



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

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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

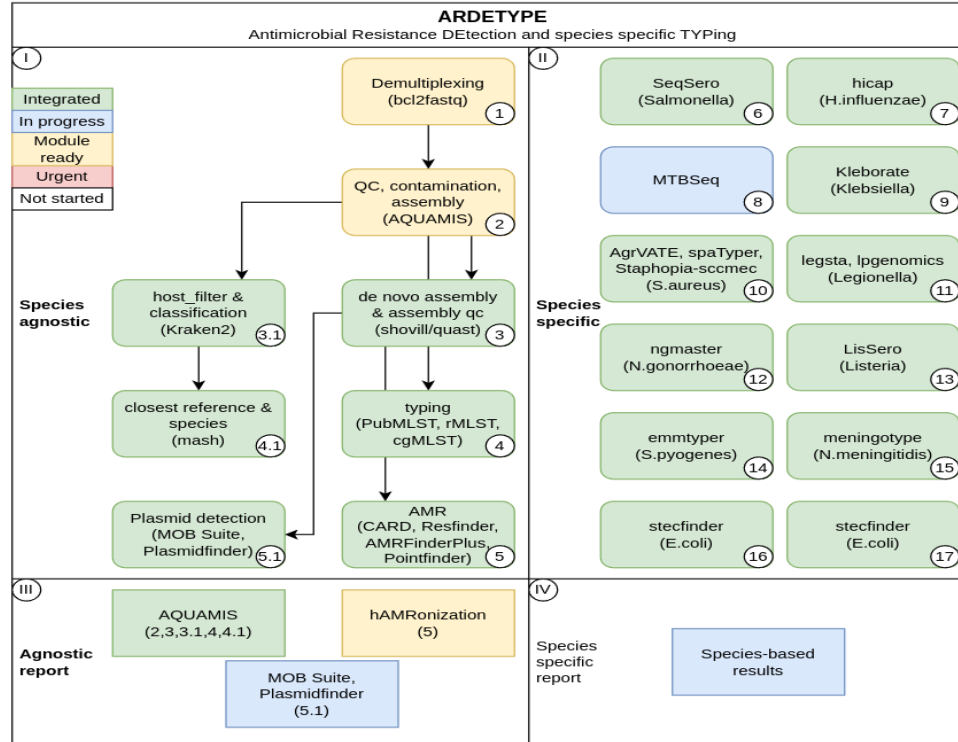
### **PCR:**

- 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



## 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

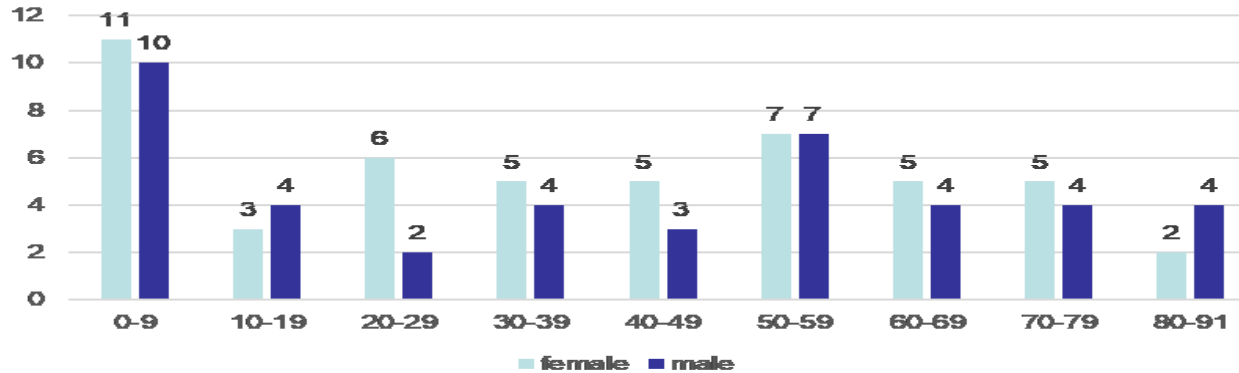
	Gadījumu skaits			Gadījumu skaits uz 100 000 iedzīvotājiem	
	2022.g.	2021g.	vidēji 2017. - 2021.g.	2022.g. *	2021.g. *
Salmoneloze	148	207	307.8	7.9	10.9
t.sk. S.Enteritidis	75	123	169	4.0	6.5
S.Typhimurium	23	42	82.6	1.2	2.2

Data from 01.01.2022 till 28.02.2022

## Demographic data

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

Age and sex distribution



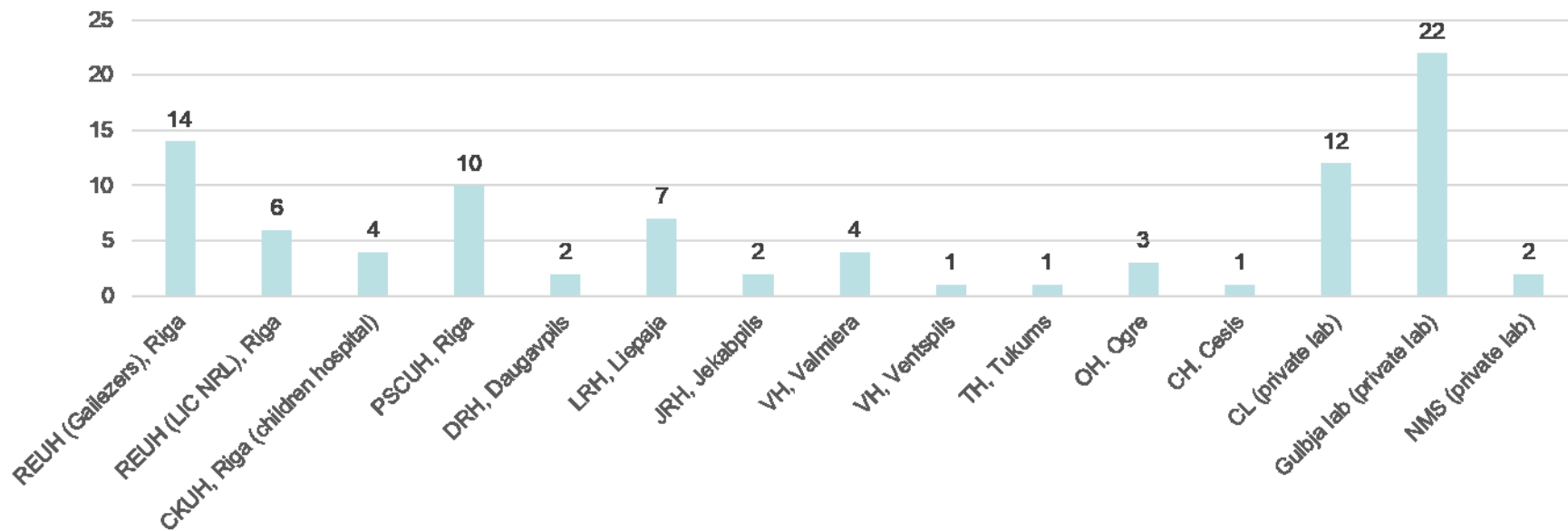
The mean age of patients at diagnosis was 38.9 years . The median age was 39 years.

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

Organisations



EUROPE > NORTH EUROPE > LATVIJA



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

Feaces – 82

Urine – 5

Blood – 3

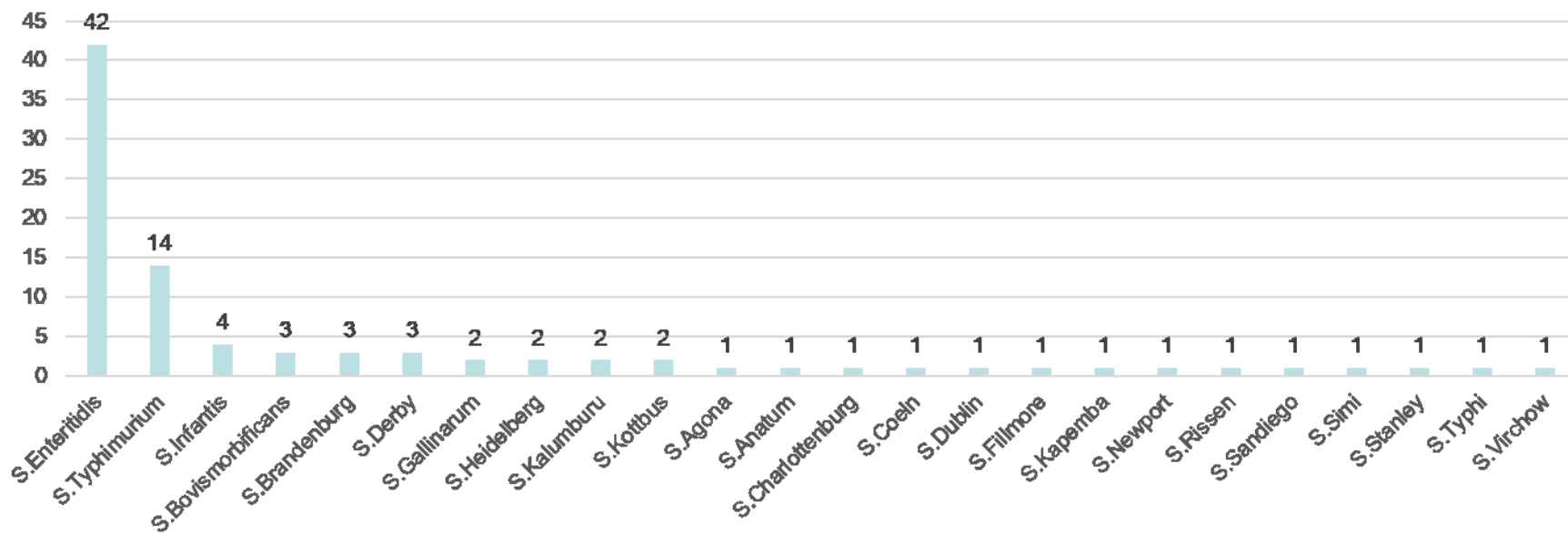
Ascites punctate – 1

Pus- 1



## Phenotypic serotyping Salmonella spp, N=24 serotypes

Salmonella spp.

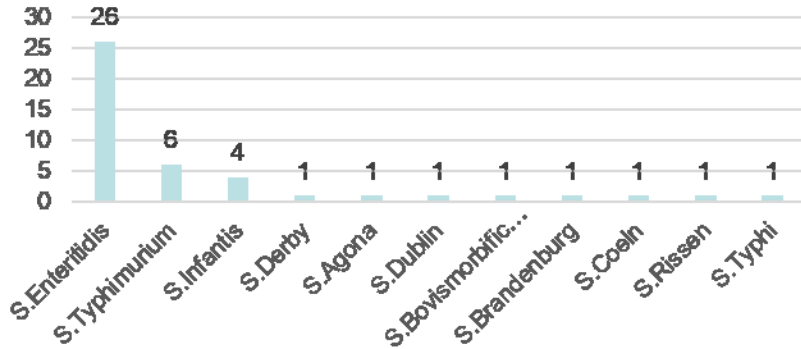


# Phenotypic and genotypic serotyping, results comparison

## 58/91 – NGS results,

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

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



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

phenotypic	genotypic
S. Typhimurium	S. Brandenburg
S. Typhimurium	S. Heidelberg
<b>S. Typhimurium</b>	<b>S. Enteritidis</b>
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 phenotypic 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	S	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	S	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	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	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	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	S	N/A	R	flrR (flrR_AF118107)	S	N/A	S	N/A	S	N/A	R	suI2 (suI2_AY034138)	dfrA1 (dfrA1_AF203818), dfrA1 (dfrA1_AJ238350), ctrA1 (ctrA1_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	flrR (flrR_AF118107), catA2 (catA2_X53796)	R	aac(3)-IId (aac(3)-IId_EU022314)	S	N/A	R	N/A	R	suI2 (suI2_AY034138)	dfrA14 (dfrA14_NF921535)
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	suI2 (suI2_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	suI2 (suI2_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.Bredelney	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	suI1 (suI1_U12338), suI2 (suI2_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	suI1 (suI1_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	flrR (flrR_AF118107)	S	N/A	S	N/A	R	N/A	R	N/A	dfrA14 (dfrA14_NF921535)

## PCR *Salmonella* results

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

*n=1 Thailand (S.stanley)*

*n=3 Turkey (S.enteritidis)*

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





# Next steps

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

**Thank you for your attention!**

