THE BENEFITS OF GENOMICS FOR MONITORING ANTIBIOTIC RESISTANCE AND DETECTING TOXI-INFECTIONS

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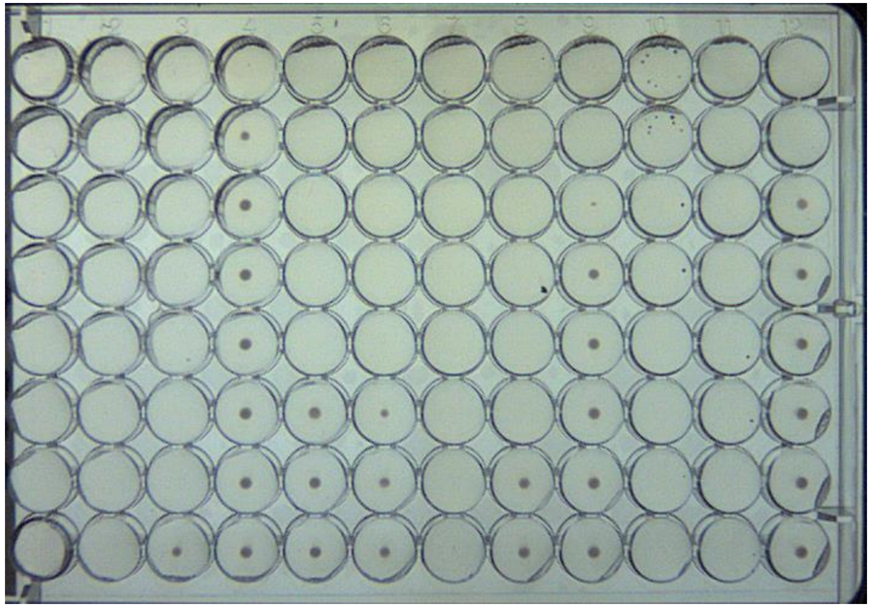
Bavo Verhaegen, Laurence Delbrassinne (Foodborne pathogens service)

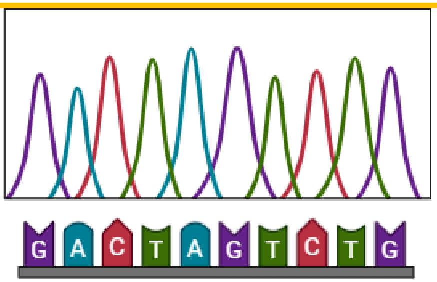
Antimicrobial resistance (AMR)

AMR: Added value phenotyping and genomics

Phenotype: expression of the resistance

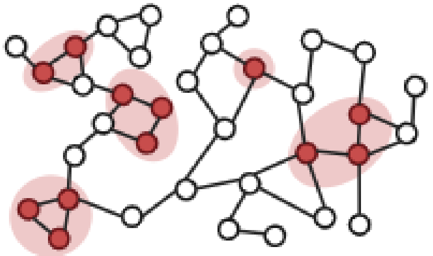


Genotype:



Sequencing

Resistance mechanisms
→ Transferable?
→ Co-selection?



Relatedness of the isolates

Toxi-infections

Foodborn outbreaks

WGS

BELGIQUE CARTE D'IDENTITE
BELGIE IDENTITEITSKAART
BELGIEN PERSONALAUSWEIS
BELGIUM IDENTITY CARD

Nom / *Name*
Prénoms / *Given names*
Listeria monocytogenes
Lieu et date de naissance / *Place and date of birth*
Belgique 20 AOUT 2013
Sexe/ *Sex*
Nationalité
Nationality BELGE
N° carte / *Card No*
000-0000000-000
Valide du - au / *Validity from - until*
31.09.2014 - 20.08.2020
Signature du titulaire / *Holder's signature*

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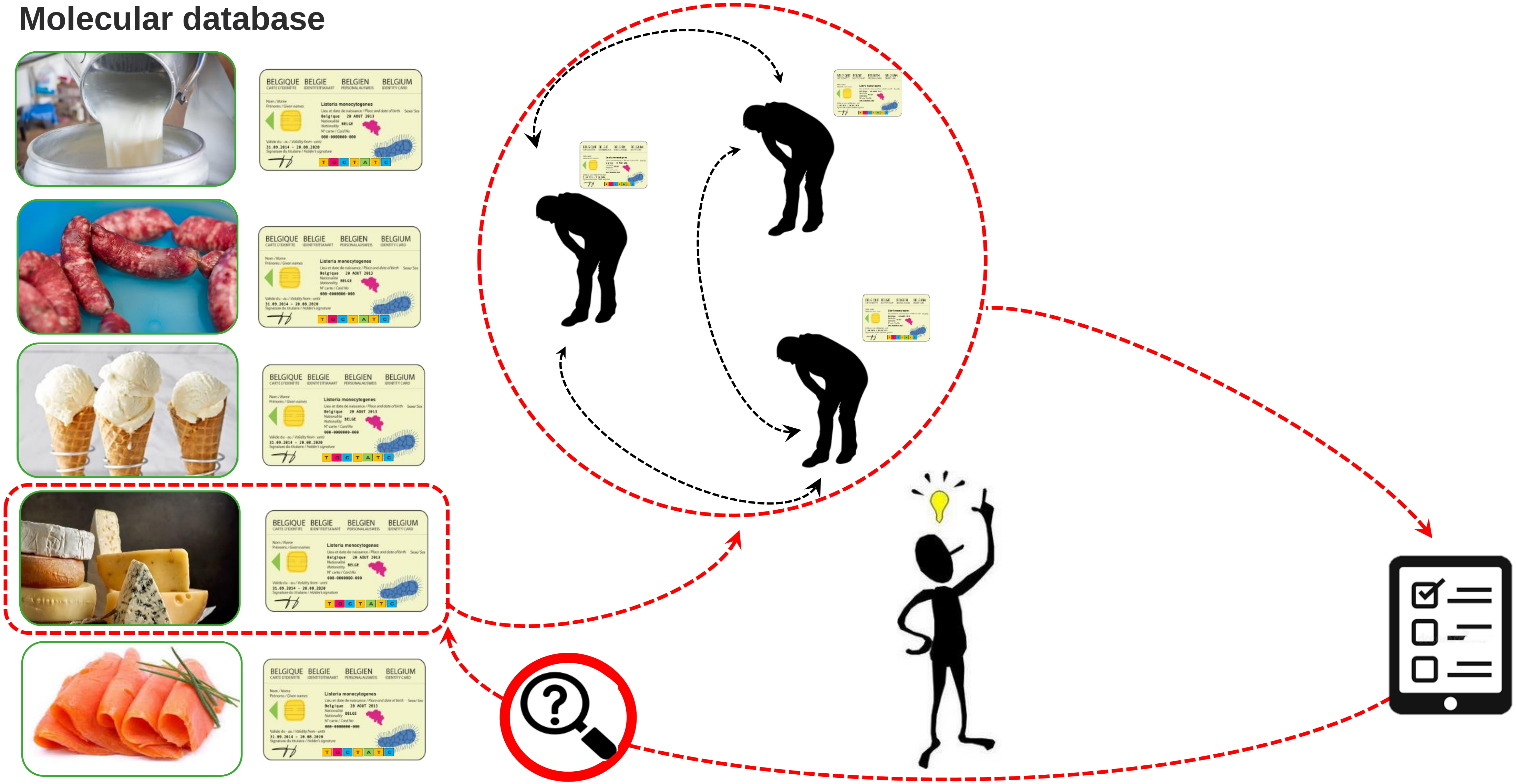
Toxi-infections: added value genomics

Molecular database



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GENOMIC MONITORING OF MRSA

Monitoring in food-producing animals in a One-health context

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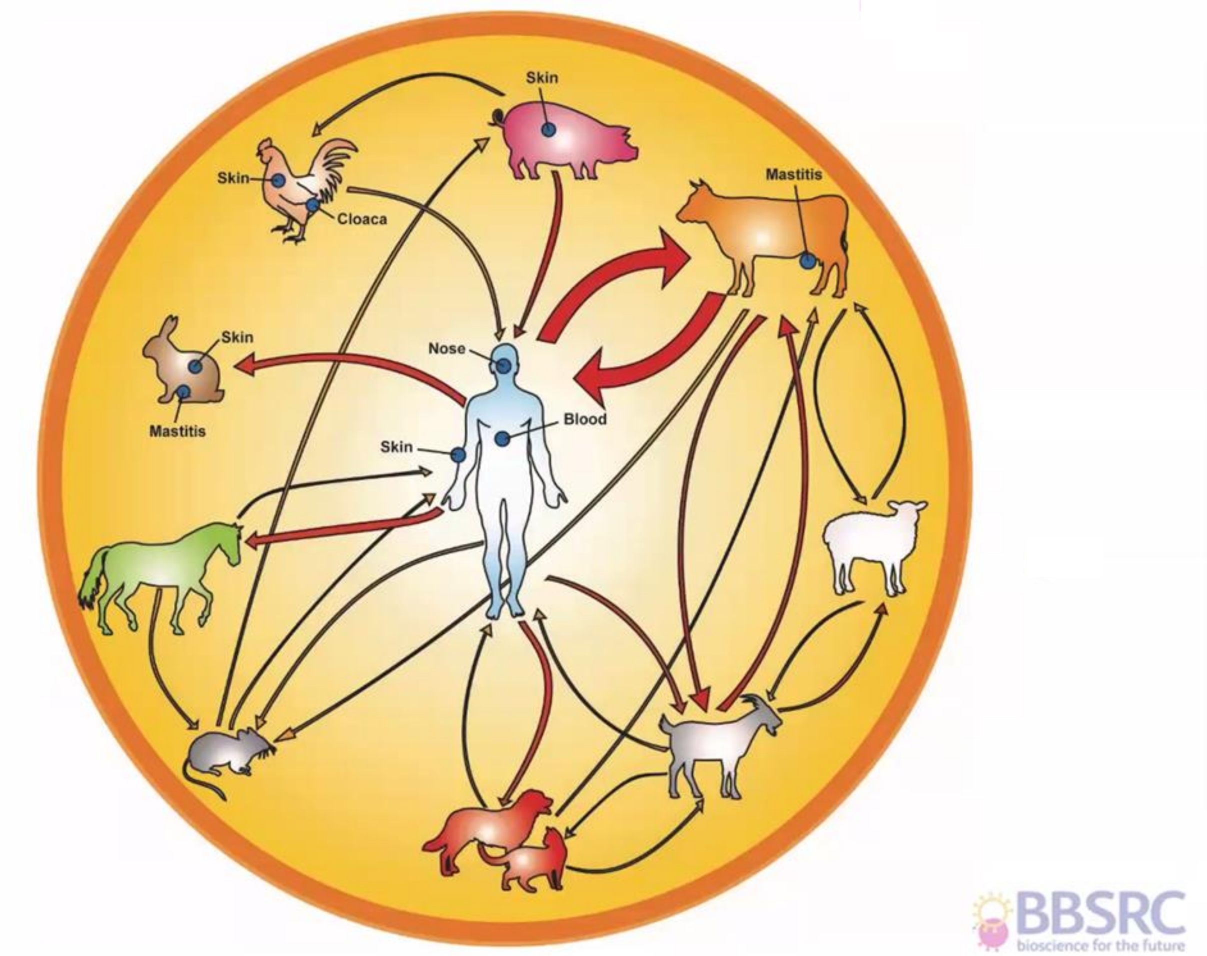
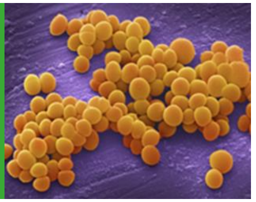
S. aureus: a multi-host pathogen

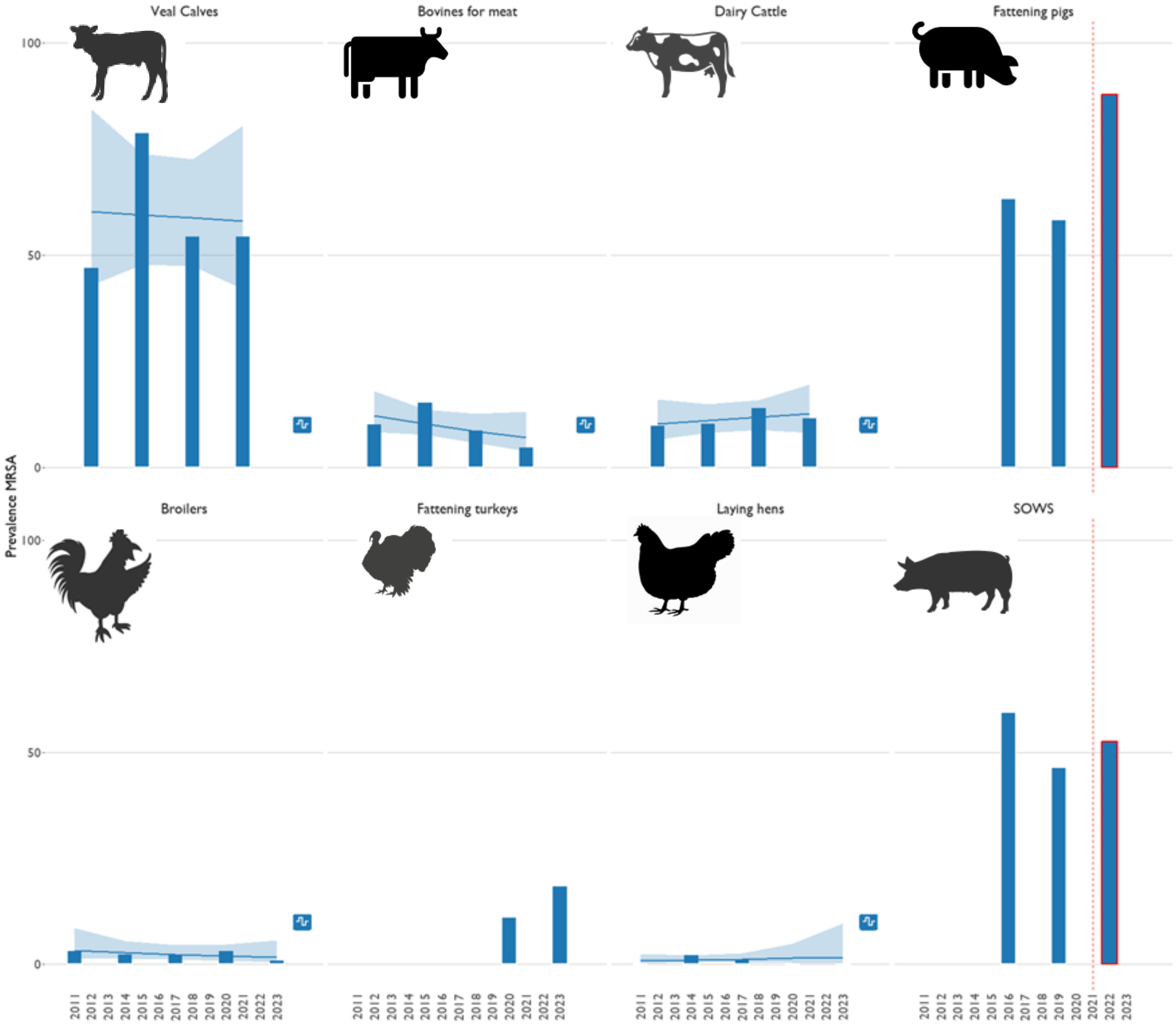


Richardson et al, *Nature Ecol Evol*, 2018

MRSA prevalence in animals in Belgium

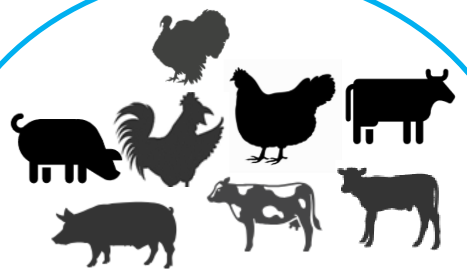


- On-farm monitoring since 2011
  - Prevalence of MRSA
  - Characterization of MRSA isolates
    - Phenotype + Basic genotype
    - In-depth genotype (WGS), since 2022
- 3-years-rotation:
  Poultry, Bovines, Porcines
- Highest prevalence (>50%) in veal calves and porcines



Source: BELMAP 2024

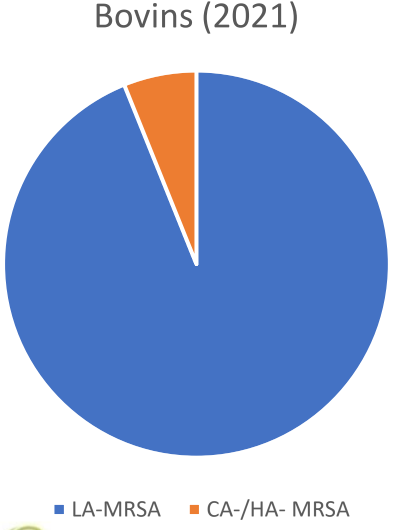
MRSA genotyping

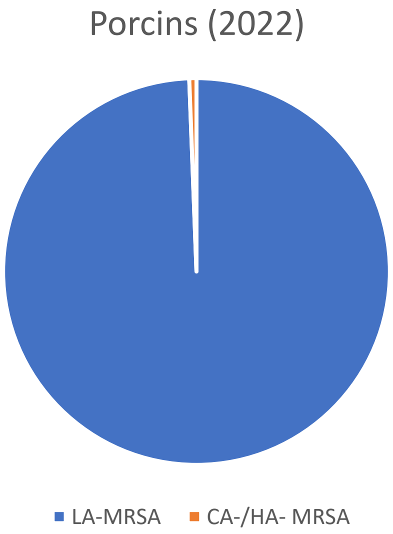


Livestock associated (LA-MRSA)

Community acquired (CA-MRSA)

Hospital acquired MRSA (HA-MRSA)





- Mostly LA-MRSA
- HA- and CA-MRSA sporadically detected in Belgium in food-producing animals:
  - porcines, bovines or poultry
  - Source not traced
  - WGS is helpful to assess genetic relatedness

Source: https://favv-afsca.be/nl/antibioticaresistentie-resultaten#sciensano

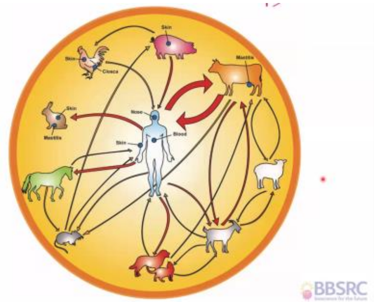
Example: HA-MRSA strain detected in bovines

Isolated from meat cattle in 2018 in Belgium:

- ST22 (CC22)/ HA-MRSA
- *tst* (toxic shock syndrome)
- *sak, chp, scn* (immune evasion cluster)
- Tet S
- SCCmec typical of LA-MRSA (IV2b/ IVa2b)

→ Hypothesis: a strain of human origin which is thought to have acquired resistance to methicillin in animals

→ Importance of genomics for the monitoring



Richardson et al, *Nature Ecol Evol*, 2018

ONE-HEALTH MONITORING OF RESISTANCE TO CRITICAL ANTIBIOTICS

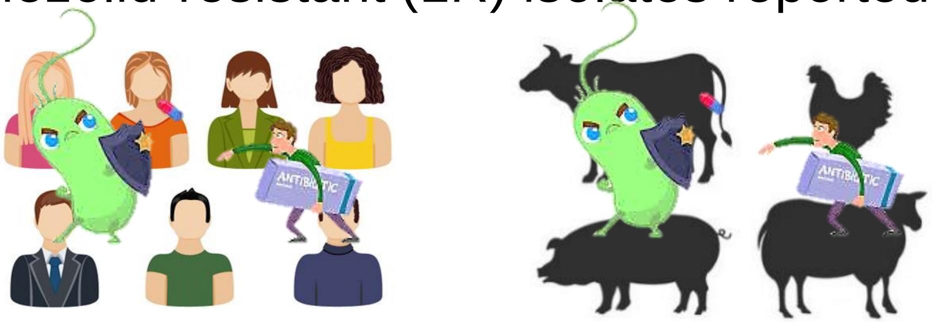
The linezolid case

Linezolid

- Linezolid (LZD):
  - a critically important antibiotic to treat human infections (MRSA, VRE)

  - not licensed for food-producing animals

  - linezolid-resistant (LR) isolates reported in European countries in H + A



Known resistance mechanisms

Known genetic determinants conferring resistance to linezolid

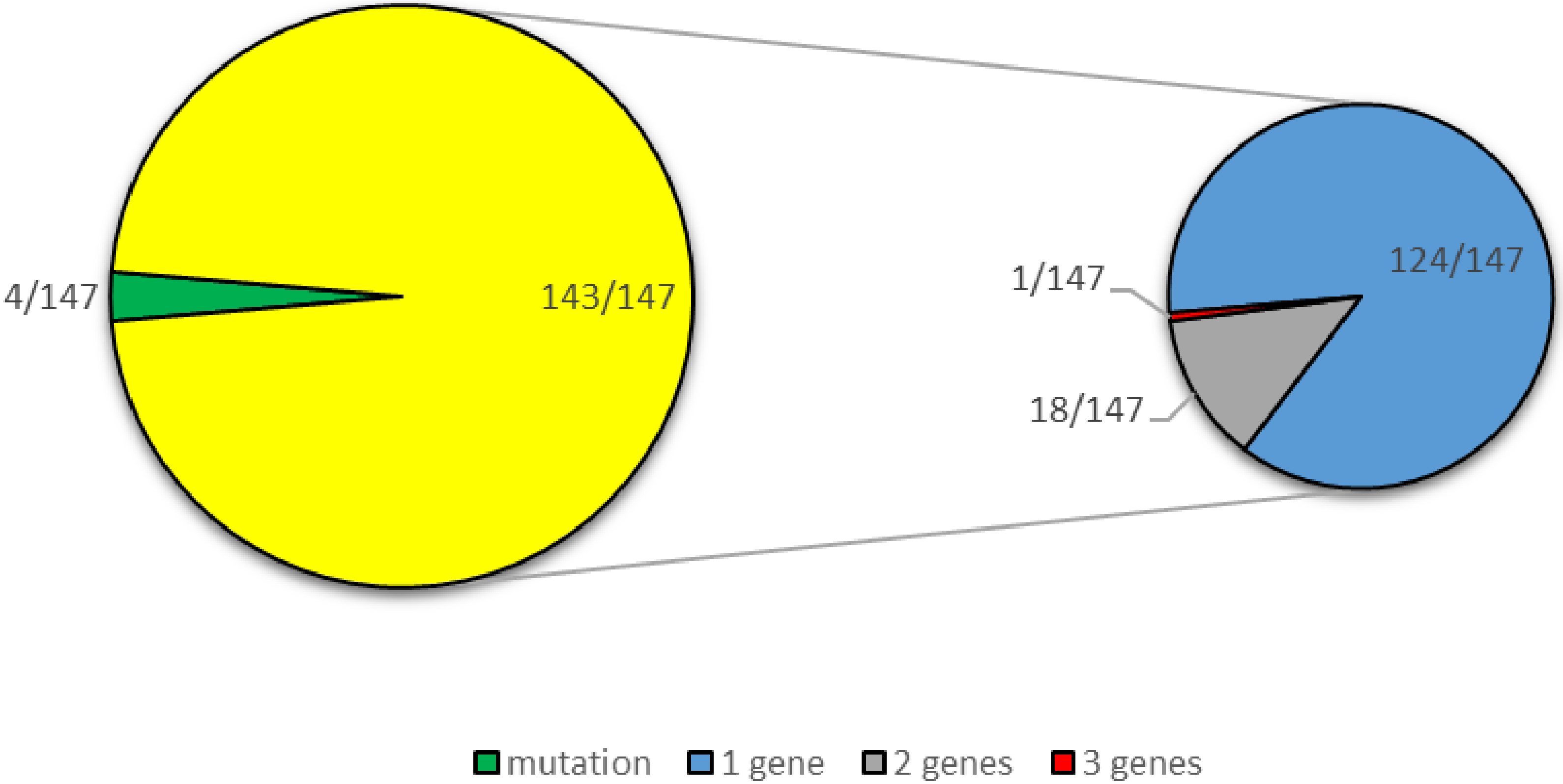
transferable

Gene	Mechanism	Antibiotic classes (Resistance to)
cfr	23SrRNA methylation	Oxazolidinones, phenicols, lincosamides, pleuromutilins, streptogramin A =« PhLOPSA »
optrA	ribosomic protection	oxazolidinones, phenicols
poxtA	ribosomic protection	Oxazolidinones, tetracycline, phenicols,
Mutations in 23SrRNA	ribosomic protection	Linezolid

Common cross-resistance to phenicols

In green, antimicrobials used (very) commonly in food-producing animals

Resistance mechanisms identified



- Most of the isolates (97.3%) carried **transferable LR genes**
- Mainly *optrA*: only gives **cross-resistance to phenicols** (and LZD)

→ **cross-selection will maintain these genes**

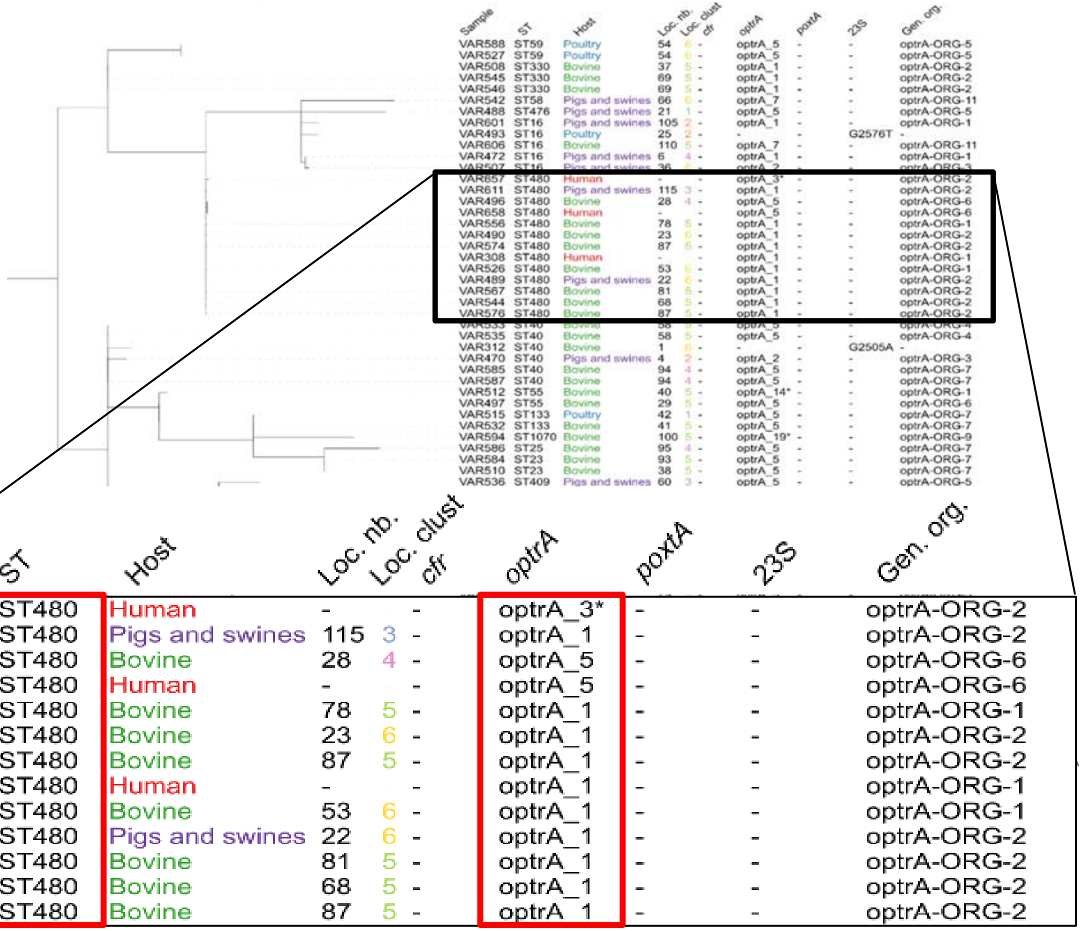
Large diversity observed among LR enterococci isolates

Very large phylogenetic differences observed along with some smaller clades containing **more closely related isolates**

- *E. faecalis*: 28 different STs (including 3 new)
- *E. faecium*: 32 different STs (including 8 new)

GENETIC DIVERSITY!

- No clear correlation between phylogenies and carried LR genes (alleles)
  → could indicate a facilitated transfer and **spread** of LR genes



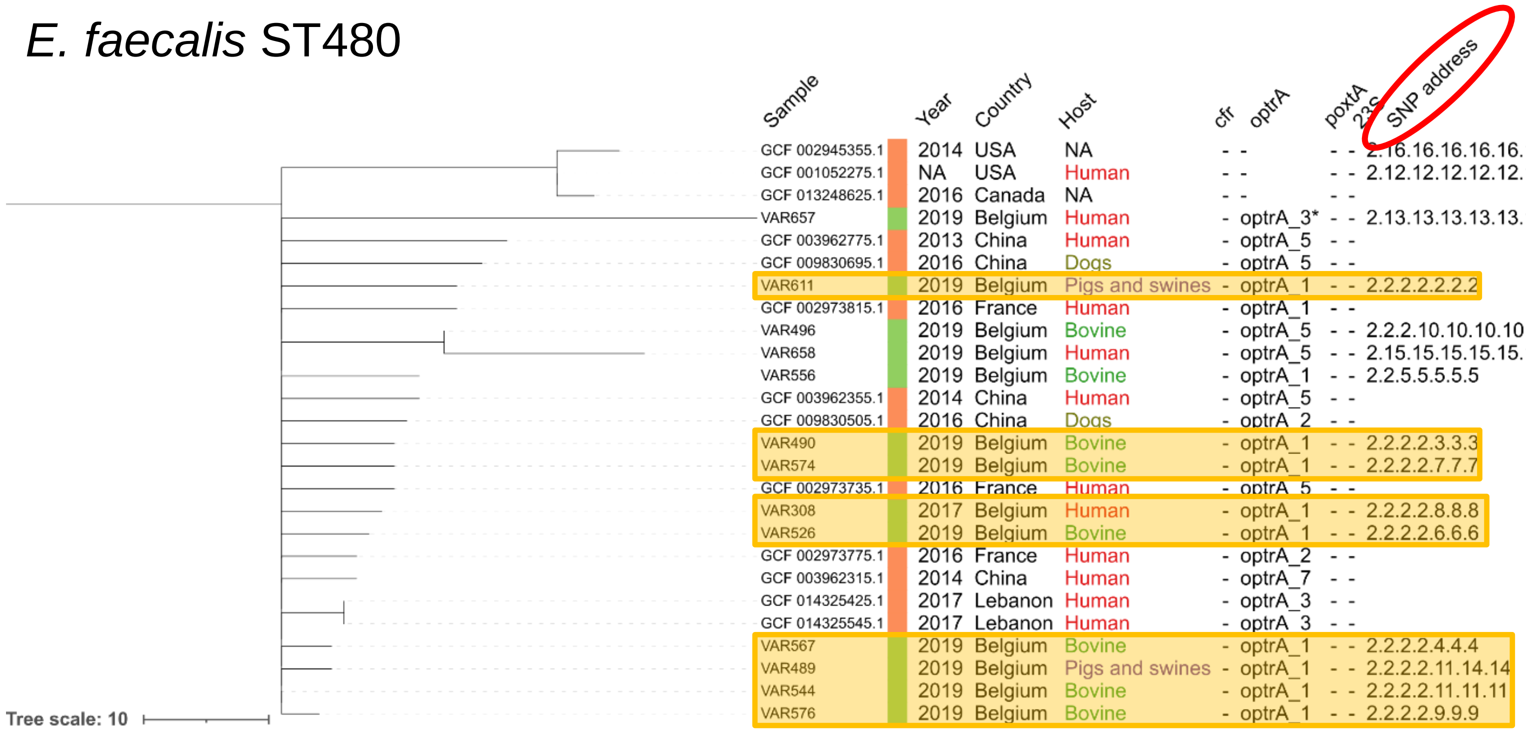
E. faecalis

Sample	ST	Host	Loc. nb.	Loc. clust	cfr	optrA	poxtA	23S	Gen. org.
VAR588	ST59	Poultry	54	6	-	optrA_5	-	-	optrA-ORG-5
VAR527	ST59	Poultry	54	6	-	optrA_5	-	-	optrA-ORG-5
VAR508	ST330	Bovine	37	5	-	optrA_1	-	-	optrA-ORG-2
VAR545	ST330	Bovine	69	5	-	optrA_1	-	-	optrA-ORG-2
VAR546	ST330	Bovine	69	5	-	optrA_1	-	-	optrA-ORG-2
VAR542	ST58	Pigs and swines	66	6	-	optrA_7	-	-	optrA-ORG-11
VAR488	ST476	Pigs and swines	21	1	-	optrA_5	-	-	optrA-ORG-5
VAR601	ST16	Pigs and swines	105	2	-	optrA_1	-	-	optrA-ORG-1
VAR493	ST16	Poultry	25	2	-	-	-	G2576T	-
VAR606	ST16	Bovine	110	5	-	optrA_7	-	-	optrA-ORG-11
VAR472	ST16	Pigs and swines	6	4	-	optrA_1	-	-	optrA-ORG-1
VAR507	ST16	Pigs and swines	36	6	-	optrA_2	-	-	optrA-ORG-3
VAR657	ST480	Human	-		-	optrA_3*	-	-	optrA-ORG-2
VAR611	ST480	Pigs and swines	115	3	-	optrA_1	-	-	optrA-ORG-2
VAR496	ST480	Bovine	28	4	-	optrA_5	-	-	optrA-ORG-6
VAR658	ST480	Human	-		-	optrA_5	-	-	optrA-ORG-6
VAR556	ST480	Bovine	78	5	-	optrA_1	-	-	optrA-ORG-1
VAR490	ST480	Bovine	23	6	-	optrA_1	-	-	optrA-ORG-2
VAR574	ST480	Bovine	87	5	-	optrA_1	-	-	optrA-ORG-2
VAR308	ST480	Human	-		-	optrA_1	-	-	optrA-ORG-1
VAR526	ST480	Bovine	53	6	-	optrA_1	-	-	optrA-ORG-1
VAR489	ST480	Pigs and swines	22	6	-	optrA_1	-	-	optrA-ORG-2
VAR567	ST480	Bovine	81	5	-	optrA_1	-	-	optrA-ORG-2
VAR544	ST480	Bovine	68	5	-	optrA_1	-	-	optrA-ORG-2
VAR576	ST480	Bovine	87	5	-	optrA_1	-	-	optrA-ORG-2
VAR533	ST40	Bovine	58	5	-	optrA_5	-	-	optrA-ORG-4
VAR535	ST40	Bovine	58	5	-	optrA_5	-	-	optrA-ORG-4
VAR312	ST40	Bovine	1	6	-	-	-	G2505A	-
VAR470	ST40	Pigs and swines	4	2	-	optrA_2	-	-	optrA-ORG-3
VAR585	ST40	Bovine	94	4	-	optrA_5	-	-	optrA-ORG-7
VAR587	ST40	Bovine	94	4	-	optrA_5	-	-	optrA-ORG-7
VAR512	ST55	Bovine	40	5	-	optrA_14*	-	-	optrA-ORG-1
VAR497	ST55	Bovine	29	5	-	optrA_5	-	-	optrA-ORG-6
VAR515	ST133	Poultry	42	1	-	optrA_5	-	-	optrA-ORG-7
VAR532	ST133	Bovine	41	5	-	optrA_5	-	-	optrA-ORG-7
VAR594	ST1070	Bovine	100	5	-	optrA_19*	-	-	optrA-ORG-9
VAR586	ST25	Bovine	95	4	-	optrA_5	-	-	optrA-ORG-7
VAR584	ST23	Bovine	93	5	-	optrA_5	-	-	optrA-ORG-7
VAR510	ST23	Bovine	38	5	-	optrA_5	-	-	optrA-ORG-7
VAR536	ST409	Pigs and swines	60	3	-	optrA_5	-	-	optrA-ORG-5

Sample	ST	Host	Loc. nb.	Loc. clust	cfr	optrA	poxtA	23S	Gen. org.
VAR657	ST480	Human	-		-	optrA_3*	-	-	optrA-ORG-2
VAR611	ST480	Pigs and swines	115	3	-	optrA_1	-	-	optrA-ORG-2
VAR496	ST480	Bovine	28	4	-	optrA_5	-	-	optrA-ORG-6
VAR658	ST480	Human	-		-	optrA_5	-	-	optrA-ORG-6
VAR556	ST480	Bovine	78	5	-	optrA_1	-	-	optrA-ORG-1
VAR490	ST480	Bovine	23	6	-	optrA_1	-	-	optrA-ORG-2
VAR574	ST480	Bovine	87	5	-	optrA_1	-	-	optrA-ORG-2
VAR308	ST480	Human	-		-	optrA_1	-	-	optrA-ORG-1
VAR526	ST480	Bovine	53	6	-	optrA_1	-	-	optrA-ORG-1
VAR489	ST480	Pigs and swines	22	6	-	optrA_1	-	-	optrA-ORG-2
VAR567	ST480	Bovine	81	5	-	optrA_1	-	-	optrA-ORG-2
VAR544	ST480	Bovine	68	5	-	optrA_1	-	-	optrA-ORG-2
VAR576	ST480	Bovine	87	5	-	optrA_1	-	-	optrA-ORG-2

Closely related isolates from pigs, bovines and humans

E. faecalis ST480



Sample		Year	Country	Host	cfr	optrA	poxtA	23S	SNP address
GCF 002945355.1	🟧	2014	USA	NA	-	-	-	-	2.16.16.16.16.16
GCF 001052275.1	🟧	NA	USA	Human	-	-	-	-	2.12.12.12.12.12.12
GCF 013248625.1	🟧	2016	Canada	NA	-	-	-	-	
VAR657	🟩	2019	Belgium	Human	-	optrA_3*	-	-	2.13.13.13.13.13.13
GCF 003962775.1	🟧	2013	China	Human	-	optrA_5	-	-	
GCF 009830695.1	🟧	2016	China	Dogs	-	optrA_5	-	-	
VAR611	🟩	2019	Belgium	Pigs and swines	-	optrA_1	-	-	2.2.2.2.2.2.2
GCF 002973815.1	🟧	2016	France	Human	-	optrA_1	-	-	
VAR496	🟩	2019	Belgium	Bovine	-	optrA_5	-	-	2.2.2.10.10.10.10
VAR658	🟩	2019	Belgium	Human	-	optrA_5	-	-	2.15.15.15.15.15.15
VAR556	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.5.5.5.5.5
GCF 003962355.1	🟧	2014	China	Human	-	optrA_5	-	-	
GCF 009830505.1	🟧	2016	China	Dogs	-	optrA_2	-	-	
VAR490	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.3.3.3
VAR574	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.7.7.7
GCF 002973735.1	🟧	2016	France	Human	-	optrA_5	-	-	
VAR308	🟩	2017	Belgium	Human	-	optrA_1	-	-	2.2.2.2.8.8.8
VAR526	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.6.6.6
GCF 002973775.1	🟧	2016	France	Human	-	optrA_2	-	-	
GCF 003962315.1	🟧	2014	China	Human	-	optrA_7	-	-	
GCF 014325425.1	🟧	2017	Lebanon	Human	-	optrA_3	-	-	
GCF 014325545.1	🟧	2017	Lebanon	Human	-	optrA_3	-	-	
VAR567	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.4.4.4
VAR489	🟩	2019	Belgium	Pigs and swines	-	optrA_1	-	-	2.2.2.2.11.14.14
VAR544	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.11.11.11
VAR576	🟩	2019	Belgium	Bovine	-	optrA_1	-	-	2.2.2.2.9.9.9

- 9 closely related isolates (difference of 10 to 25 SNPs based on SNP address)
- Isolates from pigs, bovines and humans
- Suggesting a potential epidemiological link

Genomic research as a basis of AMCRA advise

AMCRA

AVIS

2024

USAGE PRUDENT DU FLORFÉNICOL CHEZ LES ANIMAUX
EN VUE DE LIMITER LE RISQUE DE RÉSISTANCE AU LINÉZOLIDE

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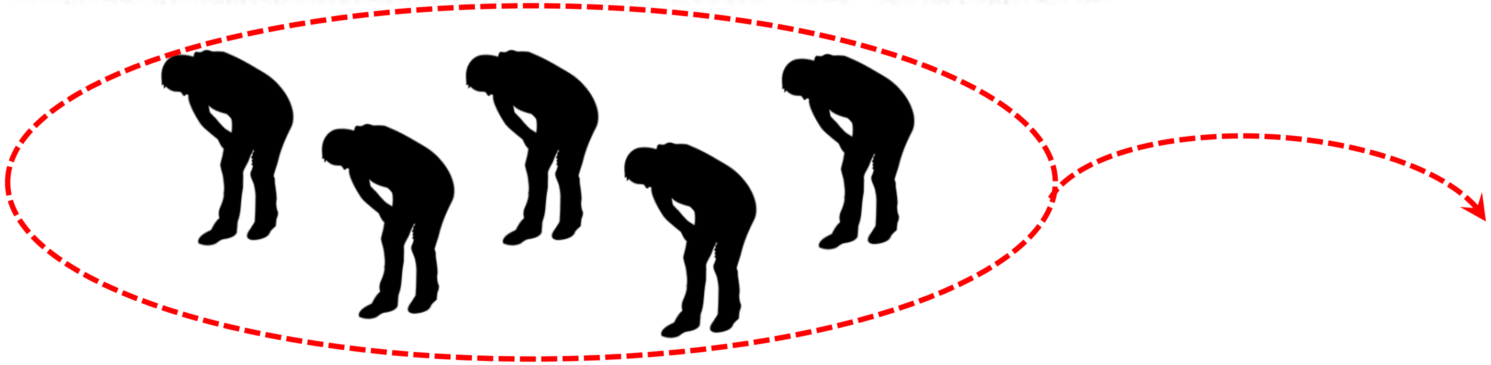
FOODBORNE OUTBREAK

Spaghetti special

- 2024: 46 ill after spaghetti night organised by youth movement → 2 hospitalisation
- mostly within 24h: Diarrhea, fever, abdominal cramps

Epidemiological study

- Lime Survey
- Retrospective cohort study



spaghetti dinner (meat or veg)
tiramisu (homemade)
ice-cream (commercial)

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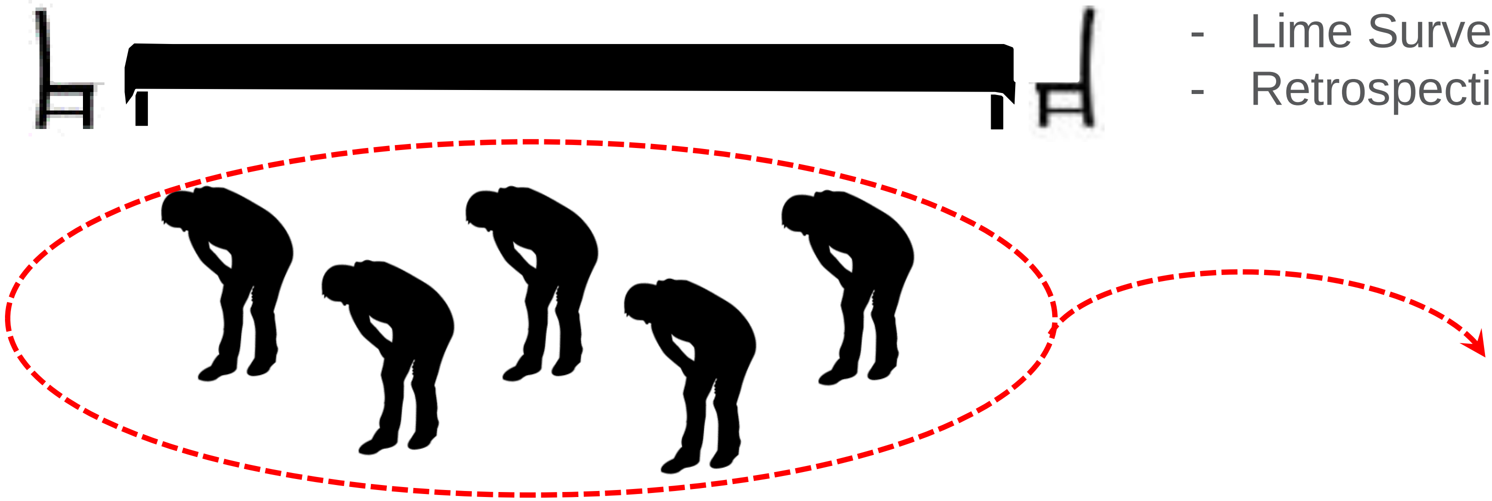

Epidemiological study

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Spaghetti special

- 2024: 46 ill after spaghetti night organised by youth movement → 2 hospitalisation
- mostly within 24h: Diarrhea, fever, abdominal cramps

Attack rates:
- 96% for tiramisu
- 85% for spaghetti

- Tiramisu (leftovers)
- Egg (same batch)

→

- qPCR++, isolation+
- qPCR+, isolation-

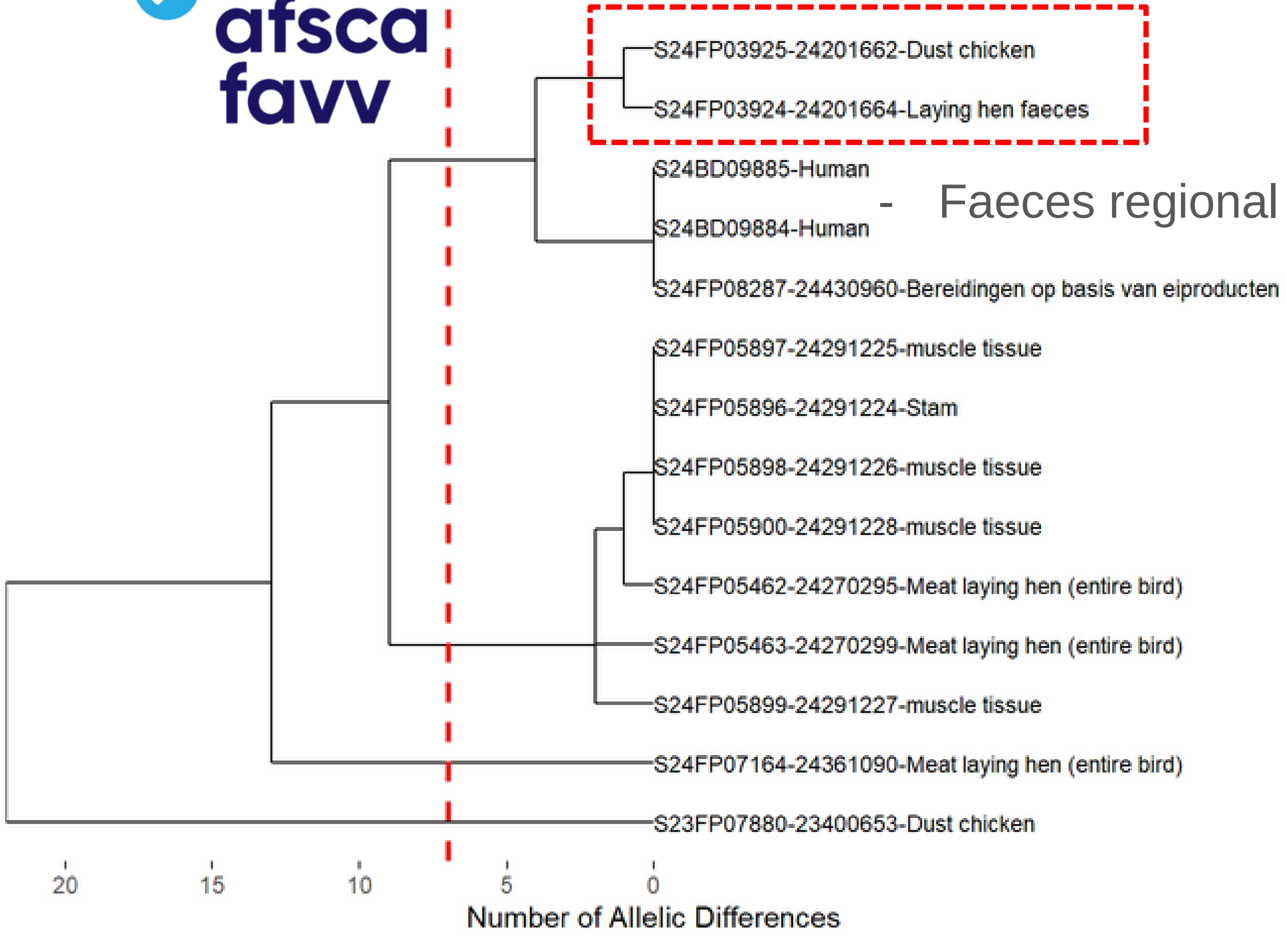
Typing: *Salmonella* Enteritidis

- Faeces regional hospital: *Salmonella* spp.
- Sent to NRC

→

Salmonella Enteritidis

Whole genome sequencing



- Faeces regional hospital: *Salmonella* Enteritidis
- Tiramisu (leftovers)

Traceability (FAVV-AFSCA)

- same hatchery
- same transport

2 farms:
- Antwerp
- Limburg

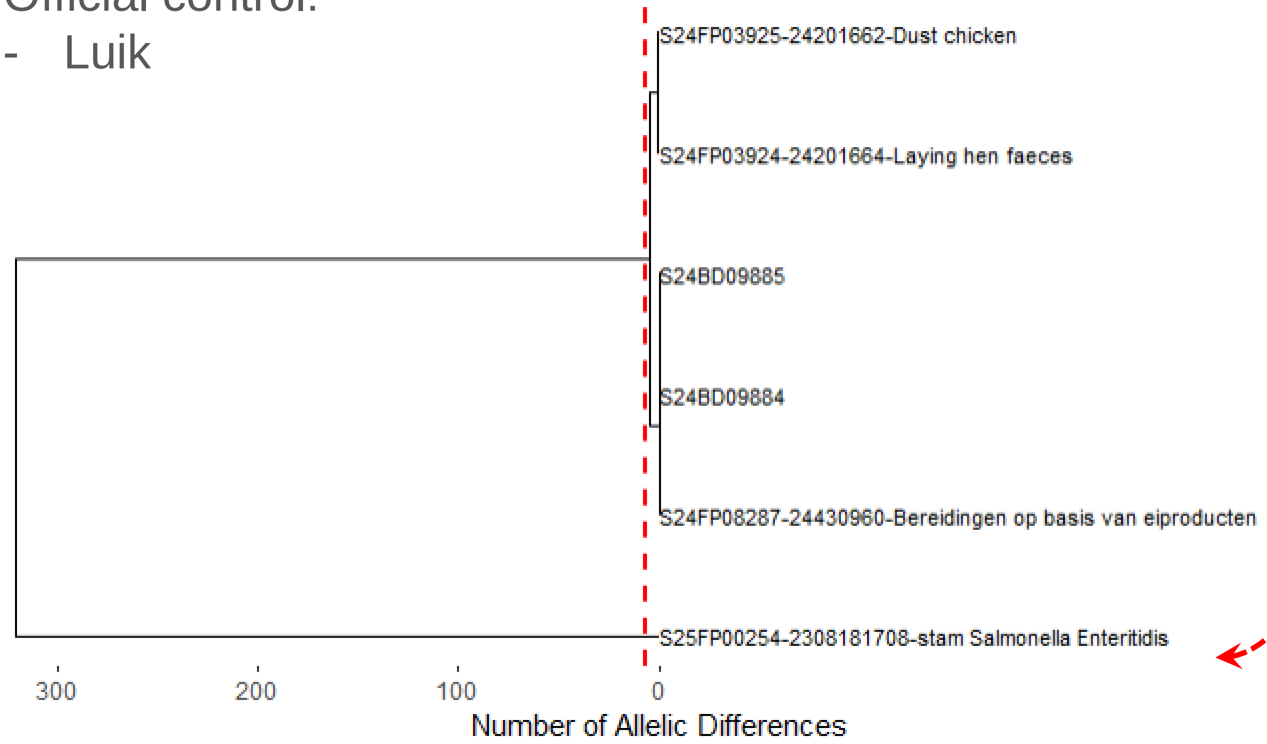
Official control:
- Luik

Autocontrol: no non conform results since 2022

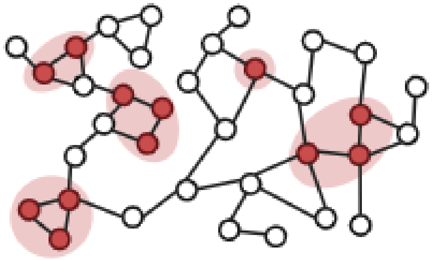
Extra sampling: no Salmonella detected

Autocontrol: one non conform

WGS



Conclusion: added value of genomics



- Link human and food/ animal strains

- Link outbreak strains to WGS database: source detection

- High resolution solution next to epidemiological data

Resistance mechanisms
→ Transferable?
→ Co-selection?

Contact

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Acknowledgements

- The AMR results were obtained thanks to the team of the NRL AMR , the FASFC , the OH-EJP and all contributors of our paper on linezolid resistance

One HEALTH EJP

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J Antimicrob Chemother
https://doi.org/10.1093/jac/dkab376

Journal of Antimicrobial Chemotherapy

Large diversity of linezolid-resistant isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019

Michaël Timmermans[1,2], Bert Bogaerts[3], Kevin Vanneste[3], Sigrid C. J. De Keersmaecker[3], Nancy H. C. Roosens[3], Carole Kowalewicz[1], Guillaume Simon[1], Maria A. Argudín[4]†, Ariane Deplano[4,5], Marie Hallin[4,5,6], Pierre Wattiau[1], David Fretin[1], Olivier Denis[6,7] and Cécile Boland[1]*

- The FBO investigation is a collaboration between NRL FBO, NRL Salmonella,

NRC Salmonella & Shigella, the FASFC , Regional Health inspection services

DEPARTEMENT ZORG

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