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Whole genome sequencing analysis of multidrug-resistant *Salmonella* Infantis isolates circulating in the German food-production chain

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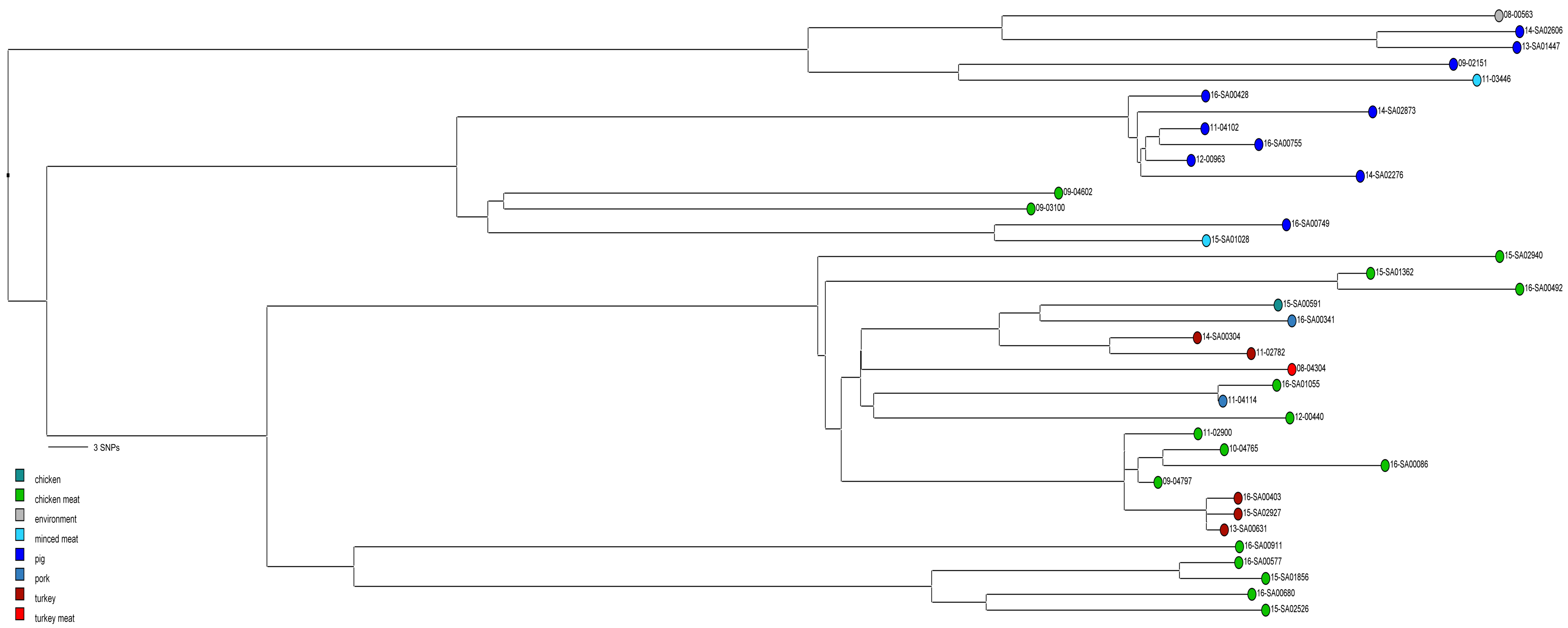
Introduction: *Salmonella* (*S.*) *enterica* is a common zoonotic pathogen causing human gastroenteritis.[1] Among them, *S. enterica* subsp. *enterica* serovar Infantis is one of the leading cause of human salmonellosis worldwide, which has its main reservoir in poultry and swine.[2] Increasing resistance against 3rd generation cephalosporins, carbapenems, (fluoro-)quinolones and/ or colistin in Enterobacteriaceae is a major public health concern.[3] Since livestock is described as a reservoir for multidrug resistant bacteria, the focus of this study was to gain an insight into the resistance mechanisms and the genetic diversity of the *S.* Infantis population from the German food-production chain. Thus we analyzed 3rd generation cephalosporin-, carbapenem-, (fluoro-)quinolone- and/ or colistin resistant *S.* Infantis isolates, from animals and food originating from the strain collection of the German National reference Laboratory for Salmonella (NRL-Salm) selected within the EngAGE-project (http://www.engage-europe.eu).

Results: ResFinder analysis revealed that 3rd generation cephalosporin resistance in 14 isolates was caused by the presence of extended-spectrum beta-lactamase (ESBL) encoding genes $bla_{CTX\text{-}M\text{-}1}$ (8), $bla_{CTX\text{-}M\text{-}15}$ (2), $bla_{CMY\text{-}2}$ (1), $bla_{ACC\text{-}1}$ (2) and $bla_{SHV\text{-}12}$ (1). The two $bla_{ACC\text{-}1}$ positive isolates (swine and minced meat) harbored an additional $bla_{VIM\text{-}1}$ carbapenemase gene. (Fluoro-)quinolone resistance in 25 isolates was caused by chromosomal mutation $gyrA_{S83Y}$ in 19 isolates, $gyrA_{D87G}$ in four isolates and by the presence of both, *aac(6')Ib-cr* and *qnrB1*-like genes in two isolates (both $bla_{CTX\text{-}M\text{-}15}$ producers). The colistin encoding resistance gene *mcr-1* was detected in one (liver, poultry) of three colistin resistant (MIC for colistin ≥ 4 mg/L) isolates. Replicons of incompatibility groups IncI1 (12) and IncH12 (4) were identified with the PlasmidFinder. The MLSTFinder analysis revealed that *S.* Infantis isolates belonged either to ST2283 (7) or ST32 (31). Isolates with similar resistance profiles (presence of $bla_{VIM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$ and mutation $gyrA_{D87G}$) grouped together in phylogenetic clusters (see figure 1).



Isolate	MLST type	Plasmids	pMLST	Resistance genes/ chromosomal mutations conferring resistance against: Beta-lactams	(Fluoro-) Quinolones	Colistin
08-00563	ST-32	IncI1,IncQ1	IncI1 [ST-26]	$bla_{TEM\text{-}1b}$	-	n.d.
14-SA02606	ST-32	IncHI2A,IncHI2,TrfA	IncHI2 [ST-1]	$bla_{CTX\text{-}M\text{-}15}$,$bla_{OXA\text{-}1\text{-}like}$, $bla_{TEM\text{-}1b}$	*aac(6')Ib-cr*, *qnrB1*-like	-
13-SA01447	ST-32	IncHI2A,IncHI2,TrfA	IncHI2 [ST-1]	$bla_{CTX\text{-}M\text{-}15}$,$bla_{OXA\text{-}1\text{-}like}$, $bla_{TEM\text{-}1b}$	*aac(6')Ib-cr*, *qnrB1*-like	-
09-02151	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
11-03446	ST-32	IncI1	IncI1 [ST-7]	$bla_{CTX\text{-}M\text{-}1}$	-	-
16-SA00428	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
14-SA02873	ST-32	IncI1,ColRNAI	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1\text{-}like}$	-	-
11-04102	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
16-SA00755	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
12-00963	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
14-SA02276	ST-32	IncI1	IncI1 [ST-3]	$bla_{CTX\text{-}M\text{-}1}$	-	-
09-04602	ST-32	IncI1	IncI1 [ST-12]	$bla_{CMY\text{-}2}$	-	-
09-03100	ST-32	IncI1	IncI1 [ST-95]	$bla_{SHV\text{-}12}$	-	-
16-SA00749	ST-32	IncHI2A,IncHI2,TrfA	IncHI2 [ST-1]	$bla_{ACC\text{-}1}$,$bla_{VIM\text{-}1}$	-	-
15-SA01028	ST-32	IncHI2A,IncHI2,TrfA	IncHI2 [ST-1]	$bla_{ACC\text{-}1}$,$bla_{VIM\text{-}1}$	-	-
15-SA02940	ST-32	n.d.		-	gyrA S83Y	-
15-SA01362	ST-32	n.d.		-	gyrA S83Y	-
16-SA00492	ST-32	IncX1,IncX4		$bla_{TEM\text{-}1b}$	gyrA S83Y	-
15-SA00591	ST-32	IncX4		-	gyrA S83Y	*mcr-1*
16-SA00341	ST-32	n.d.		-	gyrA S83Y	-
14-SA00304	ST-32	n.d.		-	gyrA S83Y	-
11-02782	ST-32	n.d.		-	gyrA S83Y	-
08-04304	ST-32	n.d.		-	gyrA S83Y	-
16-SA01055	ST-32	Col156		-	gyrA S83Y	-
11-04114	ST-32	n.d.		-	gyrA S83Y	-
12-00440	ST-32	n.d.		-	gyrA S83Y	-
11-02900	ST-2283	n.d.		-	gyrA S83Y	n.d.
10-04765	ST-2283	n.d.		-	gyrA S83Y	-
16-SA00086	ST-2283	n.d.		-	gyrA S83Y	-
09-04797	ST-2283	n.d.		-	gyrA S83Y	-
16-SA00403	ST-2283	IncI1	IncI1 [Unknown ST]	$bla_{TEM\text{-}1b}$	gyrA S83Y	-
15-SA02927	ST-2283	n.d.		-	gyrA S83Y	-
13-SA00631	ST-2283	n.d.		-	gyrA S83Y	-
16-SA00911	ST-32	n.d.		-	gyrA S83Y	-
16-SA00577	ST-32	n.d.		-	gyrA D87G	-
15-SA01856	ST-32	n.d.		-	gyrA D87G	-
16-SA00680	ST-32	IncX4		$bla_{TEM\text{-}1b}$	gyrA D87G	-
15-SA02526	ST-32	n.d.		-	gyrA D87G	-

Figure 1: Neighbor Joining phylogenetic tree and CGE results of 38 multidrug resistant *S.* Infantis isolates collected in 2009-2016 at the NRL-Salmonella in Germany from animals and thereof derived food. **n.d.: not detected; -: no phenotypic resistance**

Material and methods:

38 multidrug resistant *S.* Infantis isolates from animals or animal-derived food were analyzed by WGS. DNA was extracted using the PureLink® Genomic DNA Mini Kit (Invitrogen). Sequencing libraries were prepared using Nextera XT DNA Sample Preparation Kit. Paired-end sequencing was performed on the Illumina MiSeq benchtop (MiSeq Reagent v3 600-cycle Kit, 2 × 300 cycles - Illumina). *De novo* assembly of raw reads based on Velvet algorithms (CLC workbench), allowed MLST-type, plasmid content and resistance genes analysis using the tools available on CGE (http://www.genomicepidemiology.org). Mapping of raw reads against reference strain 15-SA1028 and subsequent phylogenetic analysis were carried out with the BioNumerics software – Neighbor Joining algorithm).

Acknowledgements: This work was supported by the German Federal Institute for Risk Assessment, BfR (1322-639) and the European Food Safety Authority (EFSA) co-funded project ENGAGE (GP/EFSA/AFSCO/2015/01/CT1).

Conclusions:

Analysis of WGS data from *S.* Infantis from the German food-production chain showed that this serovar is mainly associated with ESBL genes and plasmid families commonly found in German livestock populations. However, the detection of the $bla_{VIM\text{-}1}$ carbapenemase gene in swine and minced meat[4] and the *mcr-1* gene in poultry derived food emphasizes the threat associated with zoonotic diseases and underlines that this serovar gains special importance for public health.

References: 1) EFSA and ECDC The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA J* 2015; 13: 4329: 2) Hauser, E., *et al.* 2012. Foodborne Pathog Dis 9(4): 352-360 3) EFSA (2016). "The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014." EFSA J. 4) Borowiak, M., *et al* M., 2017. J Antimicrob Chemother DOI:https://doi.org/10.1093/jac/dkx101