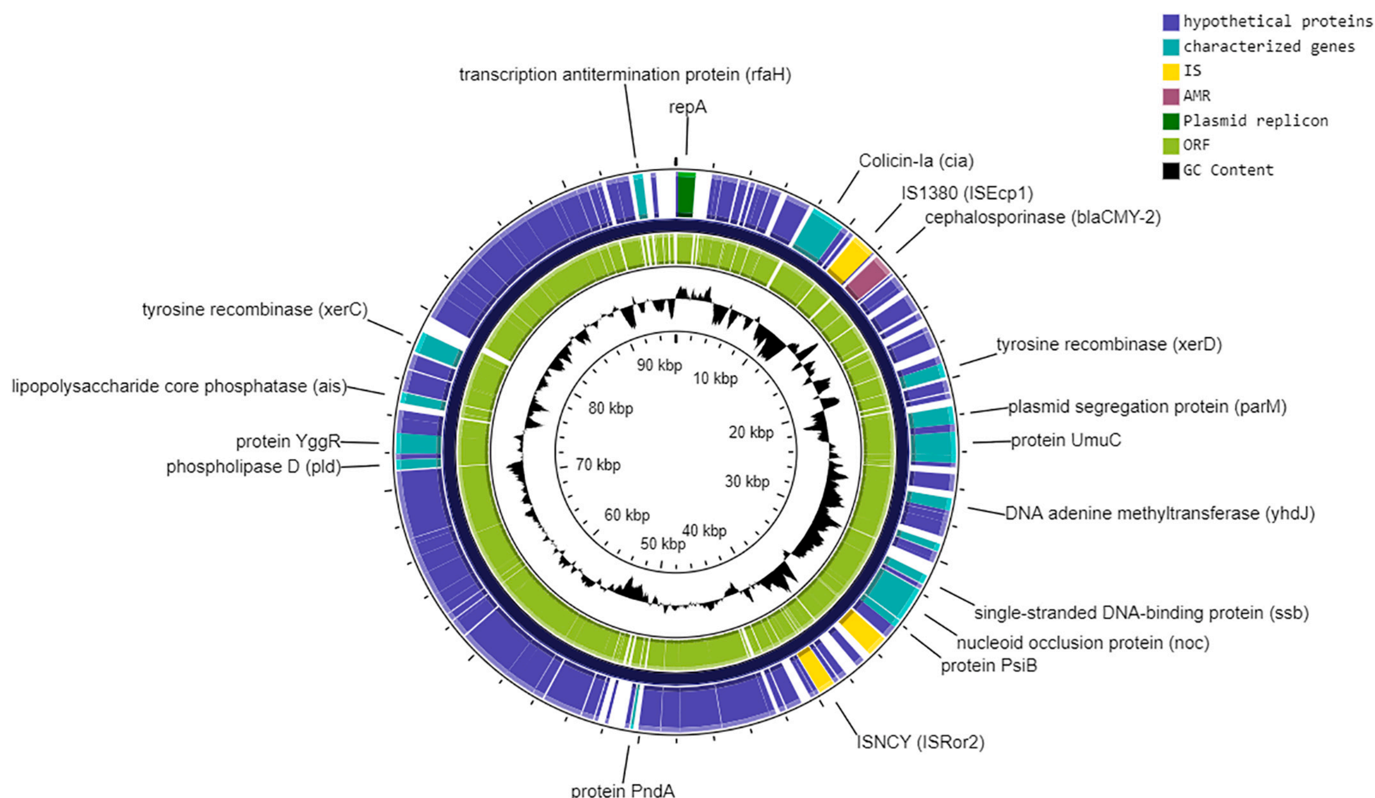

Fig. 3. Structure of small IncI1plasmid (96 kb) harbored by *S.* Infantis strain (S18/0149). IncI1 plasmid harbours resistance to cephalosporins via the bla_{CMY-2} gene (pink colour) mobilized via insertion sequence Ecp1. Plasmid was visualized and annotated automatically via CGviewer, annotation was manually verified. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in hybrid assembled sequences. Abbasoglu and Akcelik (2011) also reported the presence of phenotypic TeSmx resistance and the genes (*aadA1*, *aphA1*, *sul1*, *tetA*, *dfrA5/dfrA14* and *gyrA*) in all *S.* Infantis strains obtained from broiler chicken meat in Turkey. Similarly, a recently published study of *S.* Infantis from Turkey has identified *aadA1*, *tetA* and *sul1* genes, but failed to confirm their presence on the plasmids due to the limitations of short-read sequencing and assemblies (Acar et al., 2019). Of note, *ant(3″)-Ia* (synonymous with *aadA*), *aac(6′)-Iaa* and *mdfA* theoretically encoding aminoglycoside resistance did not correspond to phenotypic feature. This is consistent with previous reports on lack of genotype-phenotype congruence for aminoglycoside resistance in *S.* Infantis (Acar et al., 2019; Rule et al., 2019).

Additionally, only one *S.* Infantis strain was found to be phenotypically resistant to ceftazidime. For this strain the AmpC-type cephalosporinase via bla_{CMY-2} gene was identified on reconstructed IncI1 plasmid, which is not surprising as IncI1 plasmid has been described as the most common carrier of bla_{CMY-2} (Sidjabat et al., 2014). However, finding of bla_{CMY-2} was reported in only one genome of the European *S.* Infantis strains that were all identified as extended-spectrum β-lactamase coding gene, bla_{CTX-M}, carrier (Alba et al., 2020). In the current study, the sequence of the CMY-2-encoding gene was localized directly upstream of insertion sequence 1380 (ISEcp1), which has been shown to be a key player for mobilization of cephalosporin resistance genes, especially of bla_{CMY-2}, in *Salmonella* isolates (Chiu et al., 2020). Low level of resistance to cephalosporins is expected, as a recent study (Shigemura et al., 2018) has noted that the incidence of the bla_{CMY-2} gene among *Salmonella* spp. from poultry samples was decreasing virtually from 2011 to 2015 in Japan, which was attributed to discontinued use of ceftiofur in animal production.

Here, with short- and long-read sequencing of few strains and mapping with the remaining *S.* Infantis strains, we have confirmed their considerable homology with the recently published pSEI-like megaplasmid (Tate et al., 2017). Interestingly, one of the described megaplasmids herein contained more than one replication gene (IncFIB/IncX1). Such a multireplicon plasmid has been proposed to result from either non-selective divergence or plasmid recombination during the evolution (Mark Osborn et al., 2000), leading to the high alterable nature in plasmids (Compain et al., 2014).

Plasmids occurring in Turkish *S.* Infantis strains contain backbone with two virulence regions (coding fimbriae and regulatory/transport operons of *Yersinia pestis*), bacteria toxin/anti-toxin cassettes, iron transporters, mercuric and nickel resistance cassettes, which are significant regions of conserved structures. Several of those traits were also commonly noted as distinguishing elements of the pESI plasmids (Acar et al., 2019; Alba et al., 2020; Aviv et al., 2014; Tate et al., 2017), indicating the importance of these genetic features for successful dissemination of *S.* Infantis in different environments. In addition, these megaplasmids carried the genes responsible for mercury resistance, nickel and iron transporters cassette implying the important role of these features in horizontal gene transfer, indicating likely mobilization together from a common progenitor. Additionally, the megaplasmids complete sequences had several highly variable and mobile element regions. Furthermore, those variable areas harbored multiple resistance genes of different antimicrobial classes. Most of the AMR genes in the present study were plasmid-borne and were found to be not necessarily associated with pESI-like megaplasmids, which was, however, in contrary to the recently published international study (Alba et al., 2020). In the present study, the AMR genes, arsenic resistance cassettes and type IV secretion systems seemed to be swapped between the megaplasmid and the small plasmid probably due to the significant mobilization. It was shown that heavy metals act as co-selecting agents in the proliferation of antibiotic resistance in pathogenic bacteria in different environmental reservoirs (Nguyen et al., 2019). Furthermore, T4S's are membrane-associated complexes that contribute to the spread of

antibiotic resistance genes among different bacterial species (Redzej et al., 2017). They have also been noted as crucial for bacterial adaptation to environmental changes (Alba et al., 2020; Redzej et al., 2017; Thomas and Nielsen, 2005). In addition, the high occurrence of heavy metal (mercury and arsenic) resistance genes in bacterial cells has been commonly reported and linked to the environmental pollution (Zhang et al., 2018).

Fimbrial genes (*csg* – thin aggregative fimbria, *fim* – type 1 fimbria, and *lpf* – long polar fimbria) were found in the chromosomes of all analyzed *S*. Infantis genomes. This is in congruence with reports of multiple fimbrial operons in several non-typhoidal *S. enterica* serovars (Dhanani et al., 2015; Huehn et al., 2010; Worley et al., 2018), indicating these genes as being the core genome elements of *Salmonella* serovars. Additionally, we observed several toxin and virulence determinants located on the megaplasmids, which might be responsible for increased epidemic success of the *S*. Infantis ST32.

The current study clearly exhibited that *S*. Infantis strains from raw chicken meat samples have pESI-like megaplasmid carrying identical genetic structure that, as a consequence potentially provides fitness advantage for successful dissemination of this serovar in different geographic regions. Although it was limited to the strains of chicken meat samples, we hope that the findings of this study will extend the overall knowledge of this serovar. Nevertheless, further studies are needed to reveal *S*. Infantis epidemiology along the food production chain in Turkey and worldwide, as well as to identify factors of its increased epidemic success. The later might be prerequisite for efficient control of the pathogen.

Declaration of competing interest

We declare no conflict of interest.

Acknowledgments

The part of this study was presented at the II. international veterinary food hygiene congress. 24-27th October 2019, Antalya, Turkey. This project was funded by Hatay Mustafa Kemal University (BAP 18.M.032) and the work of D.W. and his team was funded by National Veterinary Research Institute (No. 4477/E-180/S/2018-1 and No. 4477/E-180/SPUB/2018/1). The authors would like to thank Assoc. Prof. Dr. Rafat Al Jassim for critical review of the manuscript.

Ethical approval

Not required

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108956.

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