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toute une vie en bonne santé

Monitoring of antimicrobial resistance, including linezolid selective monitoring, in MRSA and enterococci from food-producing animals in Belgium

Symposium Animal Health 23/09/2021

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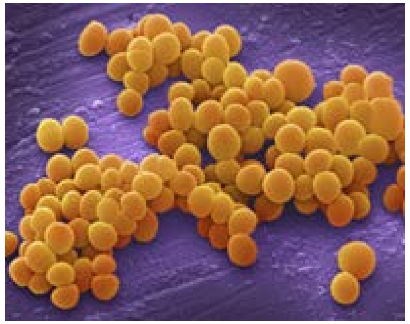
Official monitoring of MRSA and enterococci

- **MRSA:**



- Monitored **since 2011**
- **3-year rotation**: poultry, bovines, porcines
- **Nasal swabs** sampled at **farm (pooled)**
- Estimation of the MRSA prevalence
- Genotyping (CC398, spa typing)
- Antimicrobial susceptibility testing (AST)

AMR priority:
Vancomycin R
Linezolid R
MDR

- **enterococci:**

- Monitored in **2011-2013**; restarted in **2019**
- **Faeces in slaughterhouses or at farm** depending on animal categories (poultry, porcines and bovines)
- AST conducted for 2 *Enterococcus* spp: ***Enterococcus faecalis*** **and** ***E. faecium***

MRSA isolation from pool of nasal animal swabs

10 or 20 swabs

1ml

10µl

1 sample=pool of 10 or 20 nasal swabs from a single farm

300ml MH broth
6.5%NaCl
37°C 18-24h

9 ml TSB
3.5mg/l cefoxitin
75mg/l aztreonam
37°C 18-24h

Brilliance MRSA2 agar
37°C 18-24h

Single suspected colony is confirmed as MRSA by triplex real-time PCR:
- identification of *S. aureus (nuc)*
- detection of *mecA*
- detection of *mecC*

AST (EUST)
AND
Genotyping (clonal complex and spa-type)

Isolation of Enterococci from faeces or caecal animal samples

Faeces:
- Breeding hens
- Laying hens
- Veals
- Pigs

Dip a swab into faeces→**2 ml BPW**

Caecal samples:
- Broilers
- Turkey

Part the caecals with a sisser→**10 ml BPW**

Directly

Streak 2 loops (2x10µl) on **Slanetz-Bartley agar**

41.5°C 48h

Streak a single colony of presumptive Enterococcus spp. on **Columbia Sheep Blood Agar**

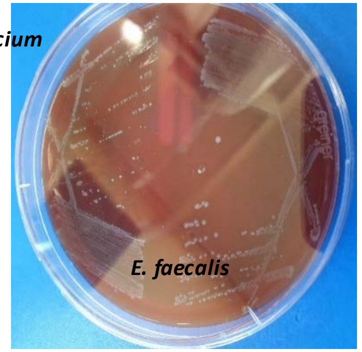
37°C 24h

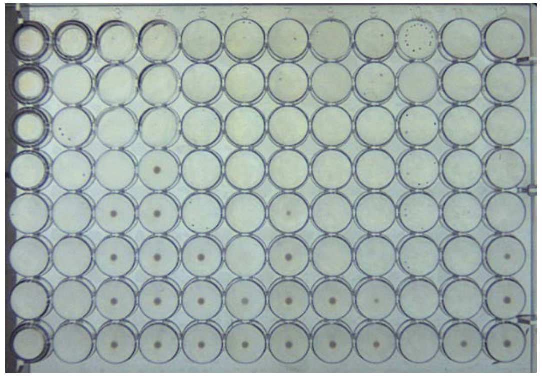
Identification by **MALDI-TOF**

Enterococcus faecalis or faecium

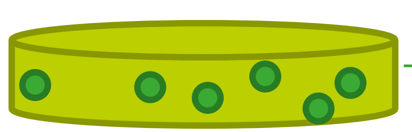
AST (EUVENC) **for 1 E. faecalis and 1 faecium isolate/sample**

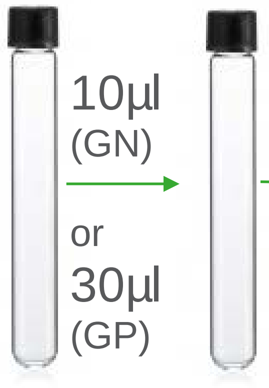
Other bacteria/Enterococcus spp.→ no additional analyses





Workflow AST: Preparation of bacterial suspension



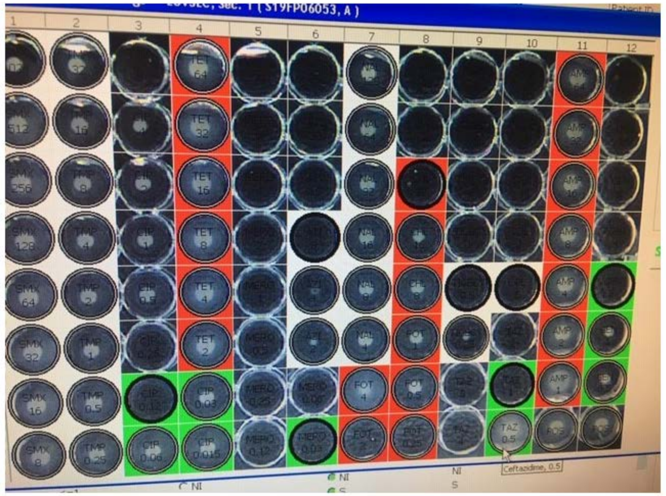


10µl (GN)

or

30µl (GP)

50µl



Sterile physiological water (0.5McFarland)

11ml cation-adjusted Mueller-Hinton broth with TES

96 well plate (18-24h at 35°C)

Lecture Vizion

OFFICIAL MONITORING OF MRSA

KEY RESULTS

Prevalence of MRSA in animals –
3-year rotation: poultry/cattle/pig since 2011

Genotyping of MRSA (CC398/spa typing): LA-MRSA or HA/CA-MRSA?

2 examples of HA/CA MRSA found in animals in Belgium:

- **HA-MRSA ST239/t037** in **poultry**:
    - 3 strains in 2011
    - 5 strains in 2014 (ST untyped)
    - 1 strain in 2017

        → clonal? Investigation to perform by WGS

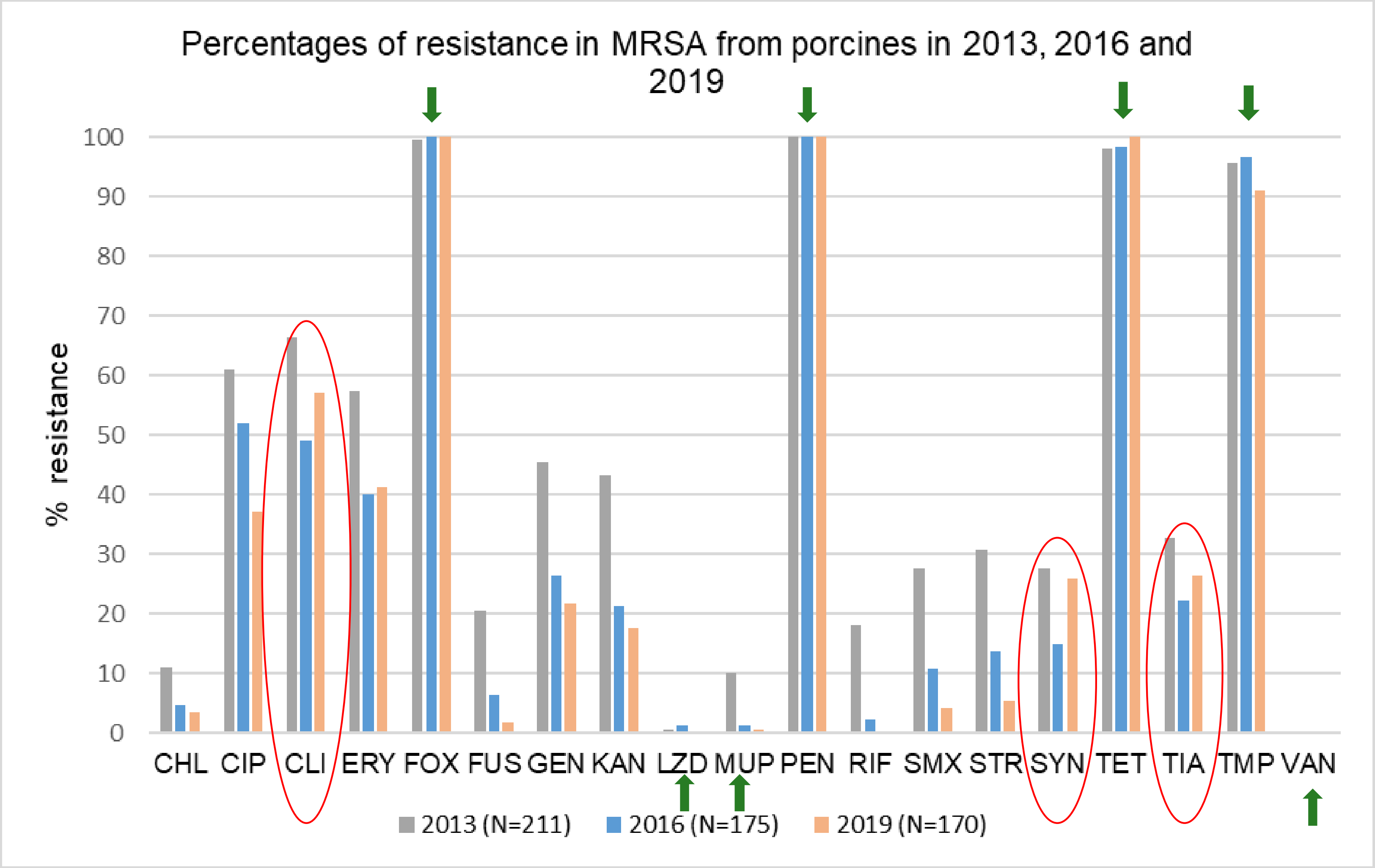
- **HA/CA-MRSA t223** in **bovine**:
    - 6 strains in 2018 (bovine for meat+dairy cows), not reported earlier in cattle in Belgium)

        → 1 of them was WGS :

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

        → Hypothesis: human strain which acquired methicillin resistance in animals

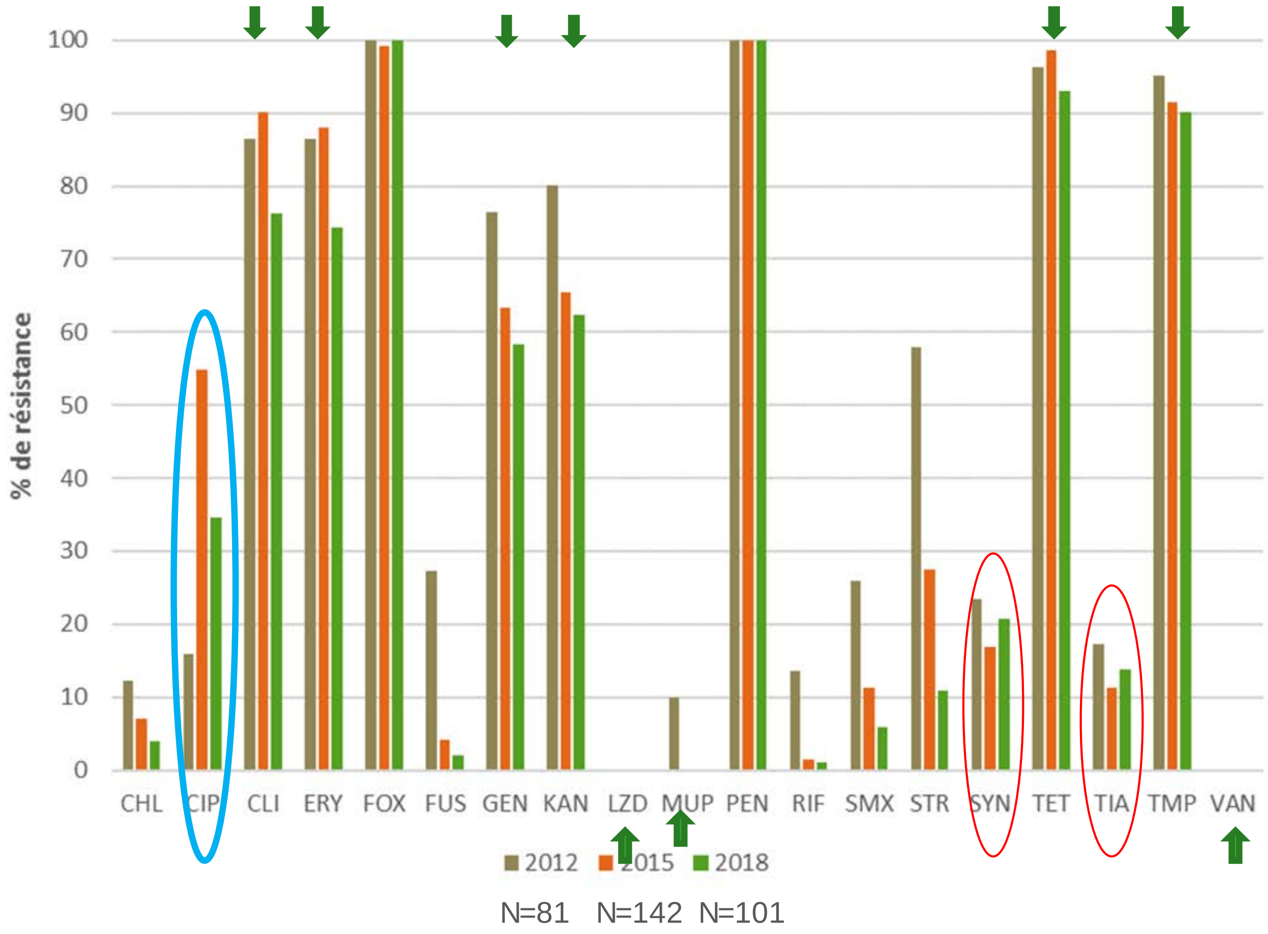
Percentages of resistance in MRSA from porcines: 2013-2016-2019



- PEN + FOX: expected R for MRSA
- TET: R typical of LA-MRSA
- TMP: R extremely high (>70%)
- VAN: No R
- LZD: R very low through the non-selective monitoring
- MUP: low R
- Point of attention:
  - CLI: very high R
  - SYN, TIA, CLI: % 2019>2016

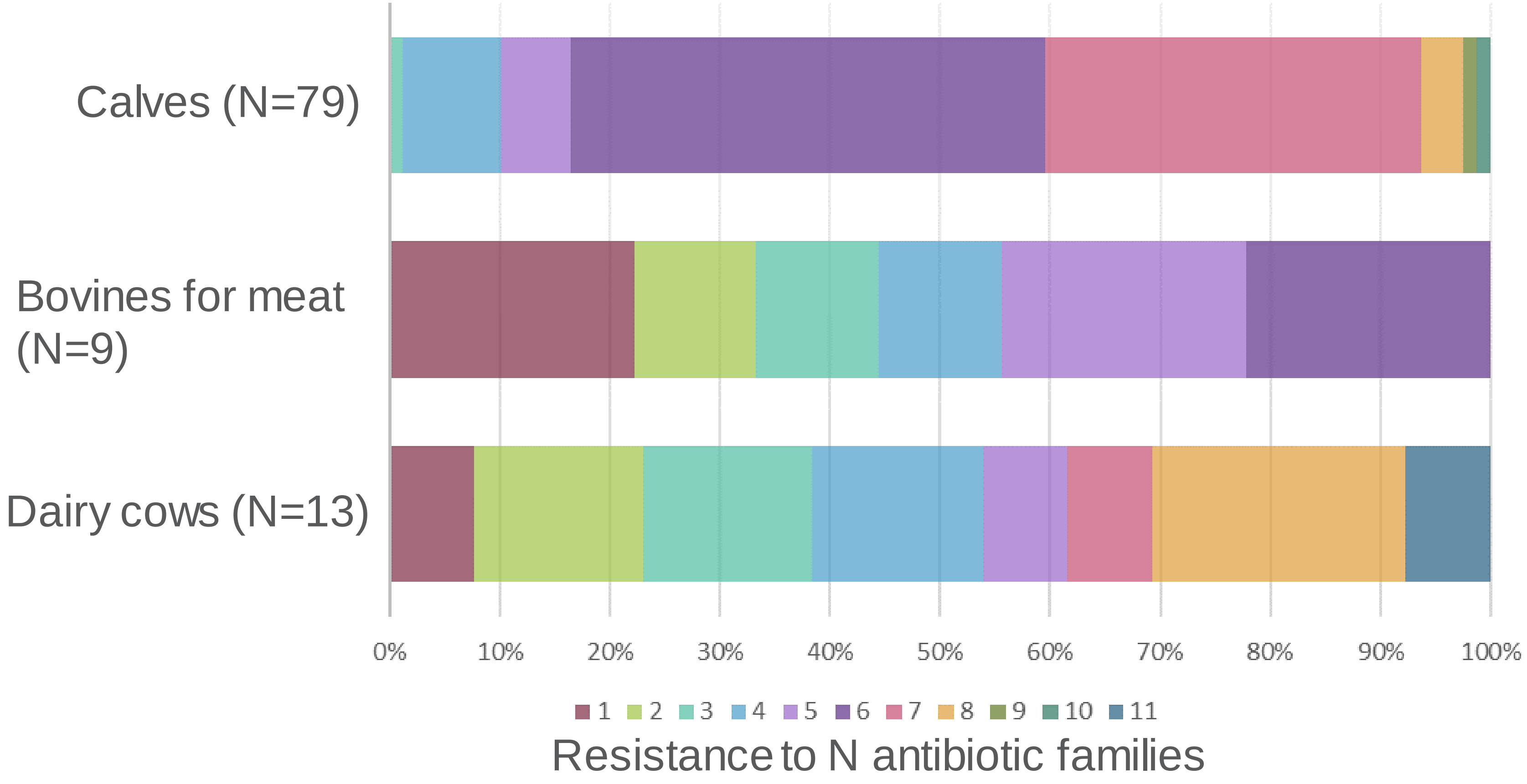
  → Cross resistance?
  under investigation through gene analysis

Percentages of resistance in MRSA from bovines: 2012-2015-2018



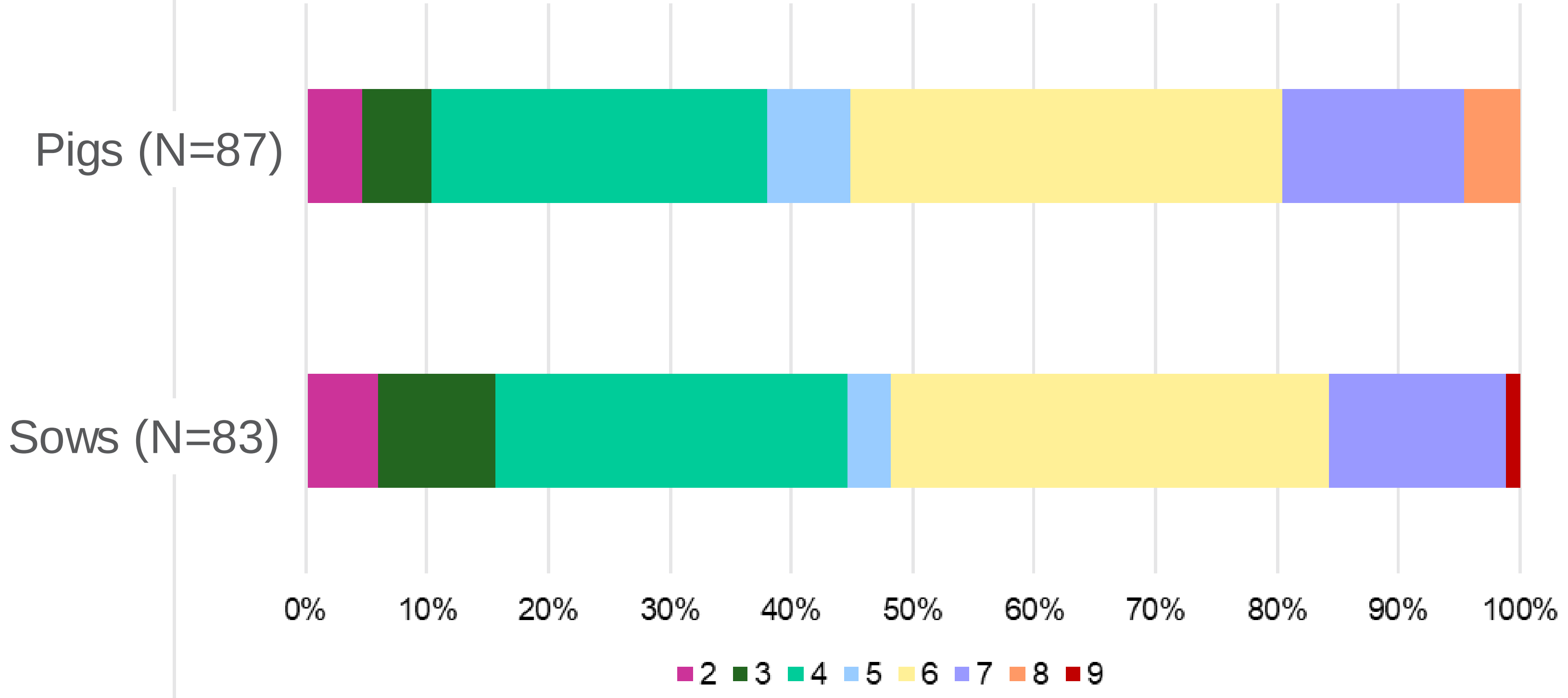
- No VAN R and LZD R
- No MUP R since 2015
- Globally: diminution in 2018 vs. 2015/2012
  except for TIA and SYN
- CIP: increased in 2015 then decreased in 2018
- R extremely high (>70%)
in 2018 for CLI, ERY, TET,TMP
- R very high (50-70%) for GEN & KAN

MDR in MRSA isolated in 2018 from bovines



MDR if ≥ 3
76.9% dairy cows
66.7% bovines for meat
100% calves

MDR in MRSA isolated in 2019 from porcines



Resistance to N antibiotic families

MDR if ≥ 3
95.4% pigs
94.0% sows

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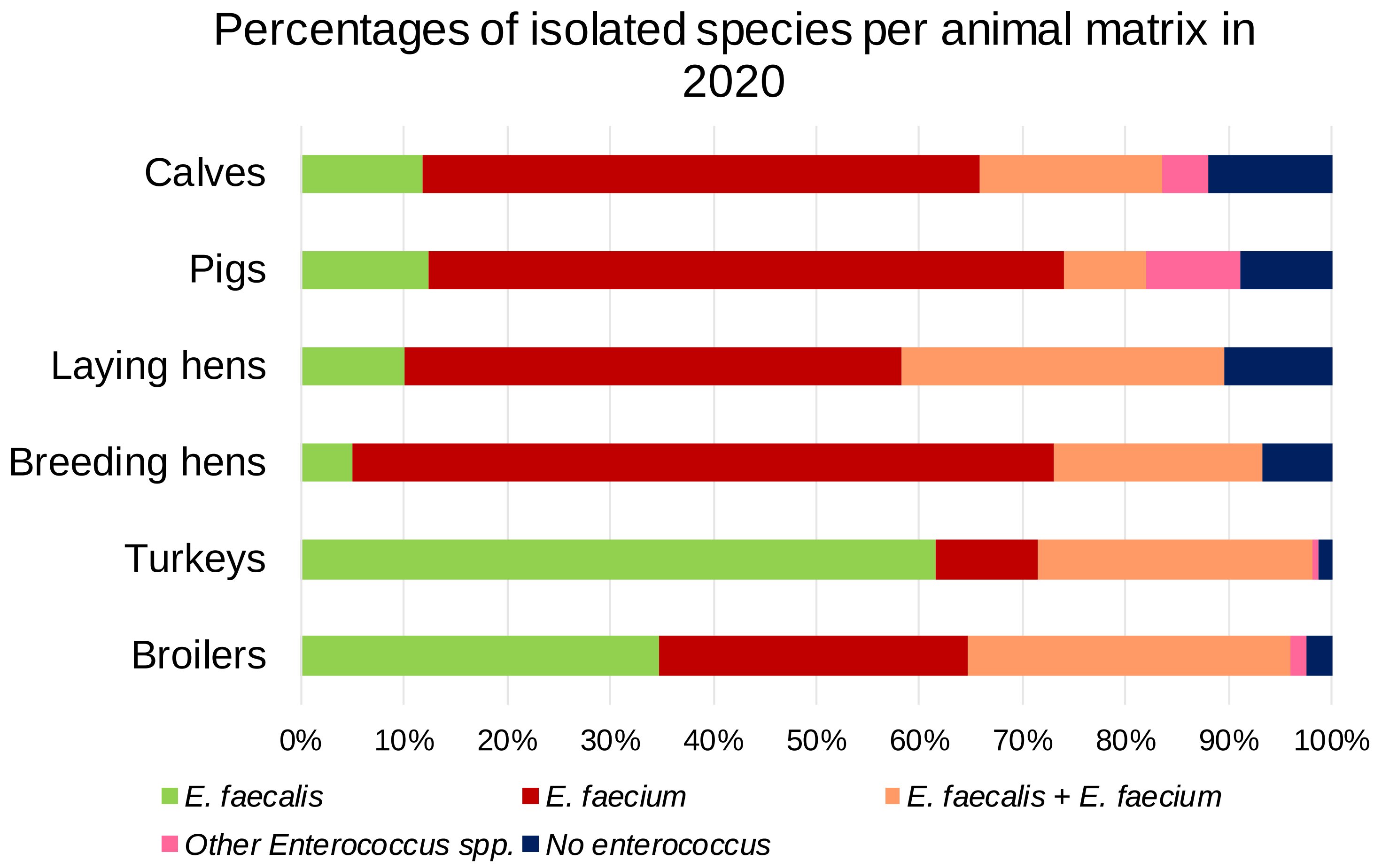
OFFICIAL MONITORING OF ENTEROCOCCI

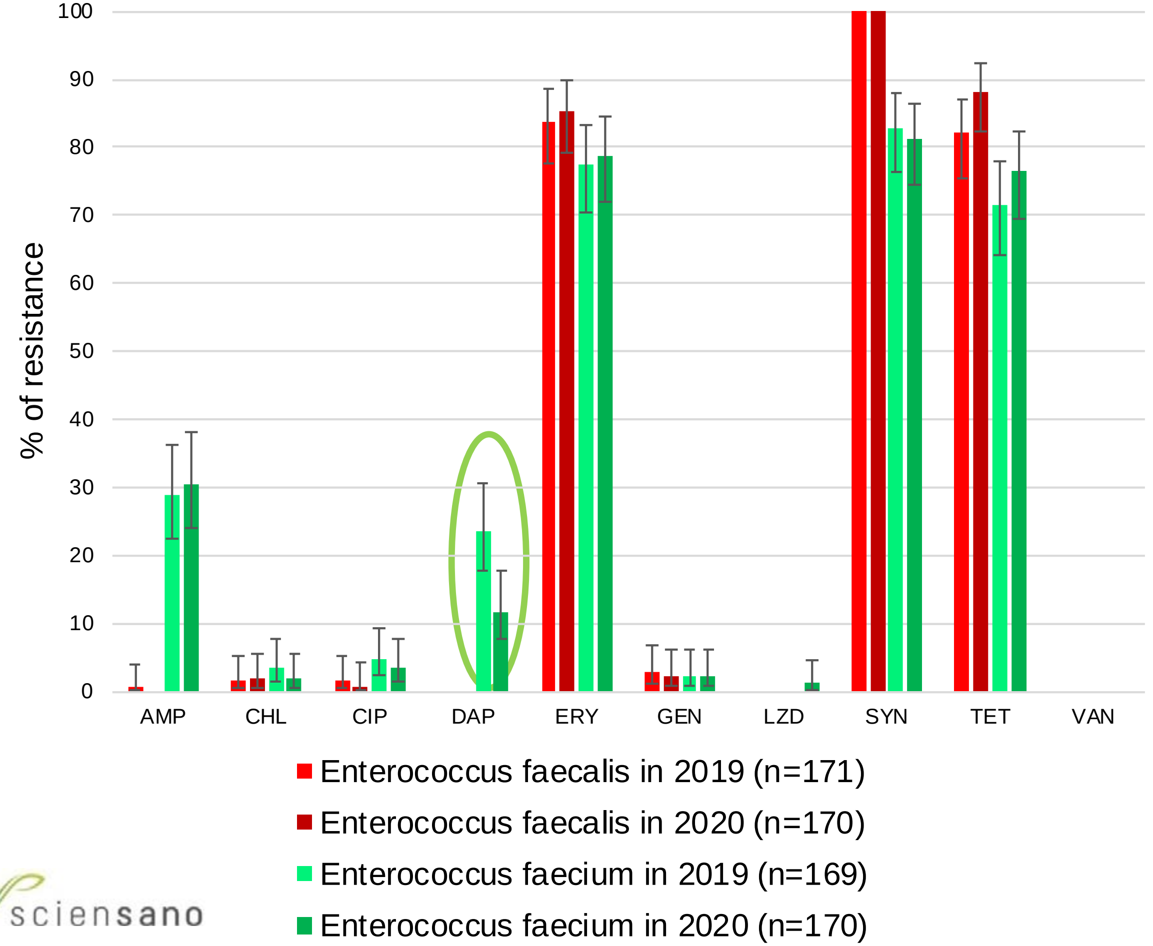
KEY RESULTS

E. Faecalis and *E. faecium* isolated from different animals

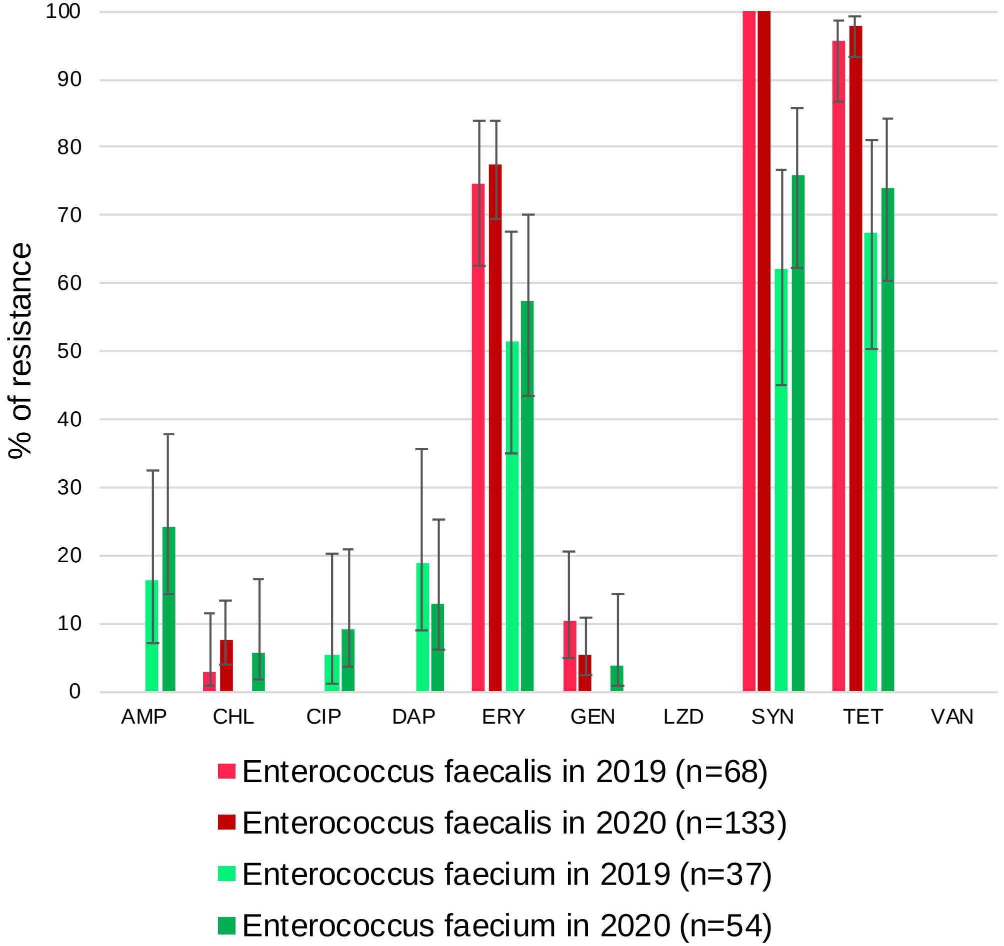


AMR in enterococci from broilers and turkeys in 2019/2020

Broilers



Turkeys



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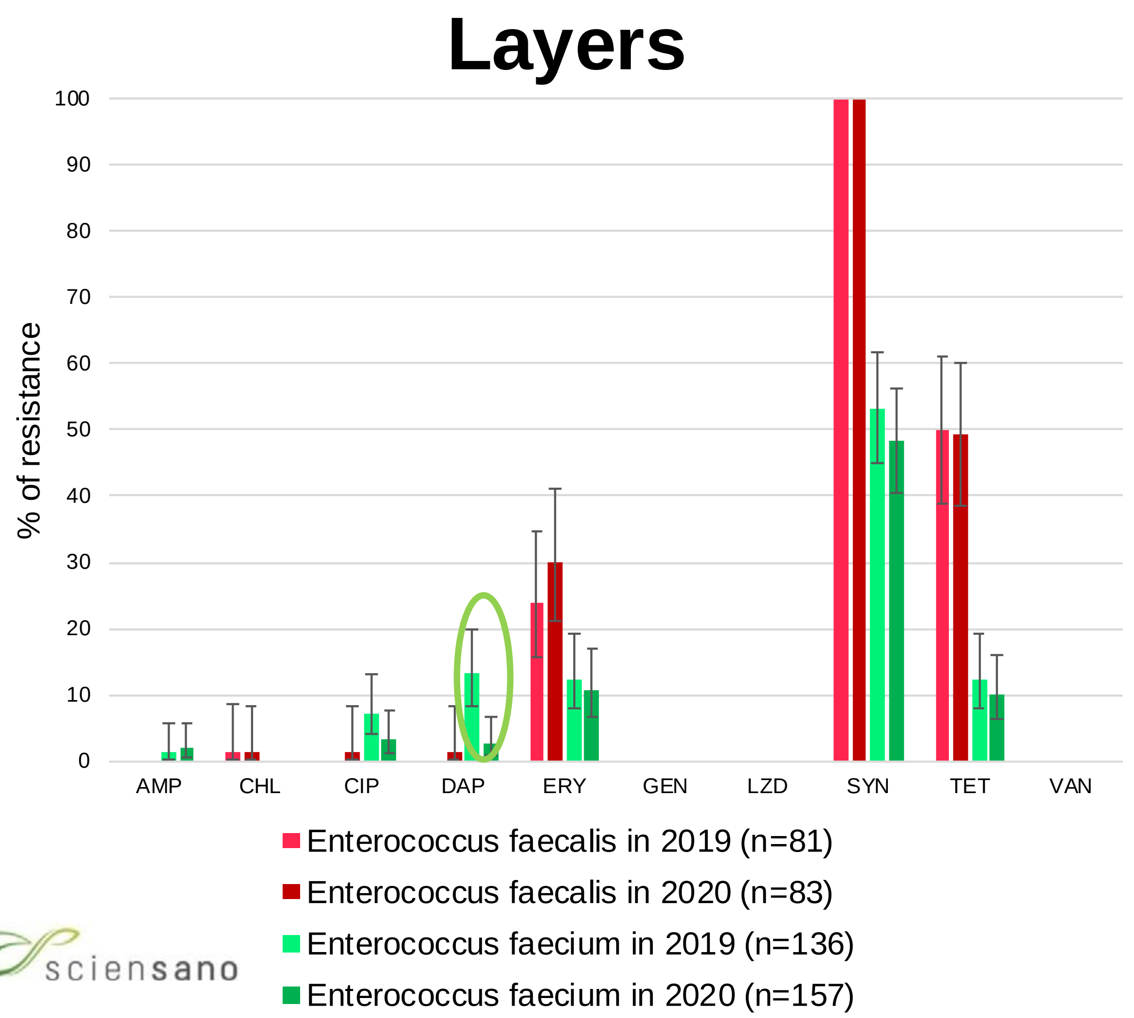
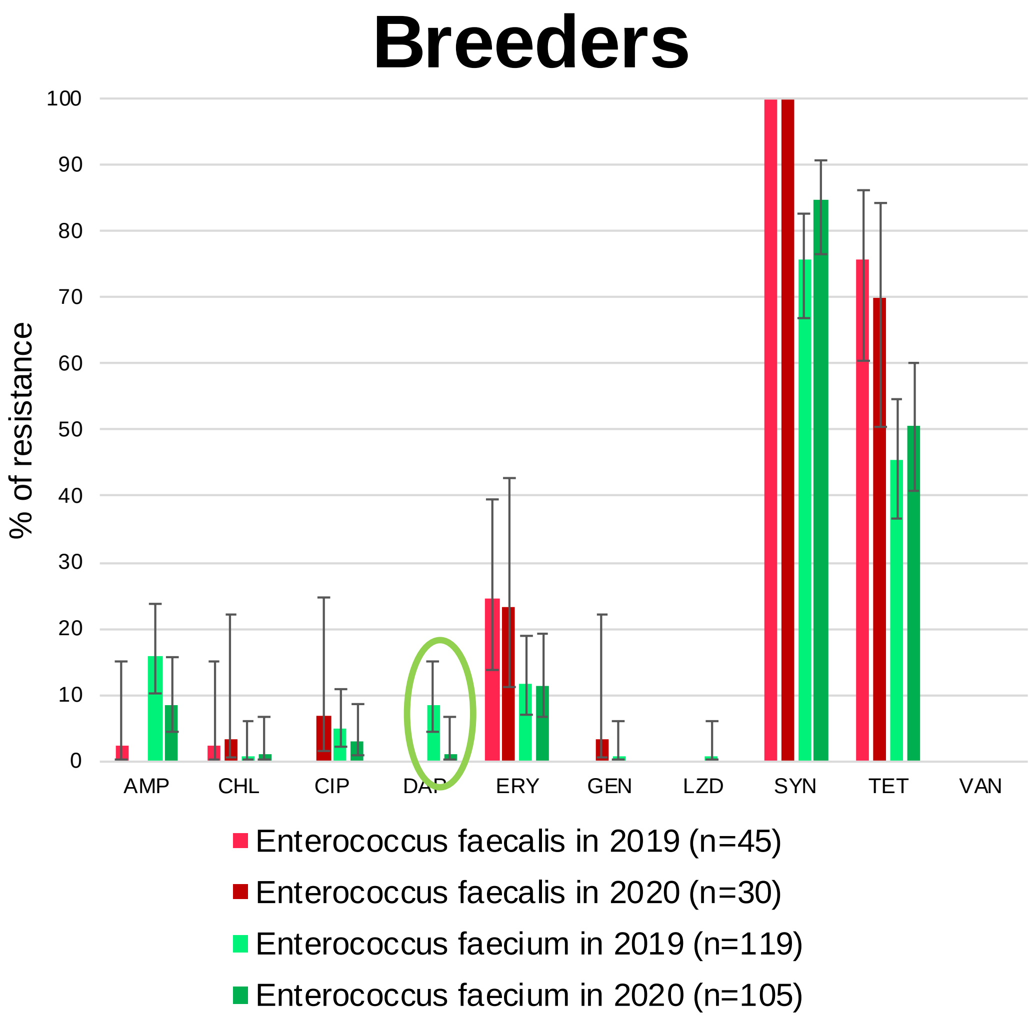
AMR in enterococci from layers and breeders in 2019/2020





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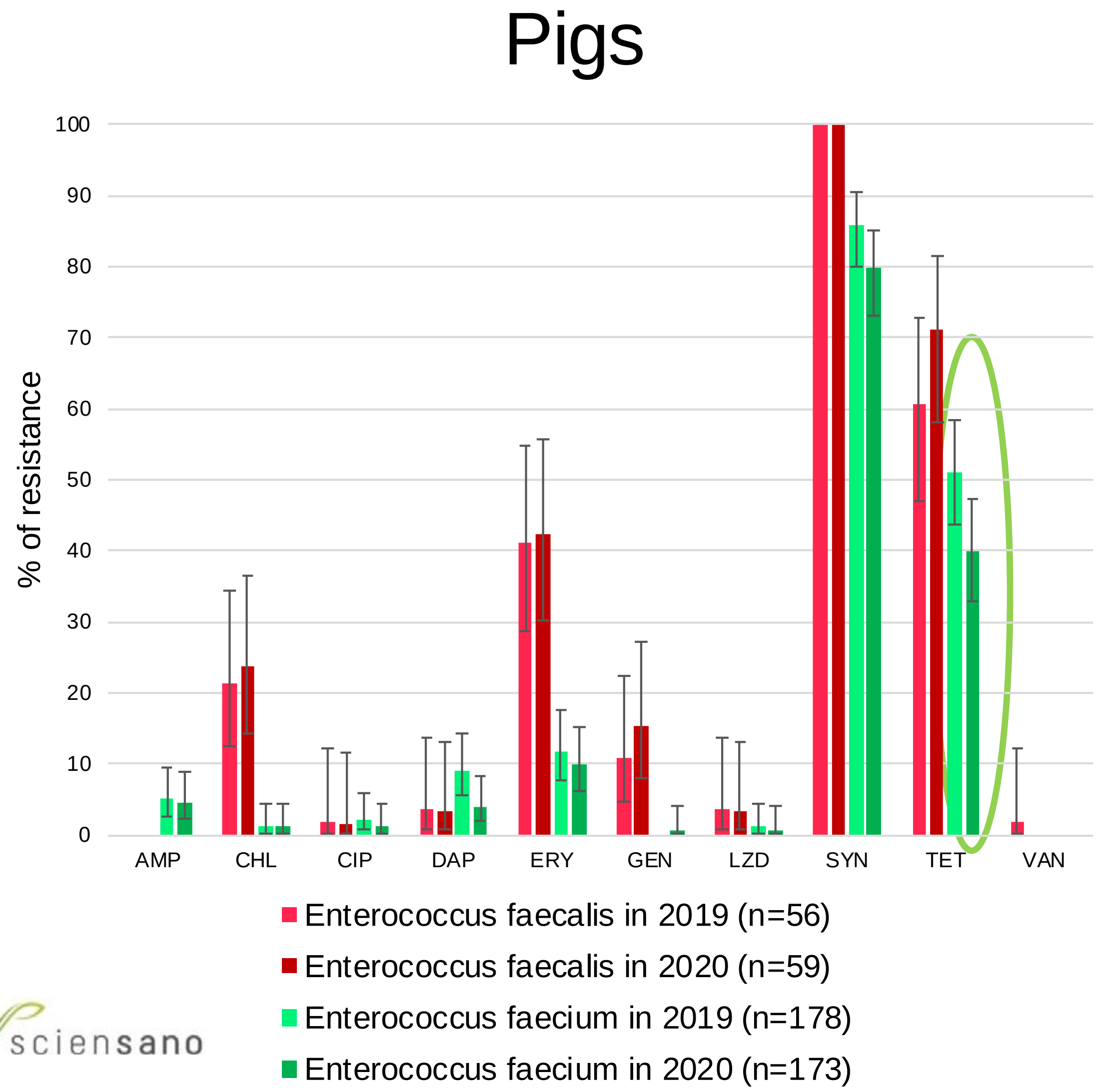
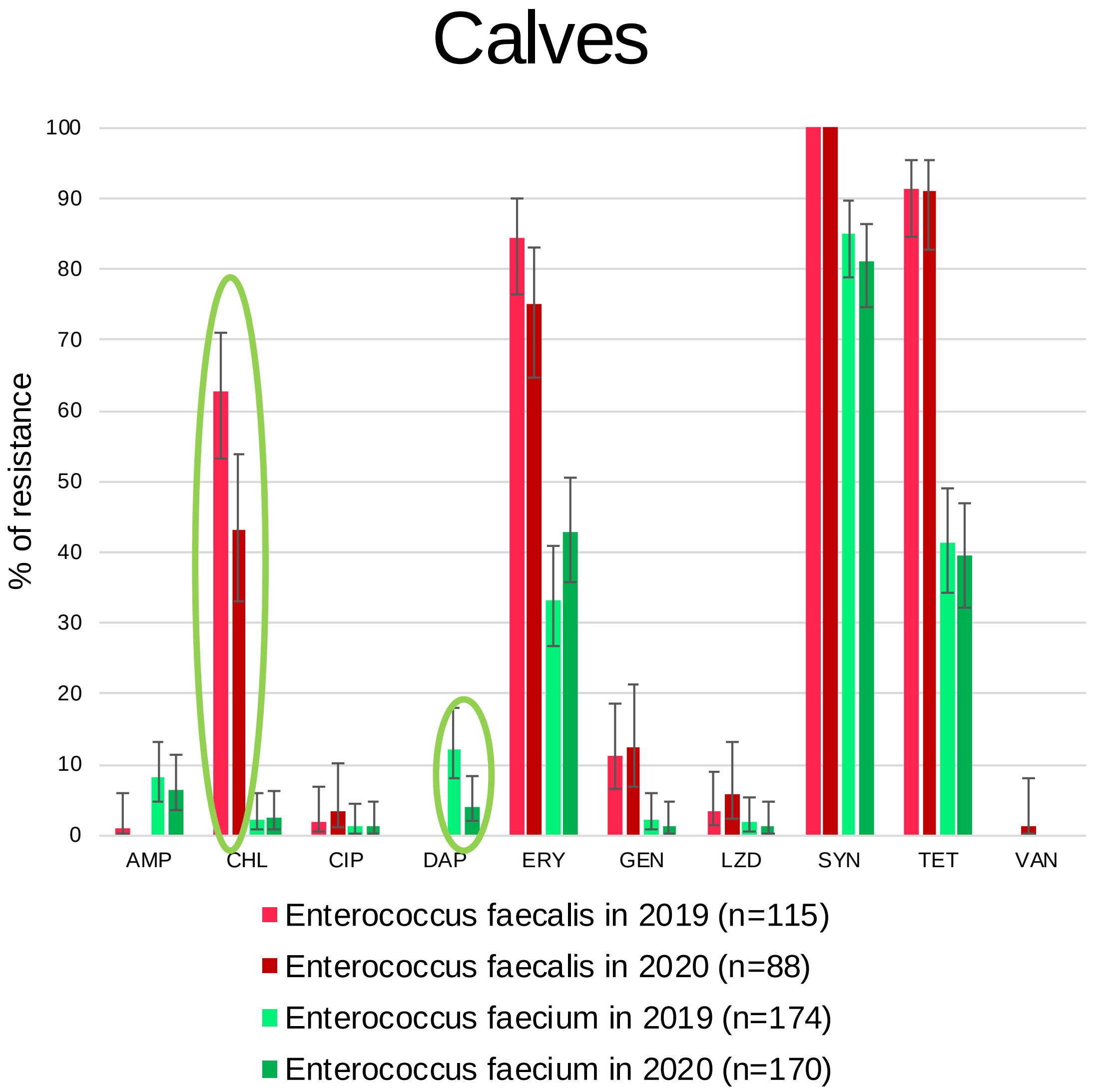
AMR in enterococci from pigs and calves in 2019/2020





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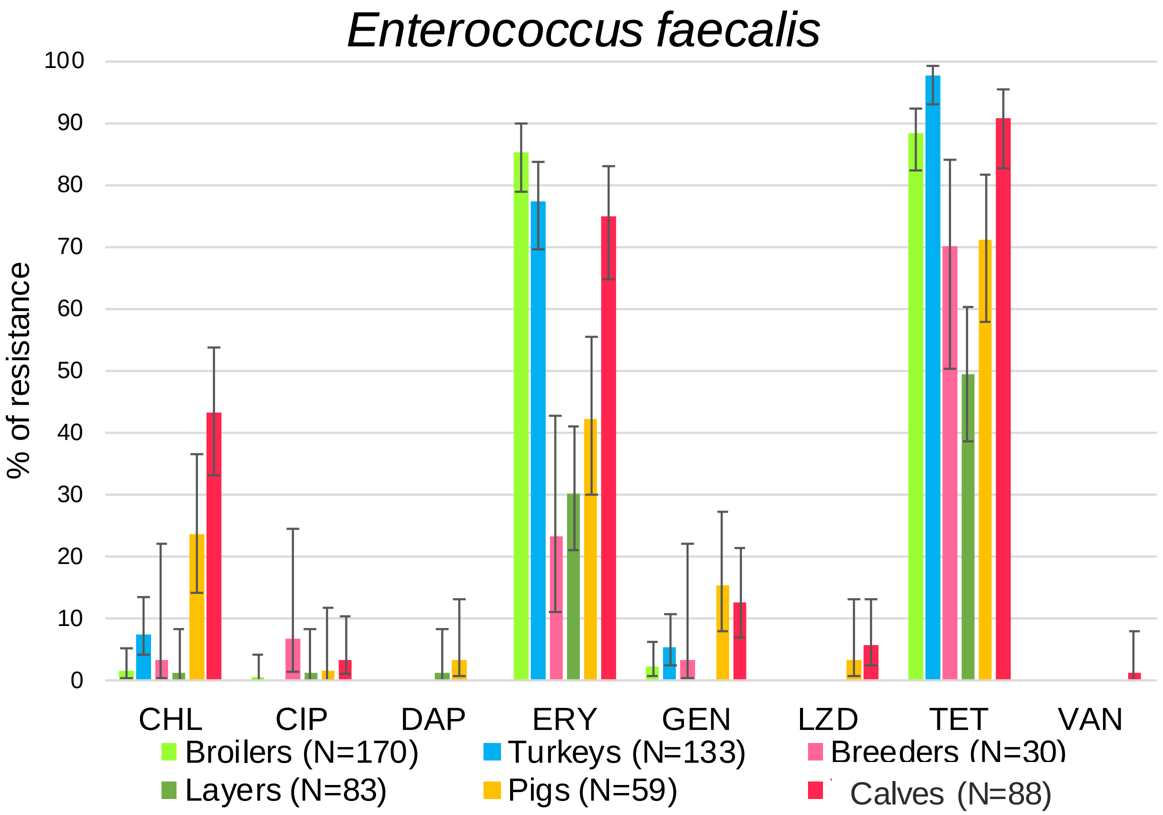
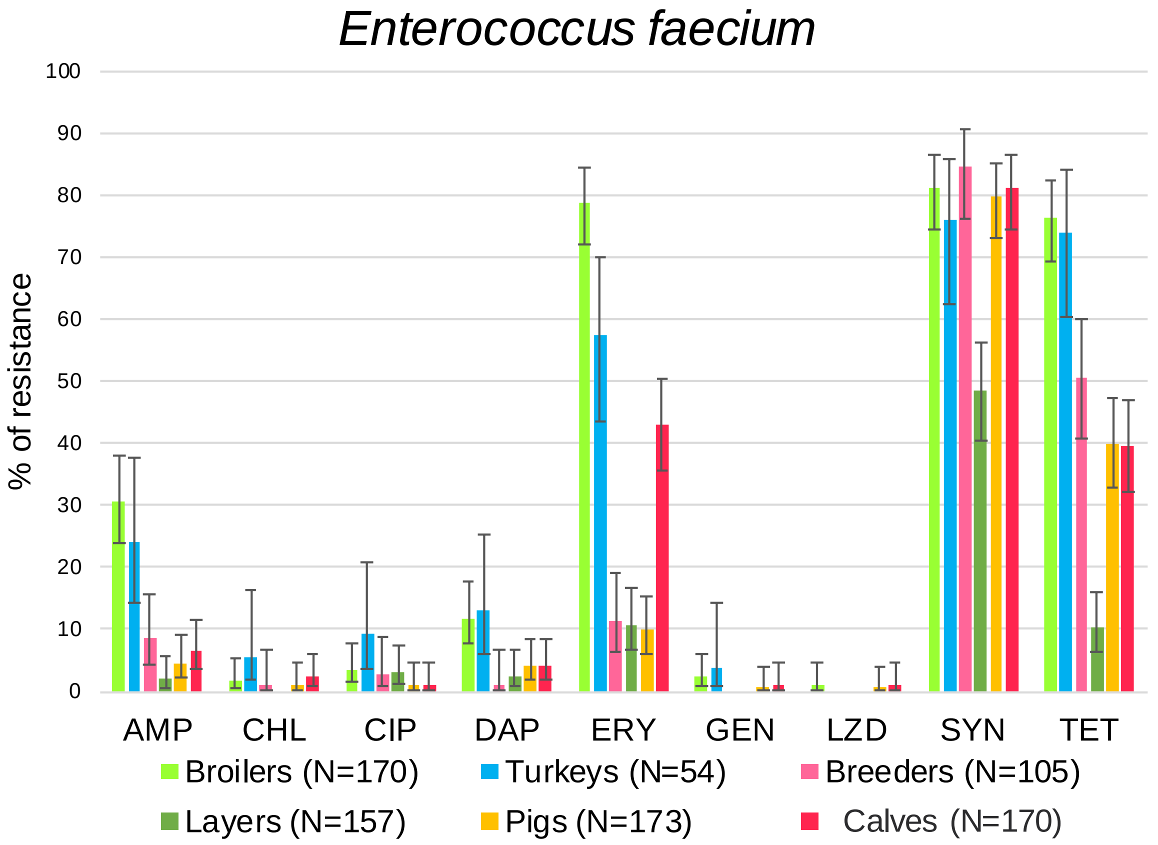
Overview of AMR in *E. faecalis* and *E. faecium* in 2020 for all animal matrices





- No ampicillin resistance in *E. faecalis* in 2020
- *E. Faecalis* intrinsically resistant to Q/D (SYN)

No vancomycin resistance *in E. faecium* in 2020

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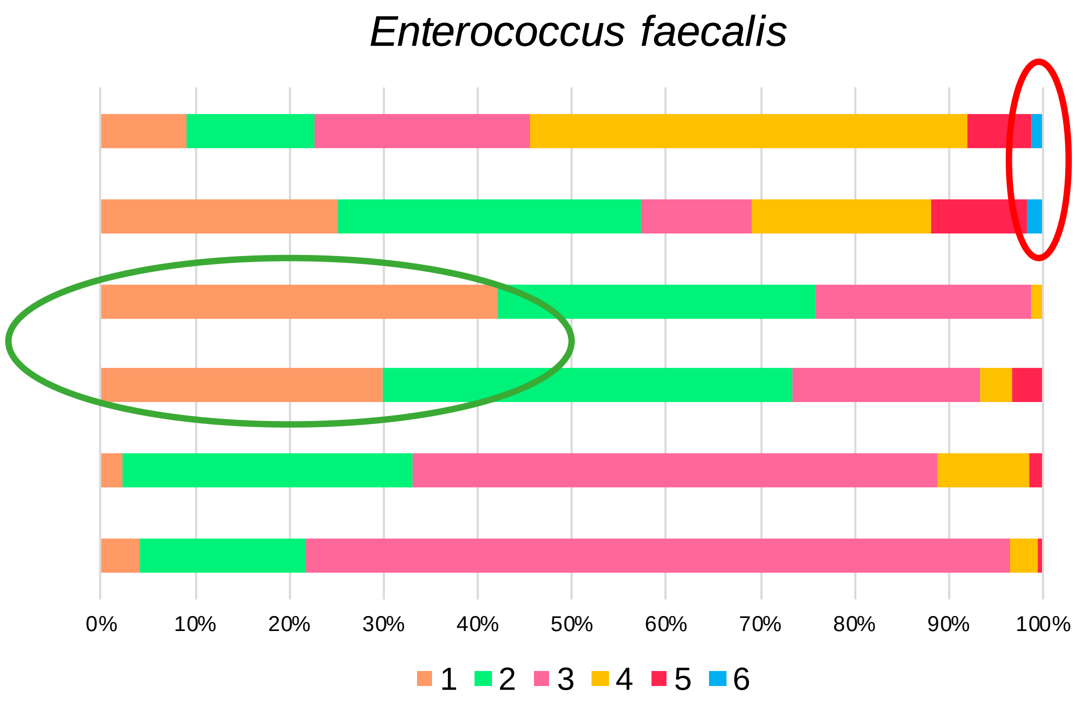
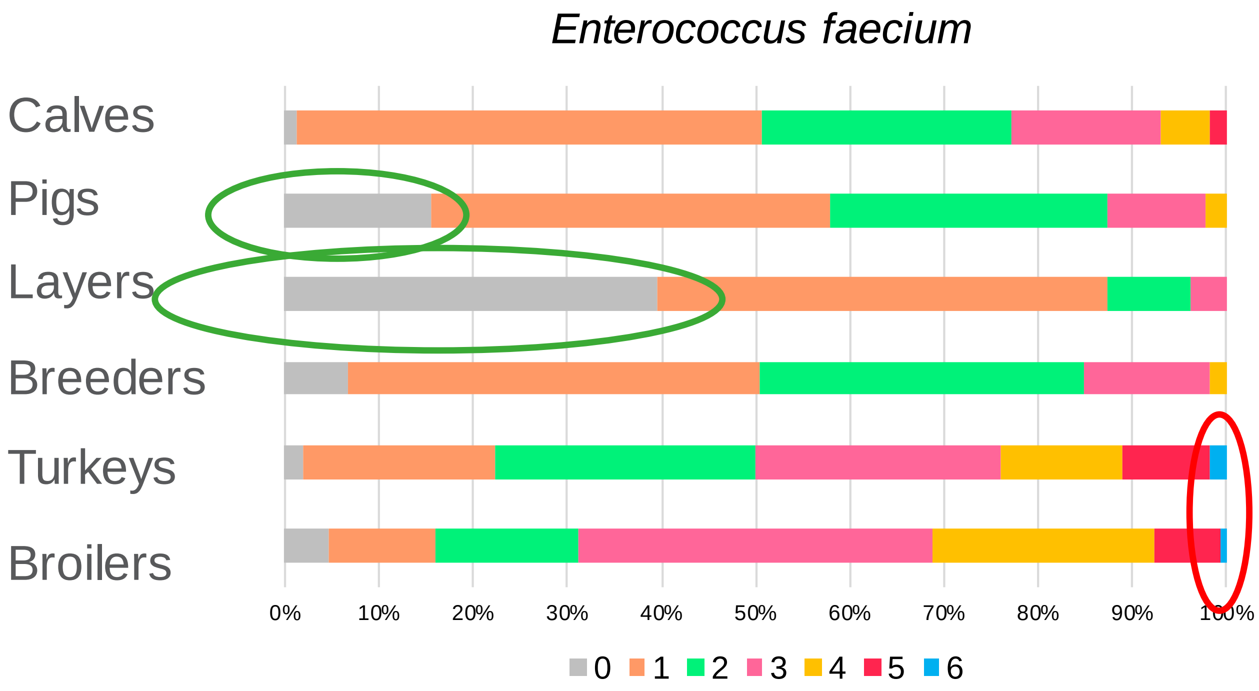
MDR in *E. faecalis and E. faecium* in 2020 in different animal categories





N≥1: *E. Faecalis* intrinsically resistant to Q/D (SYN)

E. faecalis:

- MDR (if ≥ 3) mainly in:
  - **Broilers** 78.2%
  - Calves 78.2%
  - **Turkeys** 66.9%

- In general N R faecium < faecalis Except in turkeys and broilers
- MDR (if ≥ 3) mainly in:
  - **Broilers** 68.8%
  - **Turkeys** 51.0%

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LINEZOLID SELECTIVE MONITORING

OH-EJP Projet “LIN-RES”: Molecular Basis, Origin, Transferability and Risk Factors Associated with Linezolid-Resistance in Gram-Positive Bacteria of Human and Animal Origin.

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https://onehealthejp.eu/lin-res/

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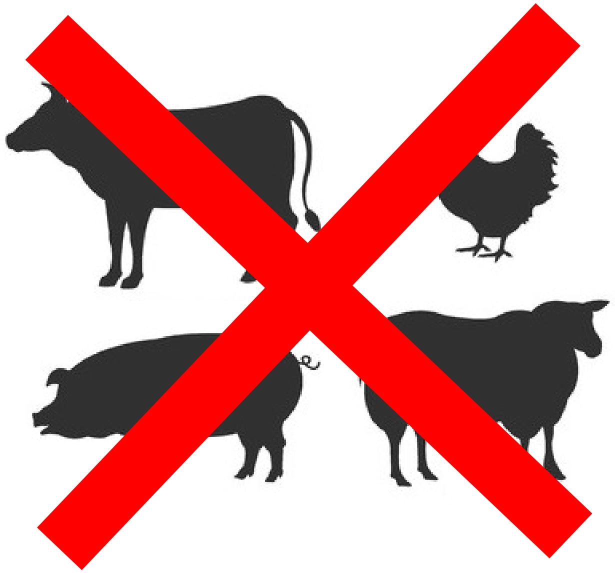
LINEZOLID: context

- Linezolid (LZD) is a critical antibiotic to treat important human infections (MRSA, VRE)

- Linezolid use in:

Not licensed for common animal use



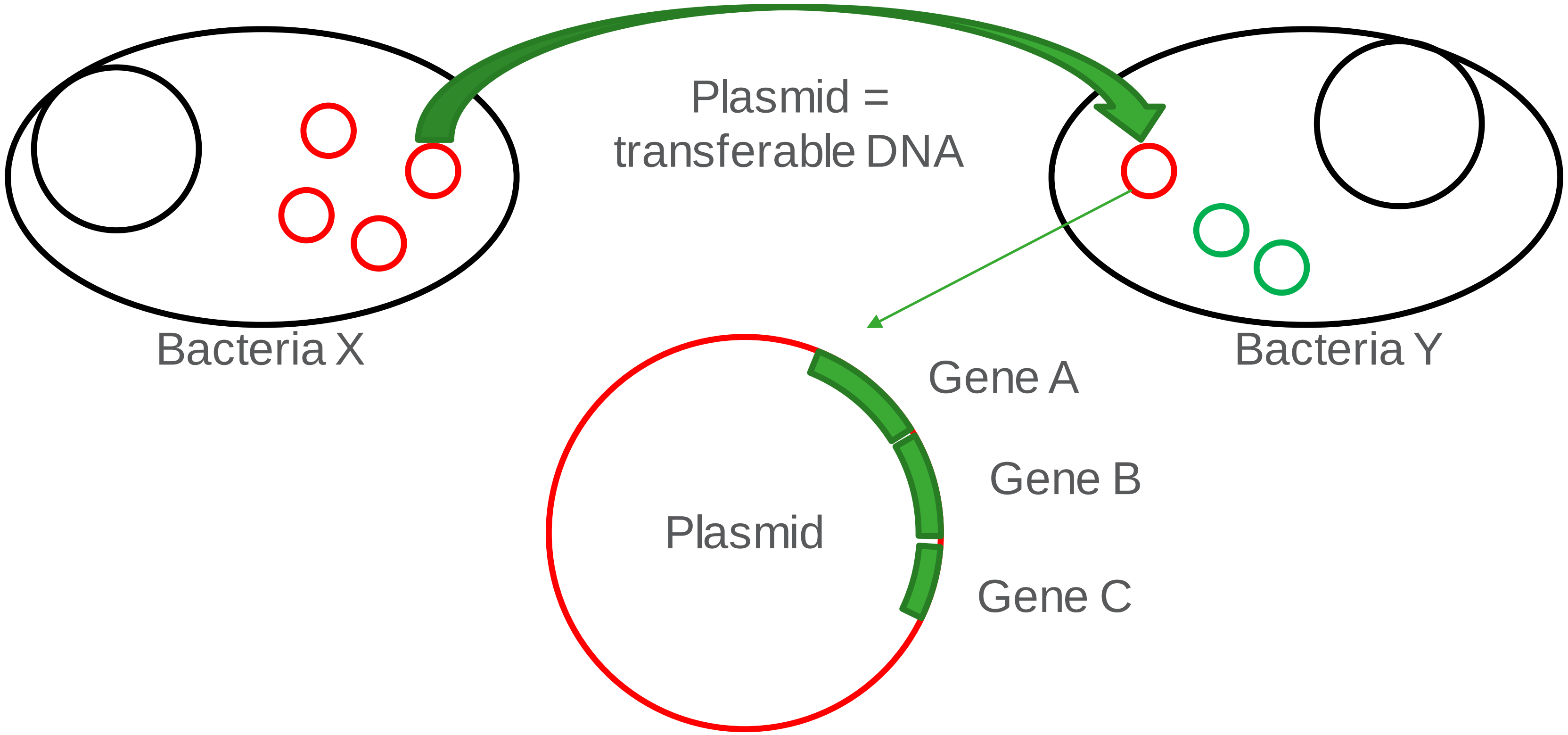
Only exceptional individual cases

LINEZOLID: context

- Resistance against linezolid found in bacteria isolated from:

LINEZOLID: context

- Two types of resistance to linezolid:
  - Mutations ➔ not transferable
  - Plasmid borne genes ➔ transferable
- Resistance to linezolid can be transferred between bacteria



➔ Bacteria are able to share genes

LINEZOLID: context

Known resistance determinants conferring resistance to linezolid

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

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LINEZOLID: context

Known resistance determinant conferring resistance to linezolid

Co/cross-selection of linezolid resistance
through
the use of non-critical antibiotics?

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

LINEZOLID: context

- Resistance to linezolid found in bacteria isolated from:

AIMS →

Occurrence of resistance (%) ?

Genetic context?

Consequences ?

Linezolid selective monitoring: methodology

- 2 main groups of bacteria: Gram-negative and Gram-positive
  - Linezolid ➔ Gram-negative ✗
  - Linezolid ➔ Gram-positive ✓

- **Selective isolation on medium supplemented with 4mg/L linezolid** of Gram-positive bacteria from samples collected through the official monitoring of MRSA (nasal swabs) and Enterococci (faecal samples) in 2019

- Growing bacteria in presence of linezolid (after 48h of incubation) ?
  - Yes ➔ resistance ➔ isolation, identification, broth micro-dilution (during 24h **and** 48h) and whole genome sequencing (WGS)
  - No ➔ no further analysis

Linezolid selective monitoring: results

- Percentage of linezolid resistance in 2019 with the selective monitoring (medium supplemented with linezolid):

Animal origin	Number of samples tested	Number of samples LZD-R	% LZD-R samples
Faecal samples monitoring			
Broilers	295	14	4.7
Turkey	86	0	0.0
Laying Hens	205	2	1.0
Breeding Poultry	163	10	6.1
Veal calves	293	48	16.4
Pigs	283	31	11.0

Animal origin	Number of samples tested	Number of samples LZD-R	% LZD-R samples
Nasal swabs samples monitoring			
Sows	78	16	20.5
Fattening pigs	70	18	25.7
TOTAL	148	34	23.0

LZD-R enterococci in nasal swabs

Depending on the animal origin, the percentage of resistance varied between 0% (turkey) and 25.7% (fattening pigs)

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Linezolid selective monitoring: highlights

- The percentage of linezolid resistance varied between the **different animal categories**:
  - between 0% and 6.1% in poultry
  - 16.4% in veal calves
  - between 11.0% and 25.7% in pigs

- **Transferable** linezolid resistance genes found in most isolates (97.3%, 143/147)

- **Putative link with use of florfenicol** given that
  - its use is growing almost every year in animals (BELVETSAC 2020)
  - Most of the isolates carrying other phenicol resistance gene
  - *cfr, optrA* and *poxtA* give cross-resistance to linezolid **and** phenicols

TAKE HOME MESSAGES

Official monitoring: take home messages (1)

MRSA:

- very high **prevalence** in **pigs** (diminution since 2013) and **veal calves**
- Some **HA/CA MRSA** are occasionally found in animals.

MDR:

- In most MRSA from **bovines** and **porcines**
- In most enterococci from **broilers** and **turkeys**

VAN-R:

- not observed in MRSA from porcines/bovines
- occasionally in *E. faecalis* from pigs and calves

LZD-R occasionally found through the official monitoring of

- enterococci from calves, pigs, broilers and breeders
- MRSA from porcines

MUP-R: occasionally found in MRSA from porcines, not observed in MRSA from bovines since 2015

DAP-R in enterococci→ keep attention on this.

Official monitoring: take home messages (2)

Raw evolution of AMR:

- **in MRSA from porcines and bovines**:
  - raw percentages of resistance tend to decrease for several antibiotics but not for all
  - TIA and SYN R tend to increase in both porcine and bovine MRSA
- **in enterococci:**
  - Only 2 years in a row→ **need more sampling points to draw trends analysis**
  - Only a few (decreasing) significant differences observed between 2019 and 2020

→ Future trends analysis should bring more details

Linezolid selective monitoring: take home messages

- The linezolid resistance is critical for human health and is already **well established** within the animal bacteria
- The genes responsible for linezolid resistance give resistance to other antibiotics and **cross-selection** will maintain these genes
- A **linezolid selective monitoring,** as conducted during this project, would be recommended to carefully monitor this resistance in animals
- The understanding of the **mechanisms** and the **spread** of the resistance is also important
- It is important to continue to promote a prudent use of ALL antibiotics

Thank you for your attention and...

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These results were obtained thanks to the current team and our predecessors and to the FASFC and OH-EJP :

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