LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture
et du Développement rural

Administration luxembourgeoise vétérinaire
et alimentaire

Salmonella in foodstuff and veterinary diagnostics

Dr. Alexandra SCHOOS
Veterinary inspector
Technical manager

Luxembourg Veterinary and Food Administration (ALVA)
State Laboratory of Veterinary Medicine

EURL Salmonella Workshop
22.-23.05.2023

Our missions

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- **Animal health**: Detection of notifiable diseases, zoonoses and pathogens

- **Public health:** Bacteriological quality of food of animal origin (Safety criteria and hygiene criteria)

- "**One Health**" approach: surveillance of emerging diseases

ESSAI
OLAS
OFFICE LUXEMBOURGEOIS D'ACCREDITATION ET DE SURVEILLANCE
NUMERO D'ACCREDITATION: 1/054
ISO/IEC 17025

Our lab in numbers

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4	12	1	1	2	1	1	2
Veterinarians	Technicians	Quality manager	Secretary accountant	Receptionist	IT	Metrologist	Technical and logistics assistant

Year	Requests	Samples	Analyses	*Salmonella*
2020	10.184	222.374	245.227	2.769
2021	11.388	258.198	289.706	2.950
2022	10.083	270.282	305.572	2.690

Year	Requests	Samples	Analyses	*Salmonella*
2020	240	1.167	5.923	655
2021	179	870	5.272	544
2022	251	1214	7.534	841

Samples and methods

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- Serology (Swine Salmonella Ab Test IDEXX) in **meat juice** samples of slaughter pigs
- Culture (ISO 6579-1*, MSRV, XLD, RSal) and identification (Kauffmann-White*) on **organs, feces and boot socks** of different species

- Culture (BRD 07/11-12/05*, RSal, ISO 6579-1* on request) and identification (Kauffmann-White*) in all **foodstuff of animal origin** from retail, processing plants, dairy and border control point

- EU 517/2011 : laying hens
- EU 200/2012 : broilers
- EU 1729/2020 : AMR-monitoring

*accredited

In 2021…

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Species	Sample type	Detected	Not detected	
Porcine	Meat juice	404	1.644	
	Organs	0	11	S. Goldcast, S. Rissen, S. Typhimurium, monophasic S. Typhimurium, S. Brandenburg, S. Bredeney, S. Derby, S. Enteritidis, S. Infantis, S. Kottbus
	Feces/boot cover	44	179	
Bovine	Organs	1	52	S. Typhimurium, S. Havana, S. Enteritidis
	Feces	8	106	
Poultry	Boot cover/feces/dust	8	435	S. Typhimurium, S. Diarizonae, S. Salamae, S. Paratyphi B
	Organs	0	24	
Goat/sheep	Organs	1	21	
	Feces	0	3	S. Dublin

Veterinary diagnostics: antisusceptibility testing

Species	Sample type	Detected	Not detected	
Animal origin	foodstuff	4	540	S. Typhimurium, monophasic *S.* Typhimurium, S. Brandenburg

In 2022…

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Species	Sample type	Detected	Not detected	
Porcine	Meat juice	444	1524	
	Organs	0	1	
	Feces/boot cover	9	15	*S.* Goldcast, *S.* Rissen, *S.* Typhimurium, monophasic *S.* Typhimurium
Bovine	Organs	3	54	*S.* Typhimurium, *S.* Muenster, *S.* Indiana, *S.* Dublin
	Feces	8	163	
Poultry	Boot cover/feces/dust	6	400	*S.* Enteritidis, *S.* Dublin, *S.* Brandenburg
	Organs	0	30	
Goat/sheep	Organs	0	8	
	Feces	0	2	
Wild animals	Organs	0	6	

Veterinary diagnostics: antisusceptibility testing

Species	Sample type	Detected	Not detected	
Animal origin	foodstuff	1	840	monophasic *S.* Typhimurium

Data processing and reporting

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- Whole genome sequencing (Illumina®) + collection in a database (SeqSphere®) and comparison with human cases

LNS LUXEMBOURG

- efsa EUROPEAN FOOD SAFETY AUTHORITY reporting: prevalence + AMR

- National antibiotic action plan

National reference laboratory

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- *Salmonella*
- *Listeria monocytogenes*
- VTEC
- CPS – Coagulase positive *Staphylococcus*
- *Campylobacter*
- AMR

LNS LUXEMBOURG

2 labs = 1 team

Thank you for your attention!

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DU GRAND-DUCHÉ DE LUXEMBOURG

Catarina Martins
Technical Lab Manager for Food Microbiology and Molecular Biology

Laboratoire National de Santé
Food Monitoring Service / Surveillance Alimentaire
Food Microbiology Lab

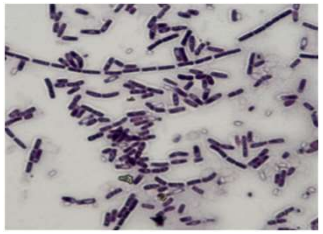
Service of *Surveillance Alimentaire*

1 – the biggest official control laboratory for food and feed in Luxembourg
2 – Accreditation according to ISO 17025 since 2003
3 – National Reference Laboratory in 14 domains

Expertise:

- Microbiological analysis (*Salmonella*, *L. monocytogenes*, STEC, *S. aureus*, *Foodborne Virus*)
- GMO: detection and quantification of genetically modified organisms
- allergens using ELISA and **PCR technology**
- pesticides in fruits & vegetables, cereals and feed
- mycotoxins and plant toxins in food and feed
- additives (food dyes, sweeteners, preservatives)
- process contaminants: furan, acrylamide, MCPD, PAHs
- food contact materials (FCM)




Service of *Surveillance Alimentaire – Food microbiology*

- +/- 2500 analyses for *Salmonella* every year
- Diversity of samples : all kind of samples except for a part of animal origin products
- 1-2 positif samples per year
- Samples are brought to us by our competent authority (ALVA)

Monitoring	• Supermarkets, baker's shops • Restaurants • Feed production sites • etc
Suspicious samples	• Recent food outbreak or complaints • Hygiene shortage suspected by sample collectors
Campaigns	• For the detection of a specific pathogen (on a specific product) • Based on previous outbreaks or recent studies

Salmonella surprise

- Approximately 30 samples of chocolate products reached our lab in march/april 2022

- Most of the suspicious lot numbers of the products weren't availabe anymore

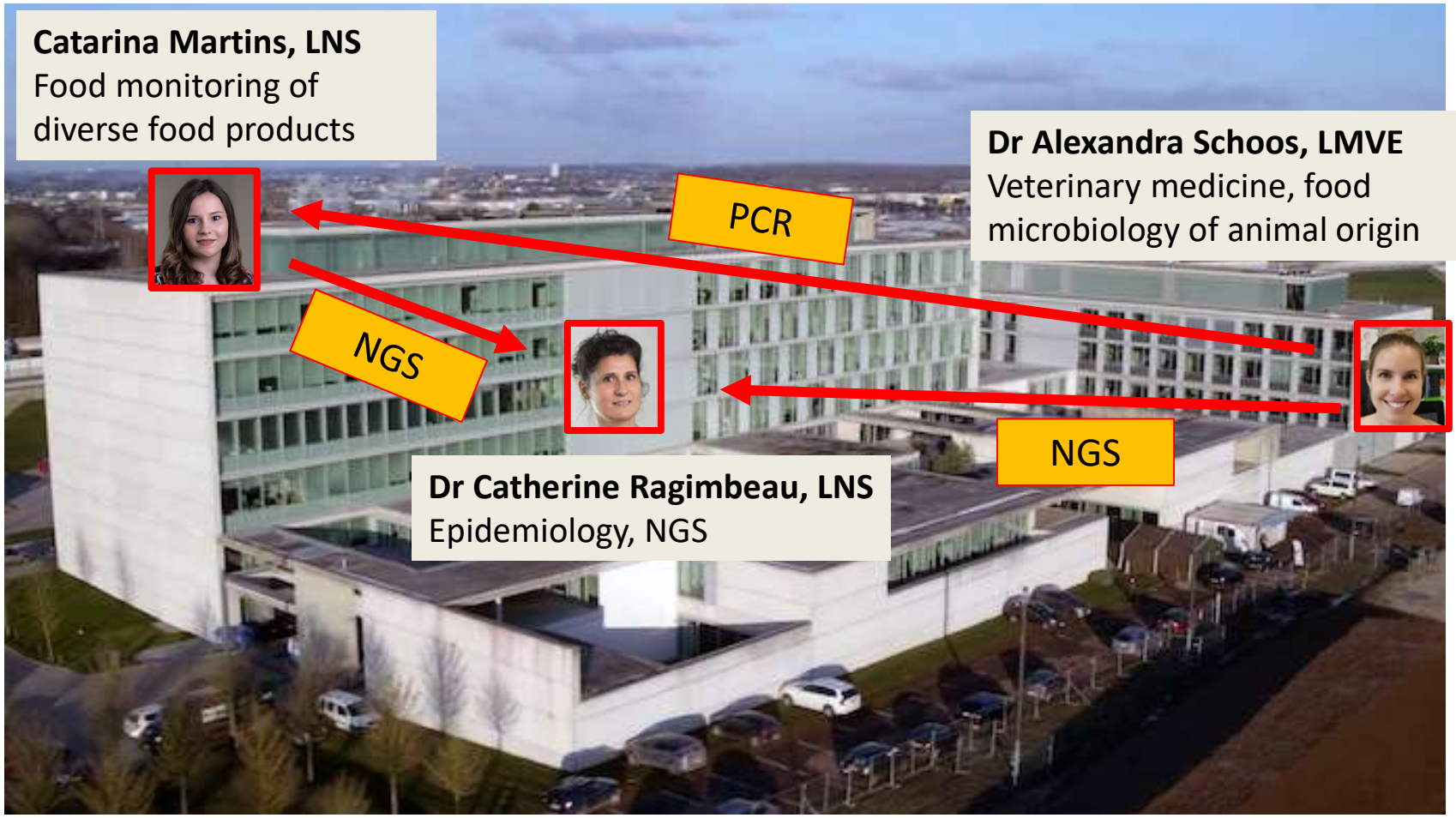
- Analyses with 2 methods :
  - Real-time PCR (iQ-Check Salmonella Kit on CFX 96)
  - ISO 6579-1 method using RVS and MKTTn
  → isolation on Compass *Salmonella* and XLD + confirmation on mini Vidas

- None of the samples was positif

- In case of positif sample : isolation of colonies to give them to Dr Ragimbeau's service for sequencing and matching with human cases

https://www.foodsafetynews.com/2022/04/kinder-chocolate-eggs-other-products-recalled-in-canada-because-of-link-to-multi-country-salmonella-outbreak/

Collaboration of the 2 luxembourgish reference labs



Thanks for your attention!

Catherine RAGIMBEAU
Scientist in bacterial Genomics
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Laboratoire National de Santé – Luxembourg
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Epidemiology and Microbial Genomics
(NGS Platform)

Microbiology Department

EPIGEM
EPIDEMIOLOGY AND MICROBIAL GENOMICS

NGS platform

Bacterial Genomics

Virology Genomics

Foodborne pathogens: integrated surveillance – One Health concept
≈1500 isolates/year

EUROPEAN FOOD SAFETY AUTHORITY

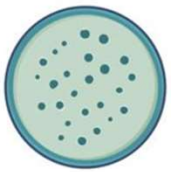
ecdc FWD-Net

Foodborne Surveillance – *Salmonella* – *Listeria* mono.– STEC – *Campylobacter* – *Yersinia enterocolitica* – One Health approach

….+ Staph aureus

Overview of the sequencing workflow

Culture



Pure culture
Solid medium
Fresh
Confirmed
< 3 subcultures

DNA extraction

Magnetic beads
Standardised
Automated
One protocol for all
Customised

Quantification

Library Prep

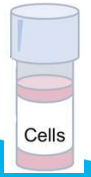
Illumina
Nextera Flex

NGS

MiniSeq Illumina

Dry-lab part

Assembly
Quality statistics
cgMLST
Virulence
Serotyping
AMR
reports



Biobanking
Storage -80°C

LNS
LUXEMBOURG

Pilot study

The complete resistance profile of the outbreak monophasic *Salmonella* Typhimurium strain linked to chocolate products.

Genomics tells us the story of the outbreak monophasic *Salmonella* Typhimurium strain linked to chocolate products.

Case definition

The agreed European Union (EU) case definition for confirmed cases was laboratory-confirmed monophasic *S.* Typhimurium with symptom onset on or after 1 October 2021 and belonging to the same five SNP single linkage cluster by SNP typing or cases who clustered within five allelic differences of another confirmed outbreak strain by core genome multilocus sequence typing (cgMLST) analysis or shared the same HC5_296366 by the EnteroBase HierCC scheme [1]. This definition therefore depended on the whole genome sequencing (WGS) methodology used at the national level in each country (i.e SNP typing or cgMLST analysis). Probable cases were those with laboratory confirmation of monophasic *S.* Typhimurium with symptom onset on or after 1 October 2021 and phenotypic antimicrobial resistance (AMR) results consistent with the outbreak strain or a multilocus variable number tandem repeat analysis (MLVA) profile 3–11–14-NA-0211.

Source: Euro Surveill. 2022 Apr;27(15):2200314.

In *silico* definition of the AMR profile (cluster 1)

The outbreak strain is **resistant** to six families of antibiotics:

- penicillins (*bla*T_{EM-1});
- aminoglycosides (streptomycin, spectinomycin, kanamycin, and gentamycin (gene combination varies); strA-strB, aac(6')-Ia, aac(3)-IId, aph(6)-Id, aadA-2, aadA-8b, aadA-12, aadA-15 and aadA-17;
- phenicols (*cmlA1*, *floR*);
- sulfonamides (sul2 with some strains having an additional sul3 gene);
- trimethoprim (*dfrA12*); and
- tetracyclines (*tetA and tetM*).

In addition, some strains contained the *lnu(F)* gene encoding resistance to **lincosamide**, but this could not be confirmed phenotypically.

The resistance to aminoglycosides, phenicols, and trimethoprim is rare in monophasic *S.* Typhimurium and could therefore be used for screening of probable cases. However, France has noted that a minor proportion of their isolates matching the outbreak sequence have lost a DNA block containing several genes, including those encoding for this rare resistance.

Source: Joint ECDC-EFSA RAPID OUTBREAK ASSESSMENT – 12 April 2022

Overview of the number of cases

Update on June 3 th 2022 – ECDC website « Threats and outbreaks of salmonellosis »

Table 1. Number of confirmed and probable cases of monophasic *S.* Typhimurium in the EU/EEA, the United Kingdom, Canada, Switzerland and the United States, as of 3 June 2022

Country	Confirmed cases	Probable cases	Total number of cases
Austria	14	0	**14**
Belgium	52	14	**66**
Denmark	4	0	**4**
France	118	0	**118**
Germany	30	5	**35**
Ireland	17	0	**17**
Italy	1	0	**1**
Luxembourg	2	0	**2**
Netherlands	3	0	**3**
Norway	1	0	**1**
Spain	2	3	**5**
Sweden	4	0	**4**
Total EU/EEA	**248**	**22**	**270**
United Kingdom	122	0	122
Total EU/EEA and UK	**370**	**22**	**392**
Canada	4	0	4
Switzerland	48	0	48
United States	1	0	1
Total	**423**	**22**	**445**

This outbreak is characterised by a high proportion of hospitalised (about 40%) cases and some cases with severe clinical symptoms such as bloody diarrhoea

In silico characterization of strains from cluster 1

- In Luxembourg:
  2 human cases linked to the outbreak = 2 strains from cluster 1

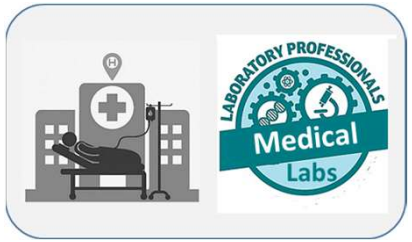
- Identification of ***qacL* gene** encoding reduced susceptibility to a variety of guanidinium including the most notable quaternary ammonium compounds (QACs)

  → **key ingredient in many cleaning and disinfection products**

- Frequency of this accessory gene in our collection of S. Typhimurium/monophasic Typhimurium isolates?

Overview of the *Salmonella* workflow – Genomic surveillance in LU

75%

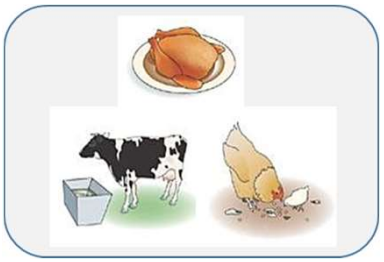


± 120 isolates/year

Routine monitoring
BAC - Epigem

± 40 isolates/year

25%



Laboratoire de Médecine Vétérinaire de l'Etat
Veterinary medecine

LNS LUXEMBOURG
Food surveillance

WGS

illumina
Nextera Flex
MiniSeq
Miseq
iSeq

ASSEMBLY

Ridom SeqSphere+
version 8.3.1
SKESA 2.4.0

Quality control criteria

- **No more than 4% of missing targets cgMLST scheme (average = 99.5%)**
- **Coverage ≥ 30 (average = 66)**

Quick overview of pipelines /bioinformatic tools

fasta files

Center for Genomic Epidemiology

PlasmidFinder 2.1 Software version: 2.0.1 (2020-07-01)

MobileElement Finder

Software version: v1.0.3 (2020-10-09)

Ridom SeqSphere+

ridom

Classification by MLST
Achtman scheme - 7 housekeeping genes

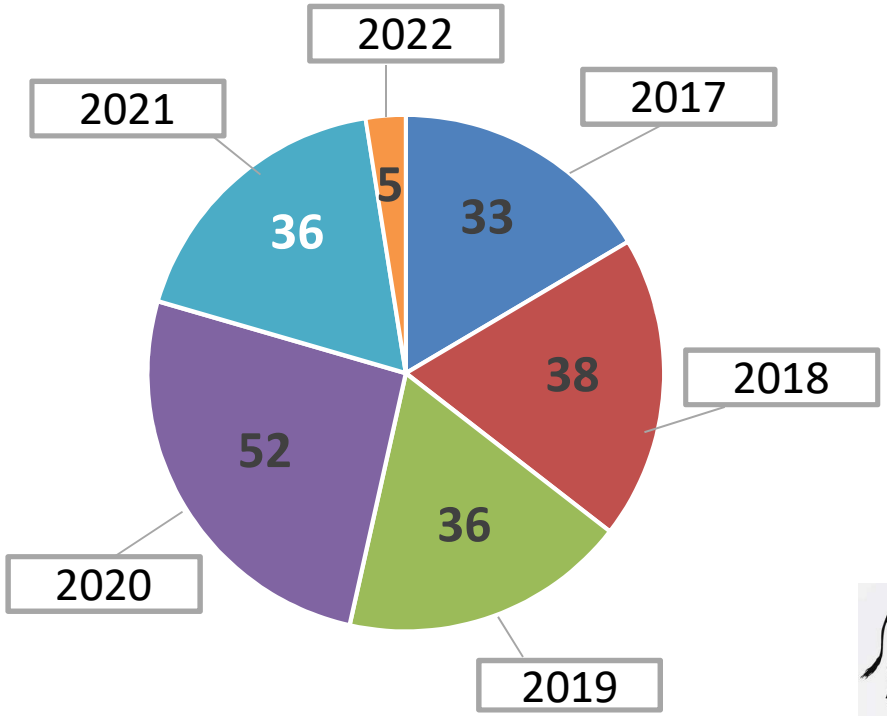
Classification: Gene-by-gene approach
***S. enterica* cgMLST- CT7 Enterobase**
3002 targets

NIH National Library of Medicine
National Center for Biotechnology Information

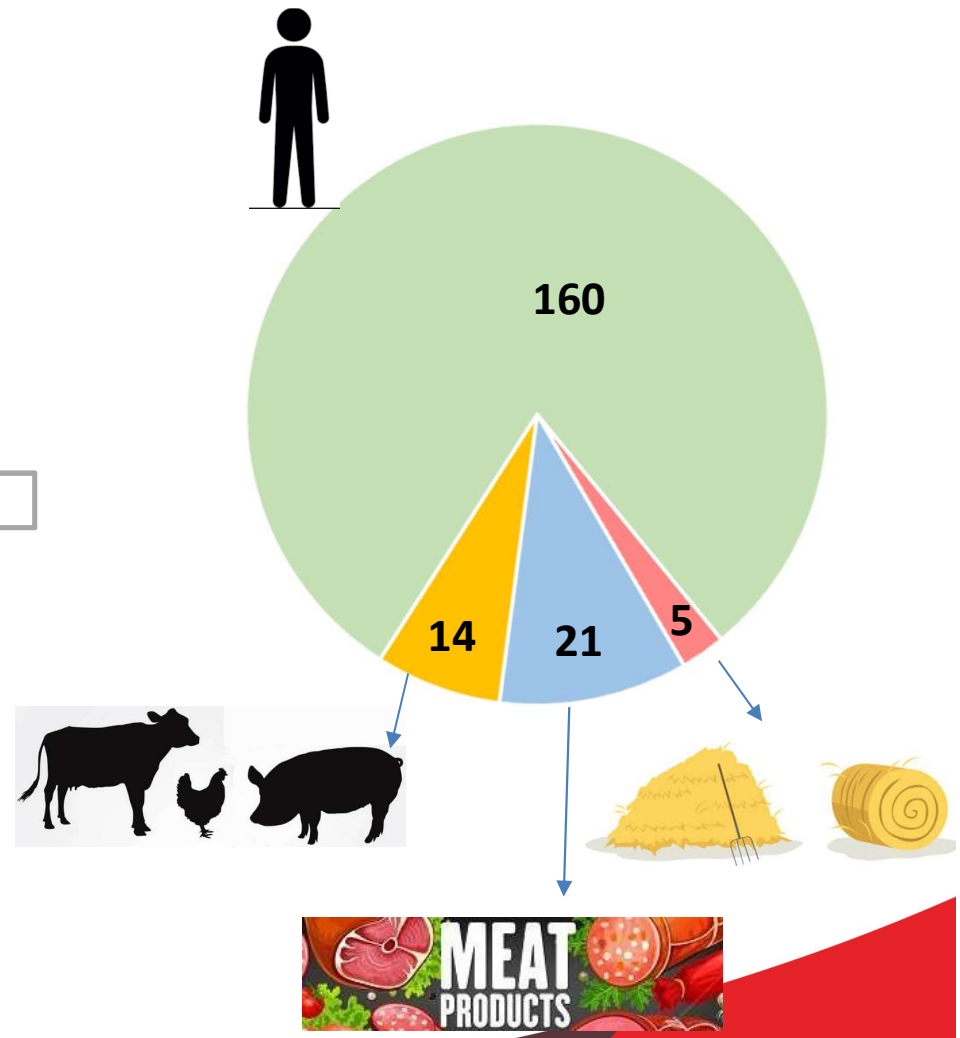
AMRFinderPlus

Collection of 200 strains *Salmonella* Typhimurium/ monophasic S. Typhimurium (ST19-ST34)

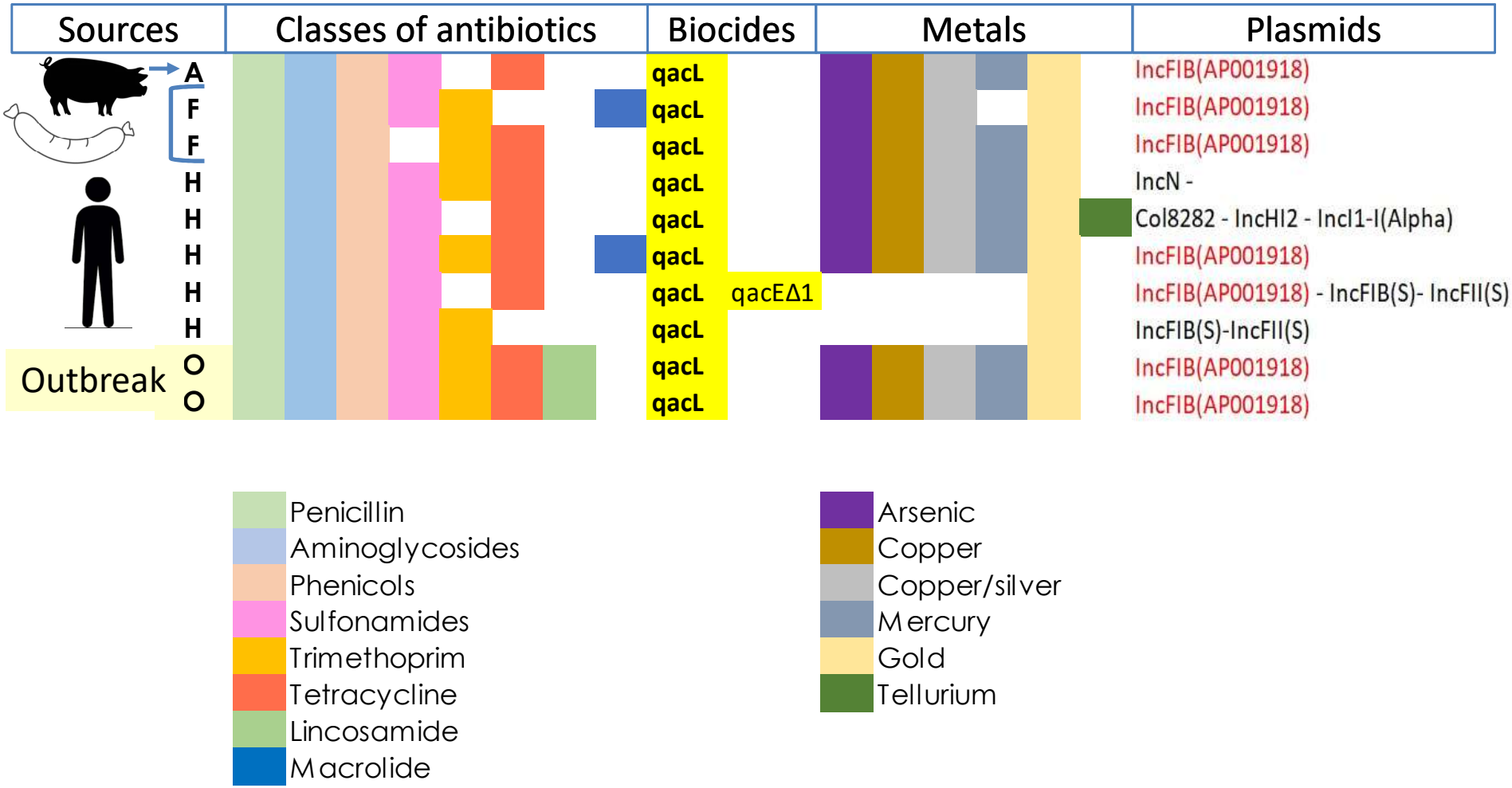
Isolated between 2017-2022 (5 years +)



One Health

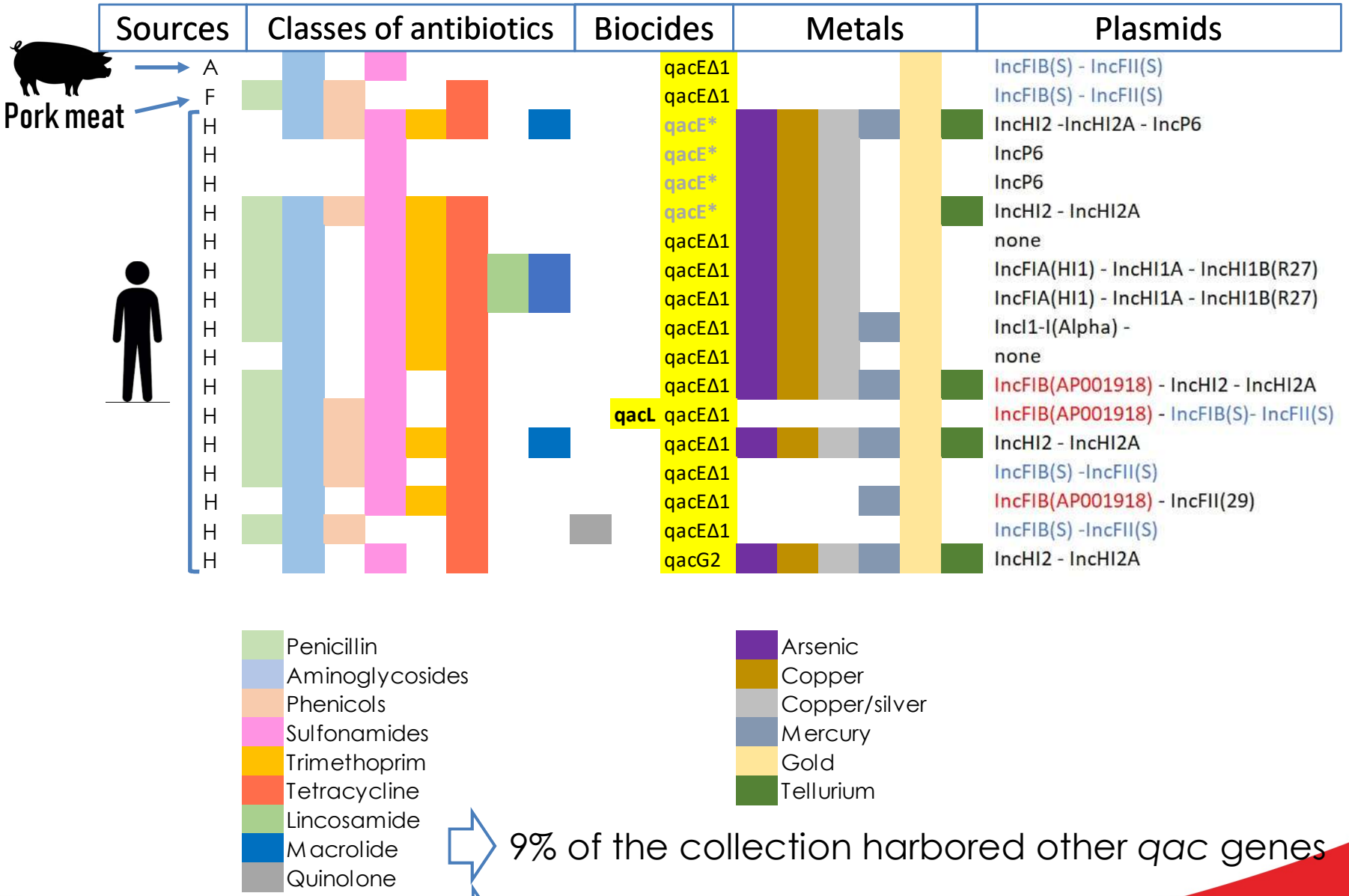


Resistance profiles in silico associated to *qacL*



- 9/200 strains (unique cgMLST types) over the 5 past years harboured qacL => **4,5%**
- 5 to 7 classes of antibiotics R + reduced succeptibilities for 5 heavy metals

Resistance profiles in silico associated to other Biocides (qacEΔ1)



9% of the collection harbored other *qac* genes

More diversity in profiles

Conclusions & Perspectives

- In silico characterization of the outbreak strain from cluster 1 highlighted its resistance profile:
  - 6+ classes of antibiotics
  - reduced susceptibilities to quaternary ammonium compounds (*qacL*)
  - reduced susceptibilities to 5 heavy metals

- *qacL* gene quite uncommon in *S.* Typhimurium/monophasic Typhimurium ≈ 4.5% of strains
  => strong interest to analyse data through integrated surveillance

- Enviromental persistence of this strains linked to biocide & heavy metals "resistance" ?
- Role of biofilm ?

- Interest for the surveillance to characterize strains in their whole profiles of resistance: antibiotics genes/mutations & genes confering an advantage to face environmental conditions

- Correlation phenotype – genotype? Perspective to test the strains with various concentration levels of biocide (quaternary ammonium)

References

Larkin L, Pardos de la Gandara M, Hoban A, Pulford C, Jourdan-Da Silva N, de Valk H, Browning L, Falkenhorst G, Simon S, Lachmann R, Dryselius R, Karamehmedovic N, Börjesson S, van Cauteren D, Laisnez V, Mattheus W, Pijnacker R, van den Beld M, Mossong J, Ragimbeau C, Vergison A, Thorstensen Brandal L, Lange H, Garvey P, Nielsen CS, Herrera León S, Varela C, Chattaway M, Weill FX, Brown D, McKeown P. Investigation of an international outbreak of multidrug-resistant monophasic *Salmonella* Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022. Euro Surveill. 2022 Apr;27(15):2200314. doi: 10.2807/1560-7917.ES.2022.27.15.2200314. PMID: 35426359; PMCID: PMC9012091.

Joint ECDC-EFSA RAPID OUTBREAK ASSESSMENT – Multi-country outbreak of monophasic Salmonella Typhimurium sequence type (ST) 34 linled to chocolate products- 12 April 2022 . https://www.ecdc.europa.eu/sites/default/files/documents/ROA_monophasic-S-Typhimurium-ST34-linked-to-chocolate_2022-00014-final_UK.pdf

15 July update: Monophasic Salmonella Typhimurium outbreak linked to chocolate products- 15 Jul 2022 https://www.ecdc.europa.eu/en/news-events/15-july-update-monophasic-salmonella-typhimurium-outbreak-linked-chocolate-products

Thank you for your attention and special thanks to…

Maria Pardos de la Gandara – Institut Pasteur FR– NRC *E. coli – Shigella – Salmonella*

Johanna Takkinen & Therese Westrell - ECDC - FWD

Thank you to all active partners involved in our integrated surveillance in Luxembourg!

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LE GOUVERNEMENT
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Administration luxembourgeoise vétérinaire
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