First experience of using NGS for *Salmonella* characterization in Latvia during FWD AMR-ReflabCap project

Riga East University Hospital Laboratory Service
Head of Laboratory “Latvian Centre of Infectious Diseases”,
National Microbiology Reference Laboratory of Latvia

Oksana Savicka

FWD AMR – RefLabCap Network meeting
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Salmonella spp. detection

Bacteriology:

1) *Salmonella, Shigella* agar (SSag) and Muller Kauffmann Tetrathionate Novobiocin Broth (MKTTn)
2) Bismuth SulfitAgar (BSA)
3) agglutination: O (somatic) antigen and H (flagellar) antigen by SSI diagnostic reagents
4) AST: Liofilchem reagents

PCR:

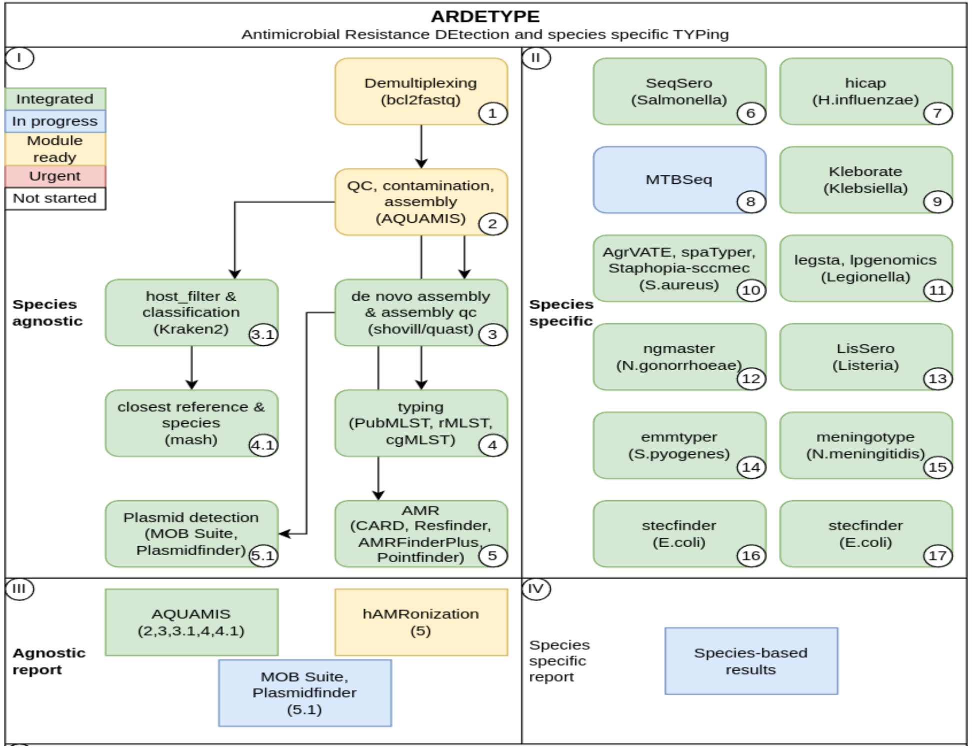
1) **Allplex GI-Bacteria(I), Seegene**: Shigella spp./Enteroinvazīvā Escherichia coli (EIEC), Campylobacter spp., Yersinia enterocolitica, Vibrio spp., Clostridium difficile toxin B, Aeromonas spp. un Salmonella spp.

2) **QIAstat-Dx Gastrointestinal Panel:** Entamoeba histolytica, Cryptosporidium spp., Giardia lamblia, Cyclospora cayetanensis, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Campylobacter spp. (Campylobacter jejuni, Campylobacter upsaliensis, Campylobacter coli), Salmonella spp., Clostridium difficile (tcdA/tcdB), Yersinia enterocolitica, Enterotoxigenic E. coli (ETEC), Enteropathogenic E. coli (EPEC), Enteroaggregative E. coli (EAEC), Shiga-like toxin-producing E. coli (STEC [enterohemorrhagic E. coli]), Shiga toxin-producing E. coli (STEC) serotype O157:H7, Enteroinvasive E. coli (EIEC)/Shigella, Plesiomonas shigelloides, Human Adenovirus F40/F41, Norovirus GI, Norovirus GII, Rotavirus A, Astrovirus and Sapovirus GI, GII, GIV and GV.

NGS:

DNA extraction Qiagen DNeasyR Blood & Tissue kit; Illumina DNAPrep Library Kit; **NGS instruments:** iSeq100, MiSeqDx, **NextSeq550DX, NovaSeq**

In-house bioinformatic pipeline



ARDETYPE
Antimicrobial Resistance DEtection and species specific TYPing

I

- Integrated
- In progress
- Module ready
- Urgent
- Not started

Species agnostic

Demultiplexing (bcl2fastq) ①

QC, contamination, assembly (AQUAMIS) ②

host_filter & classification (Kraken2) (3.1)

de novo assembly & assembly qc (shovill/quast) ③

closest reference & species (mash) (4.1)

typing (PubMLST, rMLST, cgMLST) ④

Plasmid detection (MOB Suite, Plasmidfinder) (5.1)

AMR (CARD, Resfinder, AMRFinderPlus, Pointfinder) ⑤

II

Species specific

SeqSero (Salmonella) ⑥

hicap (H.influenzae) ⑦

MTBSeq ⑧

Kleborate (Klebsiella) ⑨

AgrVATE, spaTyper, Staphopia-sccmec (S.aureus) ⑩

legsta, lpgenomics (Legionella) ⑪

ngmaster (N.gonorrhoeae) ⑫

LisSero (Listeria) ⑬

emmtyper (S.pyogenes) ⑭

meningotype (N.meningitidis) ⑮

stecfinder (E.coli) ⑯

stecfinder (E.coli) ⑰

III

Agnostic report

AQUAMIS (2,3,3.1,4,4.1)

hAMRonization (5)

MOB Suite, Plasmidfinder (5.1)

IV

Species specific report

Species-based results

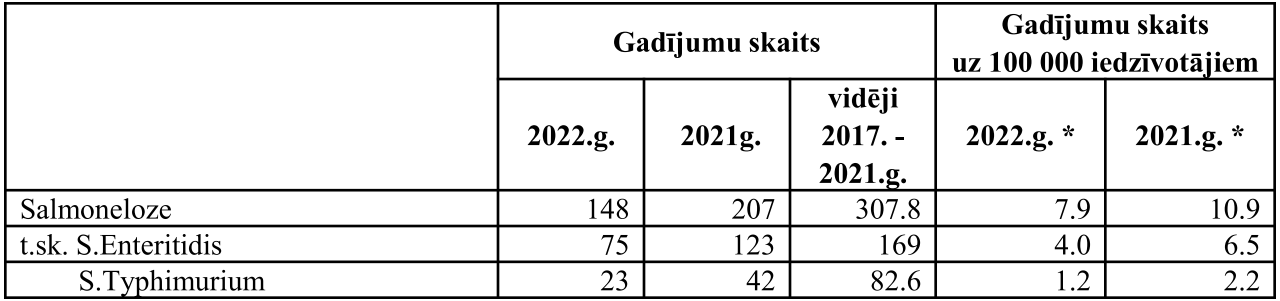
https://github.com/NMRL/Ardetype/tree/ardetype

Notified *Salmonella* cases

Centre for Disease Prevention and Control of Latvia

Epidemiological bulletin Nr.54 (1815), 01.01.2022 – 30.11.2022:

148 *Salmonella* cases

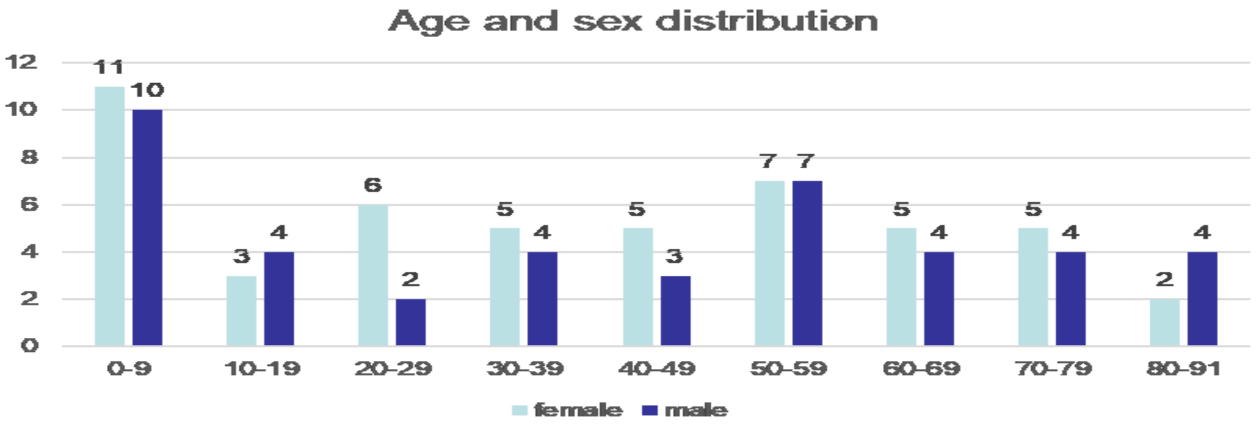


	Gadījumu skaits			**Gadījumu skaits uz 100 000 iedzīvotājiem**	
	2022.g.	**2021g.**	**vidēji 2017. - 2021.g.**	**2022.g. ***	**2021.g. ***
Salmoneloze	148	207	307.8	7.9	10.9
t.sk. S.Enteritidis	75	123	169	4.0	6.5
S.Typhimurium	23	42	82.6	1.2	2.2

Data from 01.01.2022 till 28.02.2022

Demographic data

N=91 patients, N=42 (males), N= 49 (females)

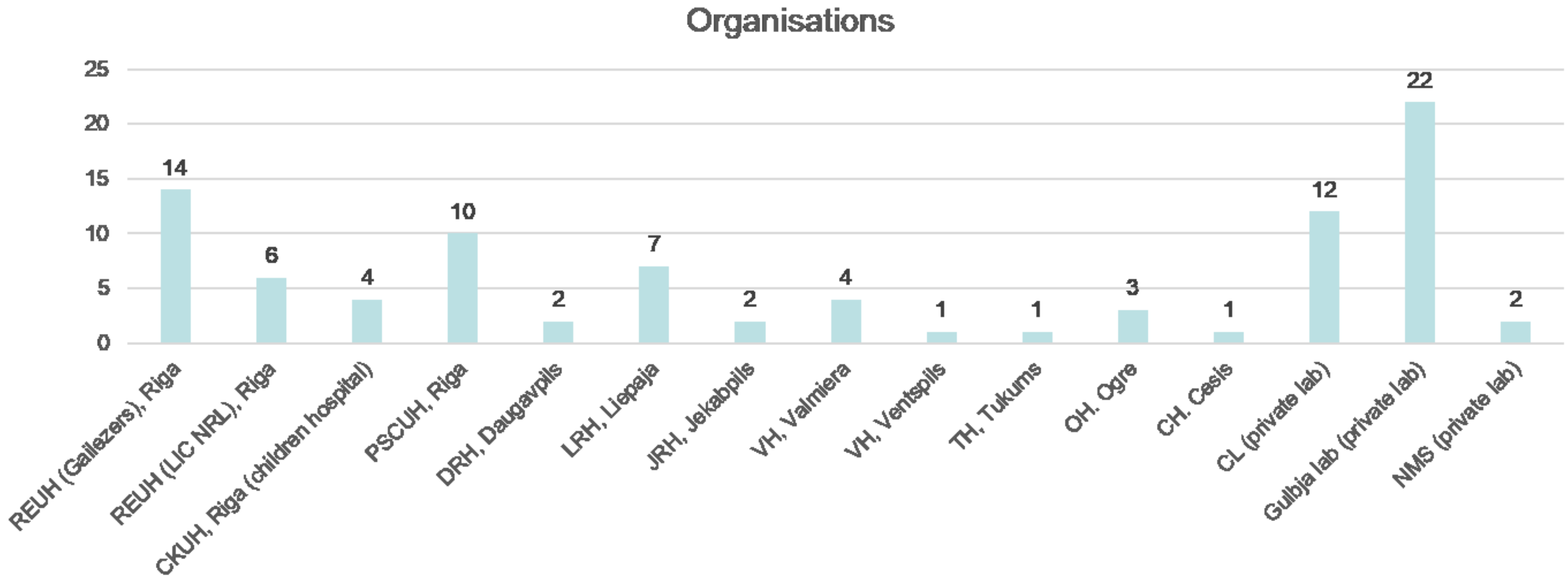


The mean age of patients at diagnosis was 38.9 years . The median age was 39 years.

The youngest patient was 0 years old, male (month-?), while the oldest patient was 91 years old, male.

28 (31%) of this group of patients were children and 63 (69%) were adults.

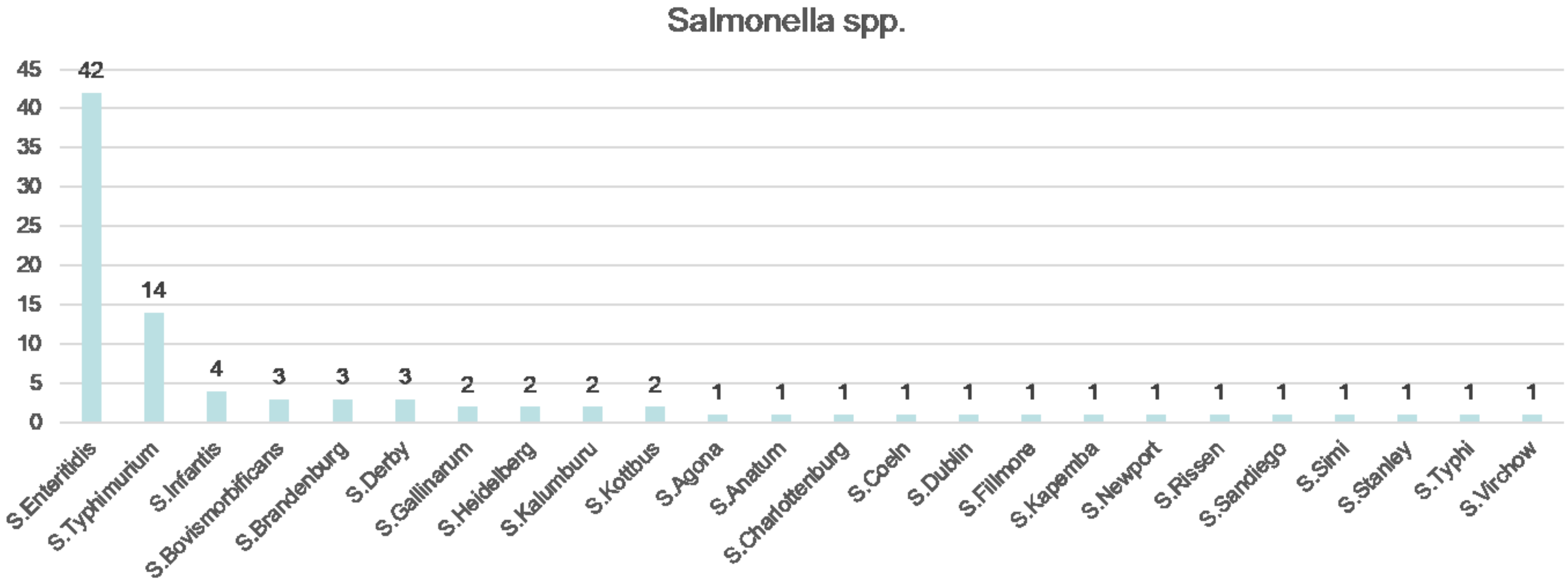
Organisations, N=15



Materials (n=92; two samples from one patient – blood and feaces)

Feaces – 82
Urine – 5
Blood – 3
Ascites punctate – 1
Pus- 1

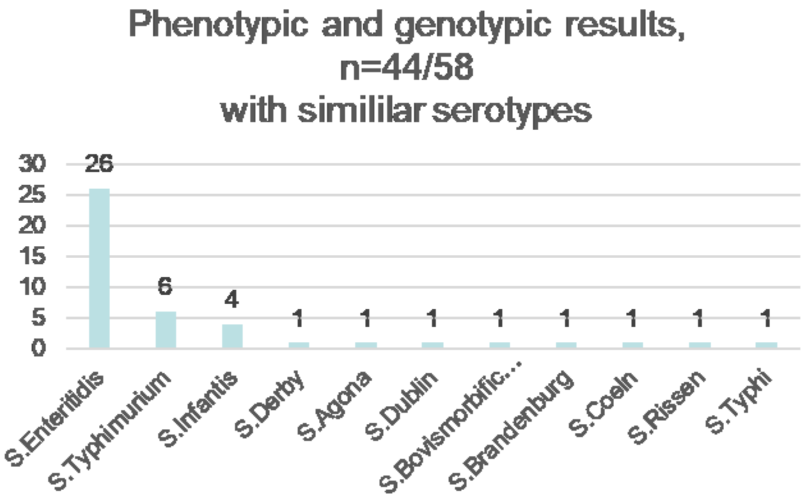
Phenotypic serotyping
Salmonella spp, N=24 serotypes



Phenotypic and genotypic serotyping, results comparision

58/91 – NGS results,

21/91 – NGS is done, not analzyed, 12/91 –NGS not done


Phenotypic and genotypic results, n=44/58 with simililar serotypes

Phenotypic and genotypic results, n=14/58 with different serotypes

phenotypic	genotypic
S.Typhimurium	S.Brandenburg
S.Typhimurium	S.Heidelberg
S.Typhimurium	S.Enteritidis
S.Derby	S.Agona
S.Derby	S.Agona
S.Brandenburg	S.Bredeney
S.Brandenburg	S.Bredeney
S.Kalumburu	S.Kottbus
S.Kalumburu	S.Bovismorbificans
S.Charlottenburg	S.Blockley
S.Gallinarum	S.Enteritidis
S.Anatum	S.Weltevreden
S.Kapemba	S.Goettingen
S.Virchow	S.Infantis

Retest by phenotipic method – S.Enteritidis

ST

Serotype	ST
S.Enteritidis	**ST 11**, n=26/58; **ST 3233**, n=1/58; **ST not detected**, n=1/58
S.Typhimurium	**ST 19**, n=2/58; **ST 34**, n=3/58; **ST** 3137, n=1/58
S.Infantis	**ST 32**, n=5/58
S.Agona	**ST 13**, n=3/58
S.Brandenburg	**ST 65**, n=2/58
S.Bovismorbificans	**ST 142**, n=2/58
S.Bredeney	**ST 897**, n=2/58
S.Typhi	**ST 2**, n=1/58
S.Dublin	**ST 10**, n=1/58
S.Heidelberg	**ST 15**, n=1/58
S.Goettingen	**ST 20**, n=1/58
S.Blockley	**ST 52**, n=1/58
S.Kottbus	**ST 212**, n=1/58
S.Weltevreden	**ST 365**, n=1/58
S.Rissen	**ST 469**, n=1/58
S.Derby	**ST 682**, n=1/58
S.Coeln	**ST 1995**, n=1/58

Phenotypic AST, N=90
N= 52 (57.8%) all with result Susceptible
N=38 (42.2%) with Resistant or Susceptible, increased exposure

Serotype	AMP ampicillin	CTX cefotaxime	CAZ ceftazidime	C chloramphenicol	GN gentamicin	MEM meropenem	PEF pefloxacin	SXT sulfamethoxazole trimetoprim
S.Brandenburg	R	S	S	S	S	S	S	S
S.Brandenburg	R	S	S	R	R	S	S	R
S.Enteritidis	R	R	R	S	R	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	R
S.Enteritidis	S	S	I	S	S	S	R	S
S.Enteritidis	S	S	I	S	S	S	S	S
S.Enteritidis	S	S	I	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	R	S	S	S	S	S	R	S
S.Enteritidis	R	S	S	S	S	S	S	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Enteritidis	S	S	S	S	S	S	R	S
S.Heidelberg	R	S	S	R	S	S	S	R
S.Infantis	S	S	I	S	S	S	S	S
S.Infantis	S	S	S	S	S	S	S	R
S.Infantis	S	S	S	S	S	S	R	S
S.Kalumburu	S	S	I	S	S	S	S	S
S.Kalumburu	S	S	I	S	S	S	S	S
S.Kapemba	R	S	I	R	S	S	S	S
S.Rissen	R	R	R	R	R	S	R	R
S.Typhi	S	S	S	S	S	S	R	S
S.Typhimurium	R	S	S	S	S	S	S	R
S.Typhimurium	R	S	S	R	S	S	R	S
S.Typhimurium	R	S	S	S	s	S	S	S
S.Typhimurium	R	S	S	S	S	S	S	S
S.Typhimurium	R	S	S	S	S	S	S	S
S.Typhimurium	R	S	S	R	S	S	R	R
S.Typhimurium	R	S	S	S	S	S	S	S
Total R:	n=15	n=2	n=2; n=7	n=6	n=3	n=0	n=23	n=7

Phenotypic and genotypic data comparison
N=33/58 (Susceptible, 100% by two methods)
N=25/58 (Resistance and partial coincidence by two methods – marked in red color)

Phenotypic	Genotypic	ampicillin		cefotaxime		ceftazidime		chloramphenicol		gentamicin		meropenem		pefloxacin		sulfamethoxazole trimetoprim		
		phen	gen	phen	gen	phen	gen	phen	gen	phen	gen	phen	gen	phen	gen	phen	gen	gen
S.Typhimurium	S.Typhimurium	R	blaTEM-1B (blaTEM-1B_AY458016)	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	R	N/A	N/A
S.Kalumburu	S.Kottbus	S	N/A	S	N/A	I	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Kalumburu	S.Bovismorbificans	S	N/A	S	N/A	I	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Infantis	S.Infantis	S	N/A	S	N/A	I	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	I	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	I	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	R	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Brandenburg	S.Brandenburg	R	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	R	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Kapemba	S.Goettingen	R	blaTEM-1B (blaTEM-1B_AY458016)	S	N/A	I	N/A	R	floR (floR_AF118107)	S	N/A	S	N/A	S	N/A	S	sul2 (sul2_AY034138)	dfrA1 (dfrA1_AF203818), dfrA1 (dfrA1_AJ238350), dfrA1 (dfrA1_X00926)
S.Rissen	S.Rissen	R	blaCTX-M-55 (blaCTX-M-55_DQ810789)	R	blaCTX-M-55 (blaCTX-M-55_DQ810789)	R	blaCTX-M-55 (blaCTX-M-55_DQ810789)	R	floR (floR_AF118107), catA2 (catA2_X53796)	R	aac(3)-IId (aac(3)-IId_EU022314)	S	N/A	R	N/A	R	sul2 (sul2_AY034138)	dfrA14 (dfrA14_KF921535)
S.Typhimurium	S.Brandenburg	R	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	N/A
S.Typhi	S.Typhi	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Infantis	S.Infantis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	sul2 (sul2_AY034138)	dfrA1 (dfrA1_X00926)
S.Typhimurium	S.Typhimurium	R	blaTEM-1B (blaTEM-1B_AY458016)	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	sul2 (sul2_HQ840942)	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Brandenburg	S.Bredeney	R	blaTEM-1B (blaTEM-1B_AY458016)	S	N/A	S	N/A	R	catA1 (catA1_V00622)	R	aac(3)-IId (aac(3)-IId_EU022314)	S	N/A	S	N/A	R	sul1 (sul1_U12338), sul2 (sul2_HQ840942)	dfrA12 (dfrA12_AM040708)
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Infantis	S.Infantis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	sul1 (sul1_U12338)	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Enteritidis	S.Enteritidis	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	S	N/A	R	N/A	S	N/A	N/A
S.Typhimurium	S.Heidelberg	R	N/A	S	N/A	S	N/A	R	floR (floR_AF118107)	S	N/A	S	N/A	R	N/A	R	N/A	dfrA14 (dfrA14_KF921535)

PCR *Salmonella* results

- 2 labs (LIC NRL, Gailezers)
- N total positive results = 24 (11 - LIC, 13 - Gailezers)
- *Salmonella* growth:

  LIC: 11/11,

  Gailzezers:10/13 (3 samples with result *Salmonella* not extracted, 1. Ct=32.7; 2. Ct=31.9; 3. Ct=25.4)

LIC: Allplex GI-Bacteria(I), Seegene: Shigella spp./Enteroinvazīvā Escherichia coli (EIEC), Campylobacter spp., Yersinia enterocolitica, Vibrio spp., Clostridium difficile toxin B, Aeromonas spp. un Salmonella spp.

Gailezers (**only for emergence clinic and patient admit)**: QIAstat-Dx Gastrointestinal Panel: Entamoeba histolytica, Cryptosporidium spp., Giardia lamblia, Cyclospora cayetanensis, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Campylobacter spp. (Campylobacter jejuni, Campylobacter upsaliensis, Campylobacter coli), Salmonella spp., Clostridium difficile (tcdA/tcdB), Yersinia enterocolitica, Enterotoxigenic E. coli (ETEC), Enteropathogenic E. coli (EPEC), Enteroaggregative E. coli (EAEC), Shiga-like toxin-producing E. coli (STEC [enterohemorrhagic E. coli]), Shiga toxin-producing E. coli (STEC) serotype O157:H7, Enteroinvasive E. coli (EIEC)/Shigella, Plesiomonas shigelloides, Human Adenovirus F40/F41, Norovirus GI, Norovirus GII, Rotavirus A, Astrovirus and Sapovirus GI, GII, GIV and GV.

Epidemiological information

96 patients

Country of residence n=95 Latvia, n=1 UK.

Symptoms: n=1 no symptoms; n=17 unknown; n=5 light form; n=69 middle severe form; n=1 severe; n=1 COVID-19; n=1 mix infection Sal.+gastroenteriditis; n=1 pulmonary congestion

Hospitalization: n=29 from outpatient departments; n=69 were hospitalized

Epidemiological information

Suspected country of origin of *Salmonella*

n=88 Latvia

n=1 UK (S.rissen)

n=1 Egypt (S.typhimurium)

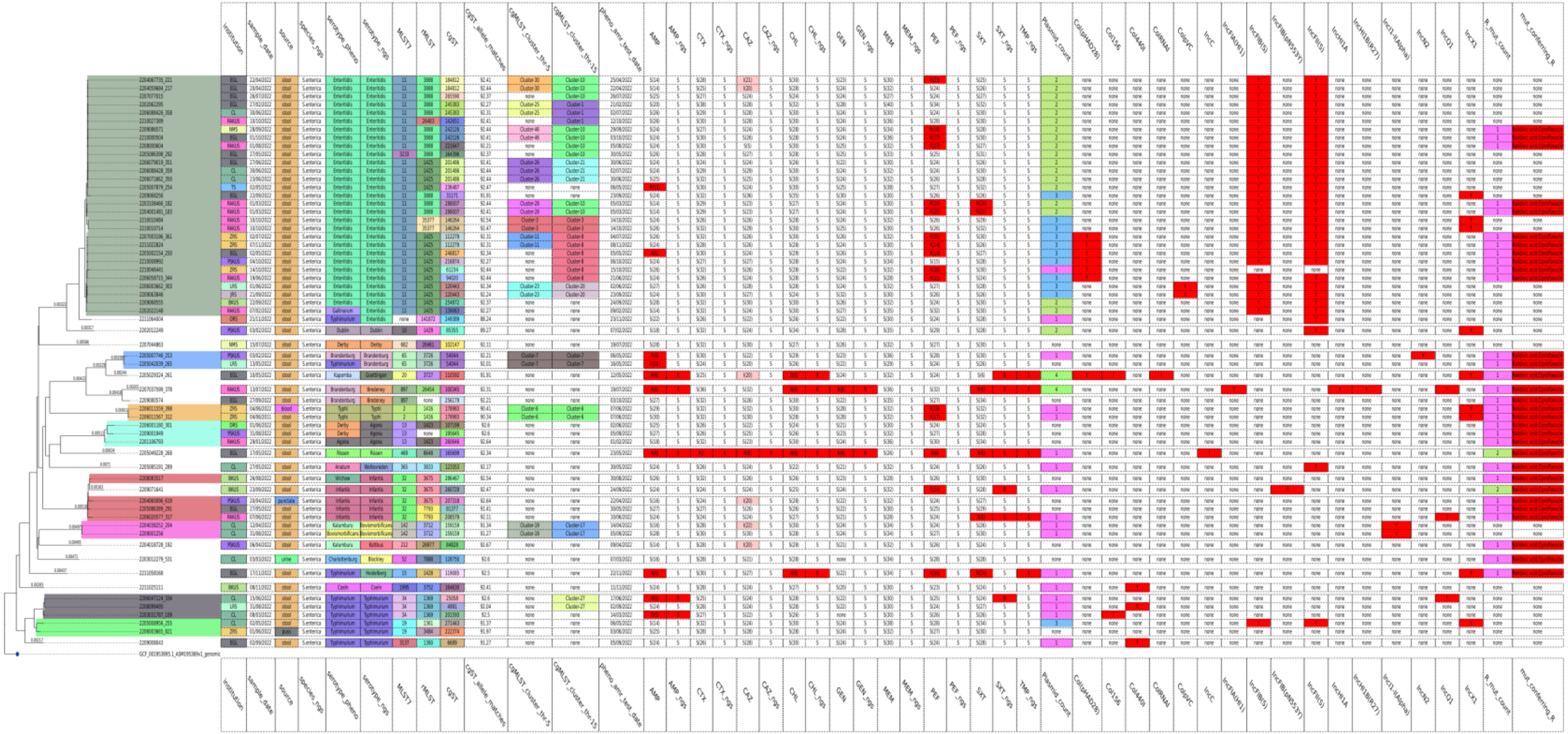
n=1 Mexico (S.typhi)

n=1 Spain (S.enteriditis)

n=1 Thailand (S.stanley)

n=3 Turkey (S.entereditis)

***Possible source of infection** – mostly chicken and eggs*



Method: QC on raw reads and contigs, denovo assembly, closest reference selection and 7-gene MLST determination conducted using AQUAMIS[1] v1.3.8 with default settings.
Tree constructed from contig-based distance matrix with Neighbor-joining method using Mashtree[2] v1.2.0 with default settings and visualized with Python-ete3[3] v3.1.2.
Branch length given in units of mash distance, approximating evolutionary distance as mutation rate between assembled contigs for each pair of isolates[4].
Clusters of isolates highlighted where all pairwise distances within cluster are less than 0.0015 and isolate similarity supported by MLST and AMR profile.
Reference included in cluster based on pairwise-distance criteria alone. Branch lengths greater than 0.0015 shown.
Accession number of closest reference genome displayed as outgroup. Resistance gene identification performed with ResFinder[5] v4.1.11 and AMRFinder+[6] v3.10.42 with default settings.
Ribosomal multi-locus sequence type[7] (rMLST) determined using PubMLST API[8,9]. Allelic distance from the reference sequence calculated using pyMLST [10].

References:
1. Deneke C, Brendebach H, Uelze L, Borowiak M, Malorny B, Tausch SH. Species-Specific Quality Control, Assembly and Contamination Detection in Microbial Isolate Sequences with AQUAMIS. Genes (Basel). 2021 Apr 26;12(5):644. doi: 10.3390/genes12050644. PMID: 33926025; PMCID: PMC8145556.
2. Katz LS, Griswold T, Morrison SS, Caravas JA, Zhang S, den Bakker HC, Deng X, Carleton HA. Mashtree: a rapid comparison of whole genome sequence files. J Open Source Softw. 2019 Dec 10;4(44):10.21105/joss.01762. doi: 10.21105/joss.01762. PMID: 35978566; PMCID: PMC9380445.
3. Huerta-Cepas J, Serra F, Bork P. ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. Mol Biol Evol. 2016 Jun;33(6):1635-8.
doi: 10.1093/molbev/msw046. Epub 2016 Feb 26. PMID: 26921390; PMCID: PMC4868116.
4. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, Phillippy AM. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol. 2016 Jun 20;17(1):132. doi: 10.1186/s13059-016-0997-x. PMID: 27323842; PMCID: PMC4915045.
5. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA,
Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020 Dec 1;75(12):3491-3500. doi: 10.1093/jac/dkaa345.
PMID: 32780112; PMCID: PMC7662176.
6. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W.
AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep. 2021 Jun 16;11(1):12728. doi: 10.1038/s41598-021-91456-0. PMID: 34135355; PMCID: PMC8208984.
7. Jolley KA, Bliss CM, Bennett JS, et al. Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. Microbiology (Reading). 2012;158(Pt 4):1005-1015. doi:10.1099/mic.0.055459-0
8. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018;3:124. Published 2018 Sep 24. doi:10.12688/wellcomeopenres.14826.1
9. RMLST species identification via restful API. PubMLST. https://pubmlst.org/species-id/species-identification-via-api. Accessed January 11, 2023.
10. https://github.com/bvalot/pyMLST

Next steps

- Collabortaion with BIOR (Institute for food safety, animal health and environment)
- To establish reporting of genotypic results

Thank you for your attention!