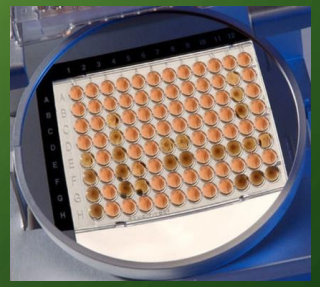
OFFICIAL SURVEILLANCE ON AMR BY CLASSICAL AND MOLECULAR METHODS IN LUXEMBOURG

M. MEO[1], D. CLAUDE[2], M. PERRIN[1], S. LOSCH[2]



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LNS LUXEMBOURG

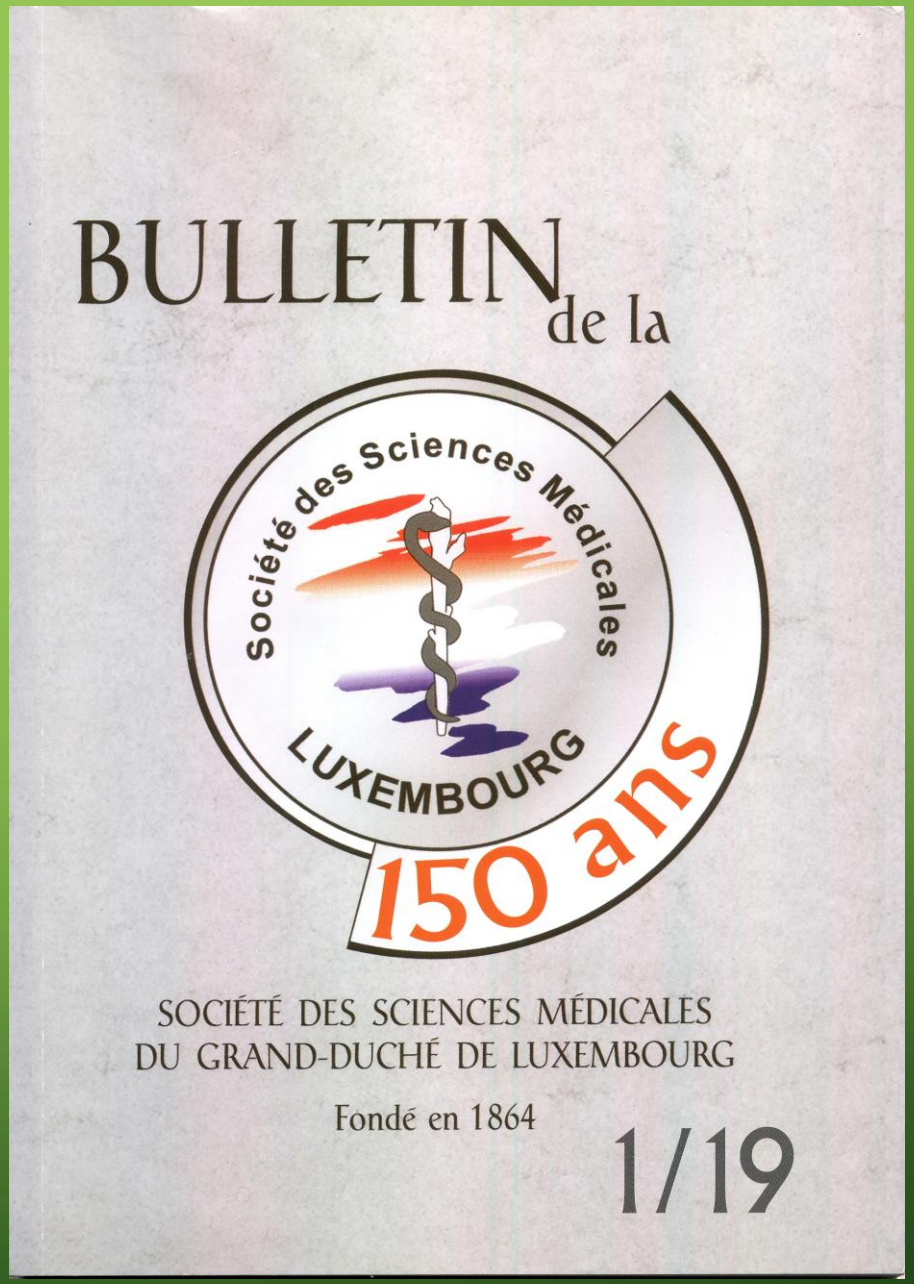
LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture,
de la Viticulture et du
Développement rural

Administration des services vétérinaires

Laboratoire de
Médecine Vétérinaire de l'Etat

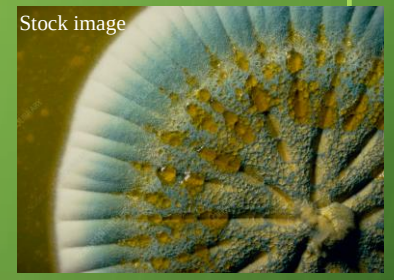
Data published:

Meo M., Claude D., Perrin M., Losch S.
2019, Bulletin de la Société des Sciences médicales1/19, p.17-38



OVERVIEW

- Introduction
- Material and Methods
- Target bacteria
- Results
- Discussion
- Conclusions



PLAN NATIONAL ANTIBIOTIQUES
ONE HEALTH

MATERIAL & METHODS : SAMPLES

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LNS LUXEMBOURG

Food:

- Any pure meat from beef, pork & poultry
- Sampling by official vets
- Bias by EU program (Decision 2013/652/EU)

Animals:

- Feces, organs, milk
- Decision 2013/652/EU
- Clinical or necropsy samples
- Sampling by practitioners or official vets

Clinical human samples:

- European surveillance of gastroenteric pathogens
- Collection by clinical labs → LNS
- ESBL transfer voluntarily

MATERIAL & METHODS

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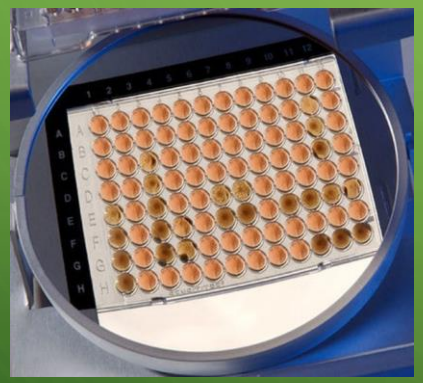
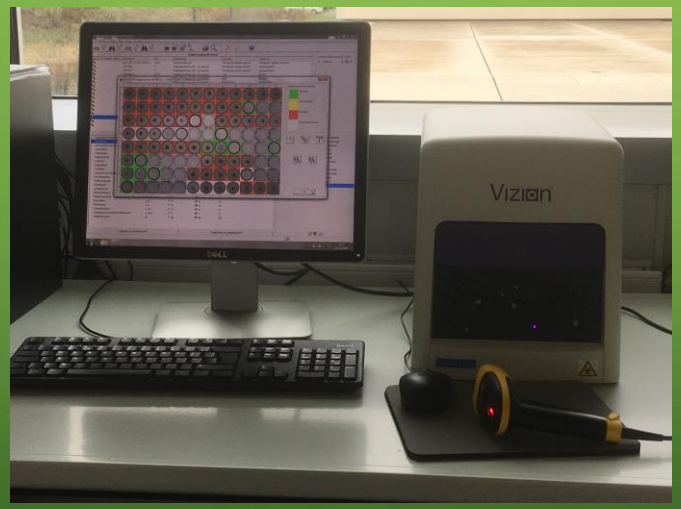
LNS LUXEMBOURG

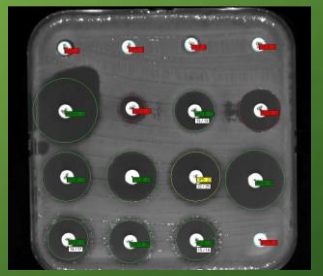
Culture → isolation → antibiogram

id. LMVE + ready strains → abg



Sensititre ®





Interpretation: CLSI, SFM, EUCAST

EUCAST

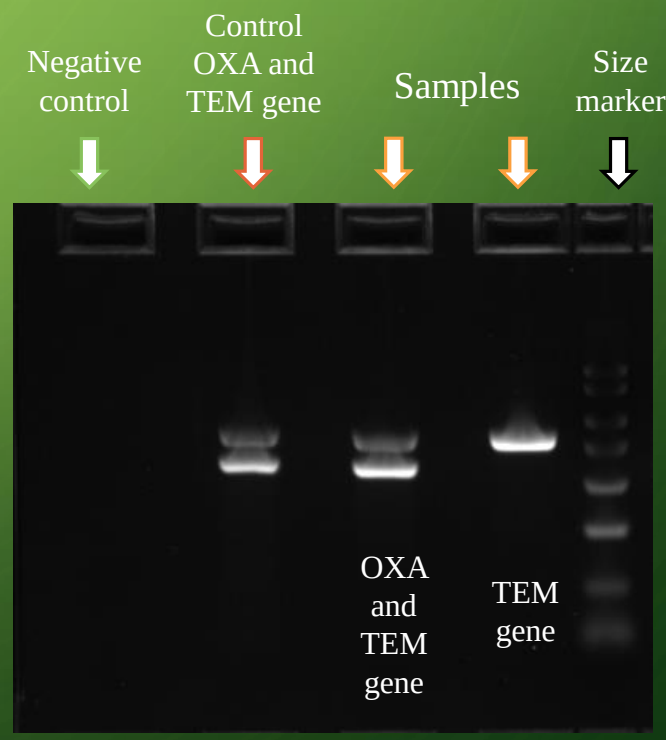
MATERIAL & METHODS

- Sample collection by clinical labs transfer to LNS for ESBL typing
- By multiplex PCR from Dallenne *et al*. and comparison of PCR product migration on agarose gel.
- wgs

Culture

DNA extraction

PCR : ESBL gene research and amplification



Molecular methods for ESBL

TARGET BACTERIA (NAP)

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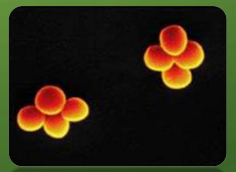
LNS LUXEMBOURG

Isolates from stool (LNS)
food / faeces or organs (LMVE)

- *Campylobacter jejuni & coli*
  (→ n°1 food borne disease EU and L: bloody diarrhea, complications)
- *Salmonella*
  (→n°2 food borne disease EU and L: diarrhea…, more or less severe illness)
- *Escherichia coli*
  (friend or enemy; diverse diseases if pathogenic)

- *(Staphylococcus aureus)*
  (→ food poisoning, skin or systemic diseases)



→ *comparison: human vs. non human of these zoonotic agents*

data 2016 – 2018

TARGET BACTERIA "BULLETIN"

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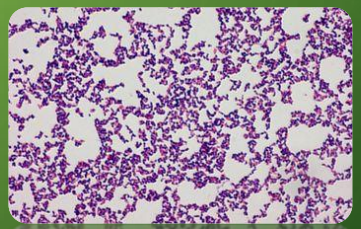
- *Pasteurella multocida*: Zoonotic agent (→ pulmonary diseases) isolated from milk or lungs
- *Trueperella pyogenes*: → suppurative mastitis, pneumonia in ruminants, pigs, horses, poultry and other species,

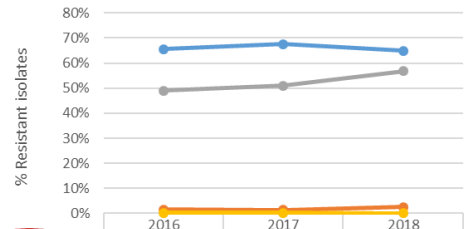


- *Campylobacter hyointestinalis* → proliferative enteritis Isolated from bovine faeces
- *Staphylococcus aureus*: milk vs food
  → skin, udder or systemic diseases, food poisoning

RESULTS: *CAMPYLOBACTER*

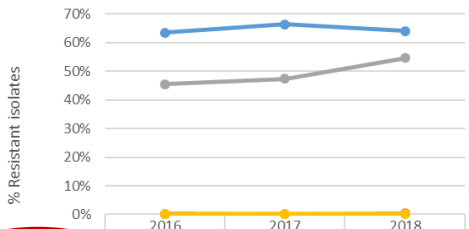

Thrillist.com

Campylobacter: resistance rates in humans



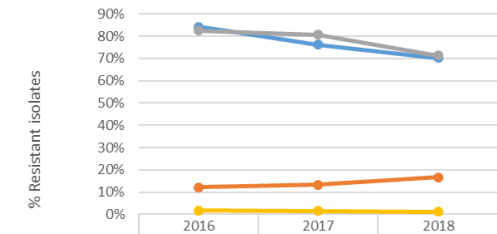
	2016	2017	2018
Ciprofloxacin	65.5%	67.5%	64.9%
Erythromycin	1.5%	1.4%	2.5%
Tetracycline	49.0%	50.9%	56.9%
Gentamicin	0.2%	0.3%	0.2%

C. jejuni : resistance rates



	2016	2017	2018
Ciprofloxacin	63.5%	66.4%	64.1%
Erythromycin	0.2%	0.0%	0.4%
Tetracycline	45.4%	47.3%	54.6%
Gentamicin	0.0%	0.2%	0.0%

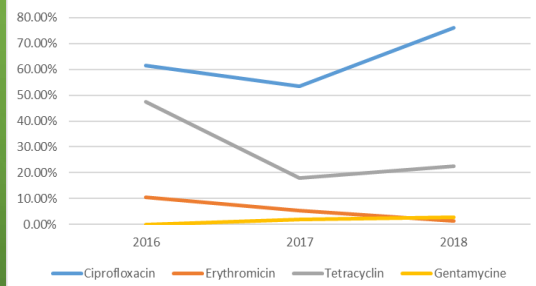
C. coli : resistance rates



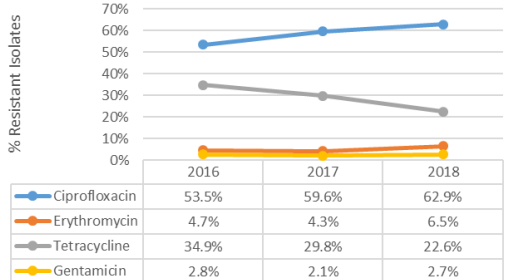
	2016	2017	2018
Ciprofloxacin	84.2%	76.1%	70.2%
Erythromycin	12.3%	13.4%	16.7%
Tetracycline	82.5%	80.6%	71.4%
Gentamicin	1.8%	1.5%	1.2%

1700 strains

Campylobacter: resistance in animals

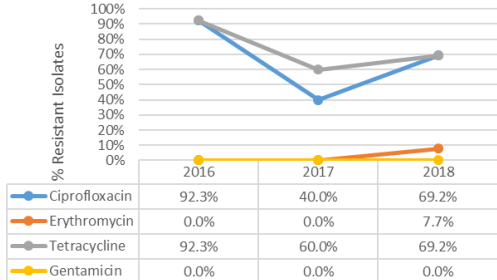


Resistance rates in animal pathology for *C. jejuni*



	2016	2017	2018
Ciprofloxacin	53.5%	59.6%	62.9%
Erythromycin	4.7%	4.3%	6.5%
Tetracycline	34.9%	29.8%	22.6%
Gentamicin	2.8%	2.1%	2.7%

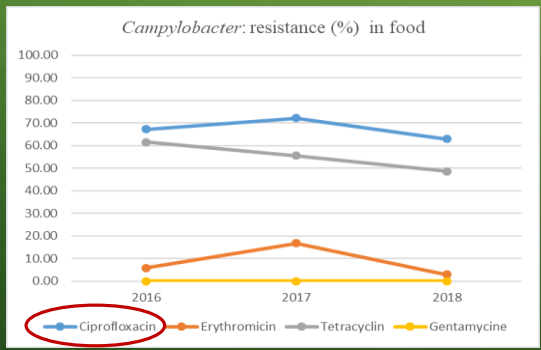
Resistance rates in animal pathology for *C. coli*



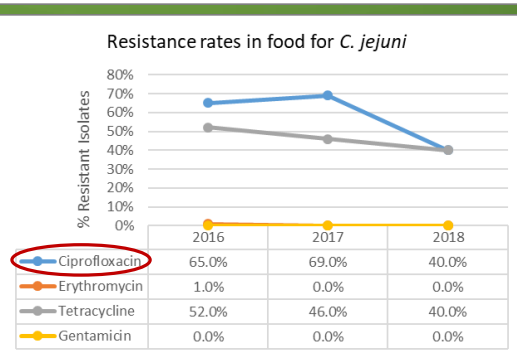
	2016	2017	2018
Ciprofloxacin	92.3%	40.0%	69.2%
Erythromycin	0.0%	0.0%	7.7%
Tetracycline	92.3%	60.0%	69.2%
Gentamicin	0.0%	0.0%	0.0%

188 strains

Campylobacter: resistance (%) in food

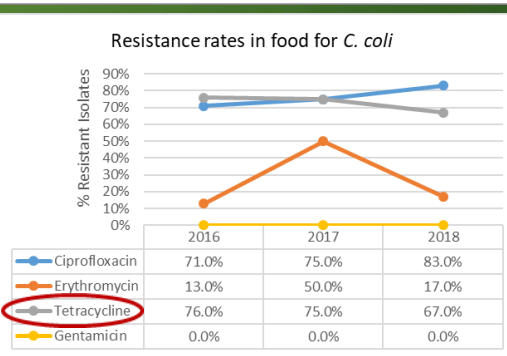


Resistance rates in food for *C. jejuni*



	2016	2017	2018
Ciprofloxacin	65.0%	69.0%	40.0%
Erythromycin	1.0%	0.0%	0.0%
Tetracycline	52.0%	46.0%	40.0%
Gentamicin	0.0%	0.0%	0.0%

Resistance rates in food for *C. coli*

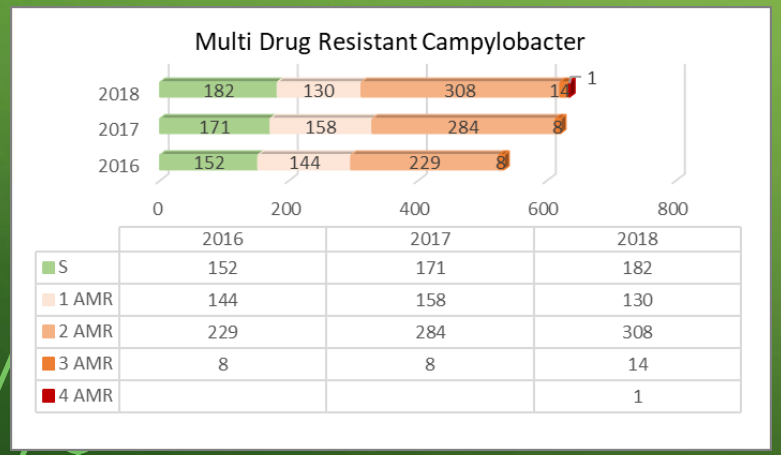


	2016	2017	2018
Ciprofloxacin	71.0%	75.0%	83.0%
Erythromycin	13.0%	50.0%	17.0%
Tetracycline	76.0%	75.0%	67.0%
Gentamicin	0.0%	0.0%	0.0%

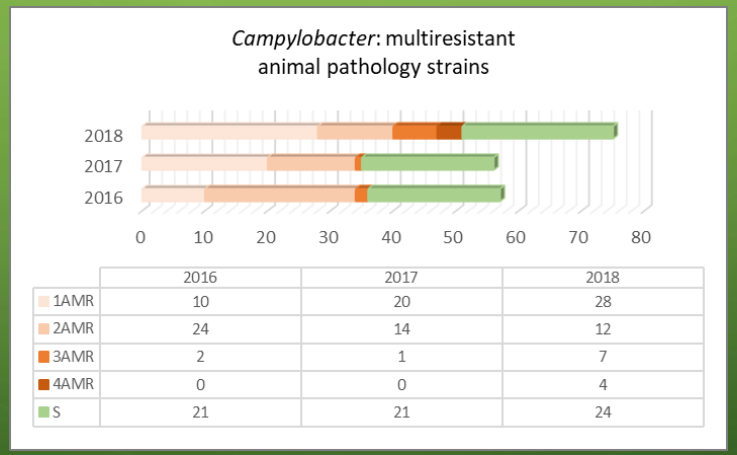
162 strains

RESULTS : *CAMPYLOBACTER*

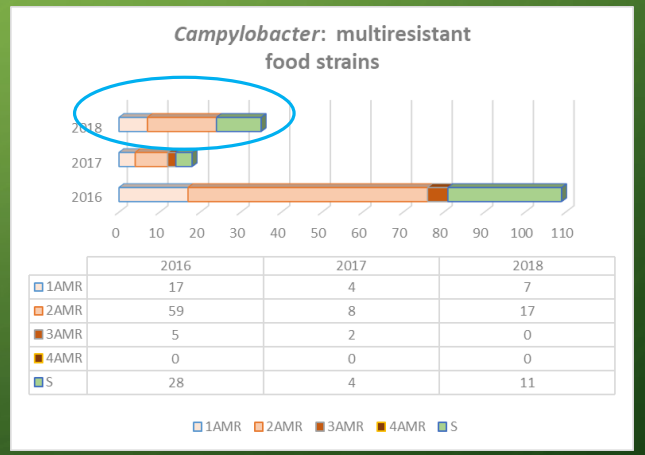
MULTIDRUGRESISTANCE



	2016	2017	2018
S	152	171	182
1 AMR	144	158	130
2 AMR	229	284	308
3 AMR	8	8	14
4 AMR			1



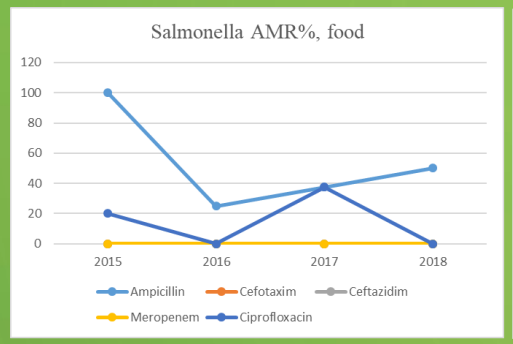
	2016	2017	2018
1AMR	10	20	28
2AMR	24	14	12
3AMR	2	1	7
4AMR	0	0	4
S	21	21	24

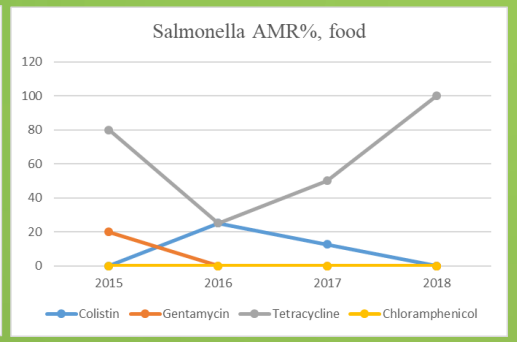


	2016	2017	2018
1AMR	17	4	7
2AMR	59	8	17
3AMR	5	2	0
4AMR	0	0	0
S	28	4	11

6 ab tested

RESULTS: *SALMONELLA*





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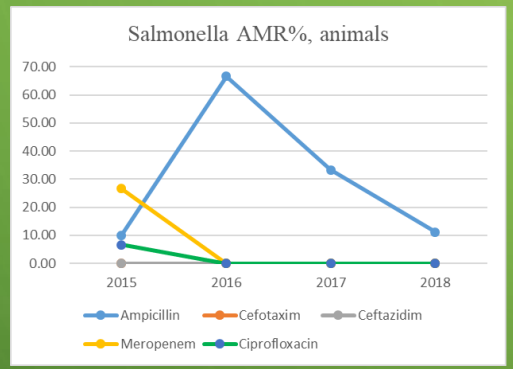
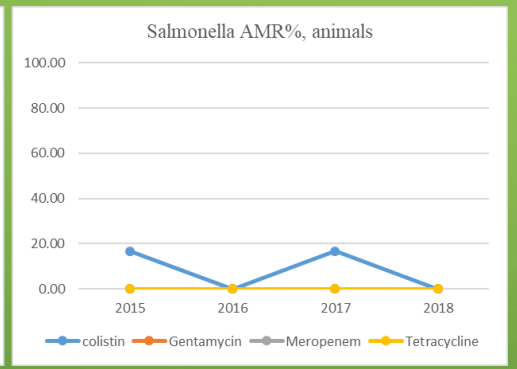
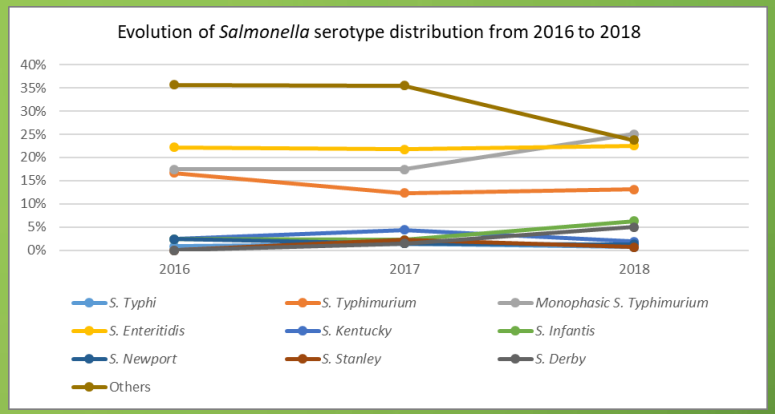




19 food + 53 animal strains tested 2015-2018
14 food + 23 animal strains tested 2016-2018

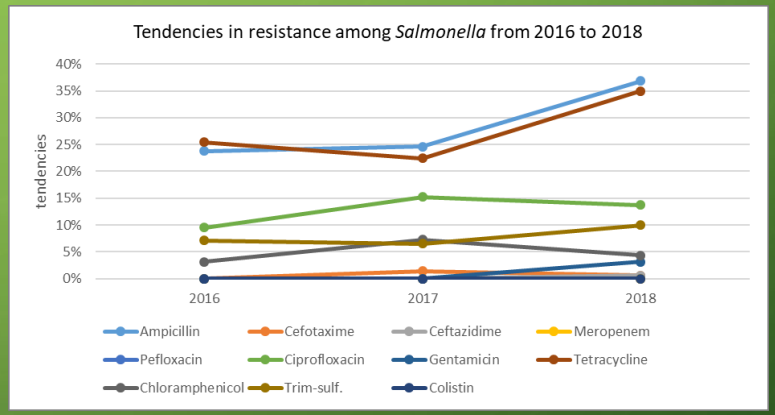
Colistin resistant S. in food:
Enteritidis (2016), Typhimurium (2017)

Colistin resistant S. in animals:
Enteritidis, Give, Livingston (2015)
Dublin (2019)



424 strains

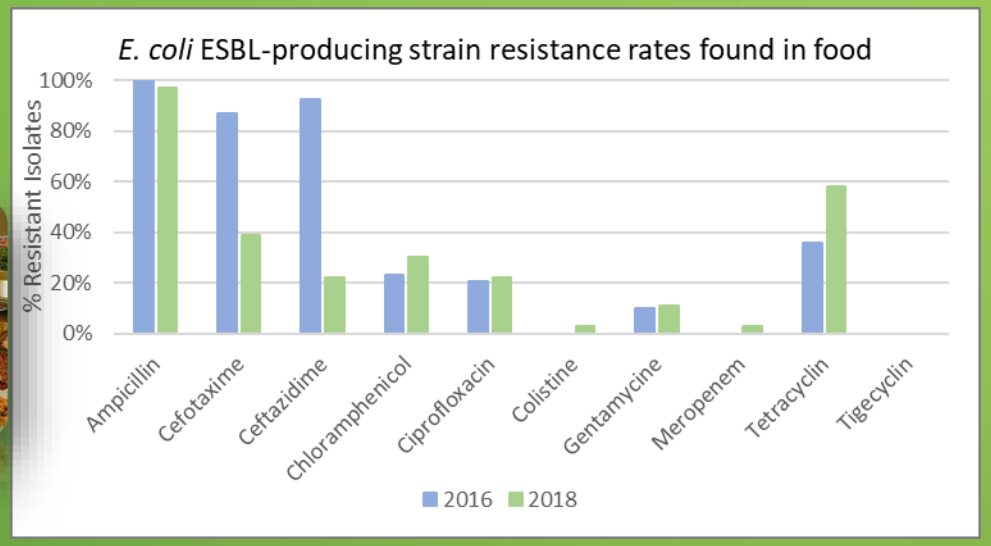
LNS LUXEMBOURG

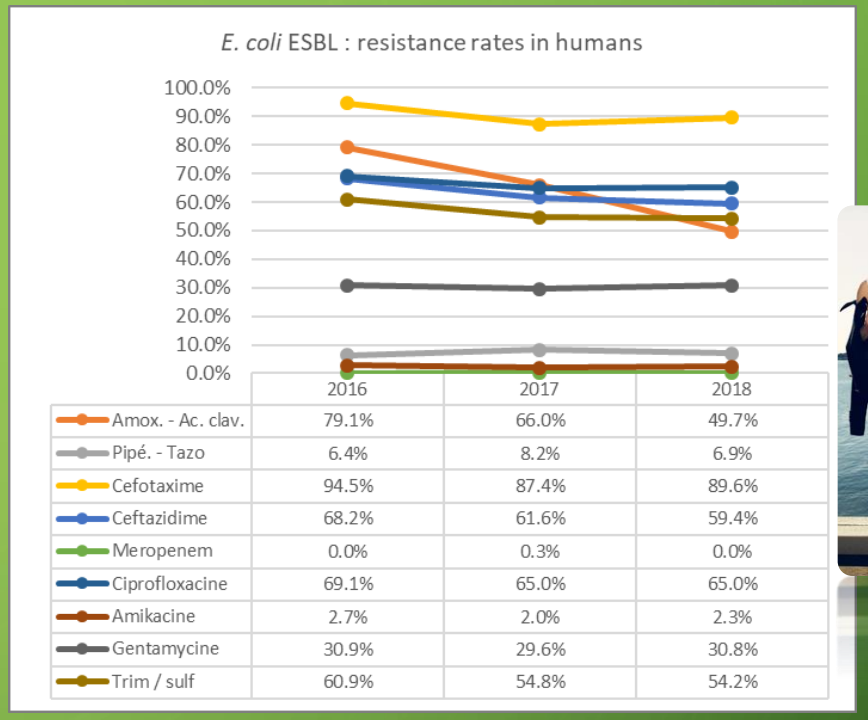


S. enteritidis, *S. Typhimurium* (bi-mono ph.) !!!

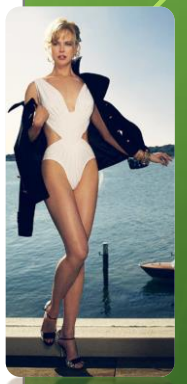
S. Kentucky, Infantis, Derby, Newport, Stanley
Every year isolated

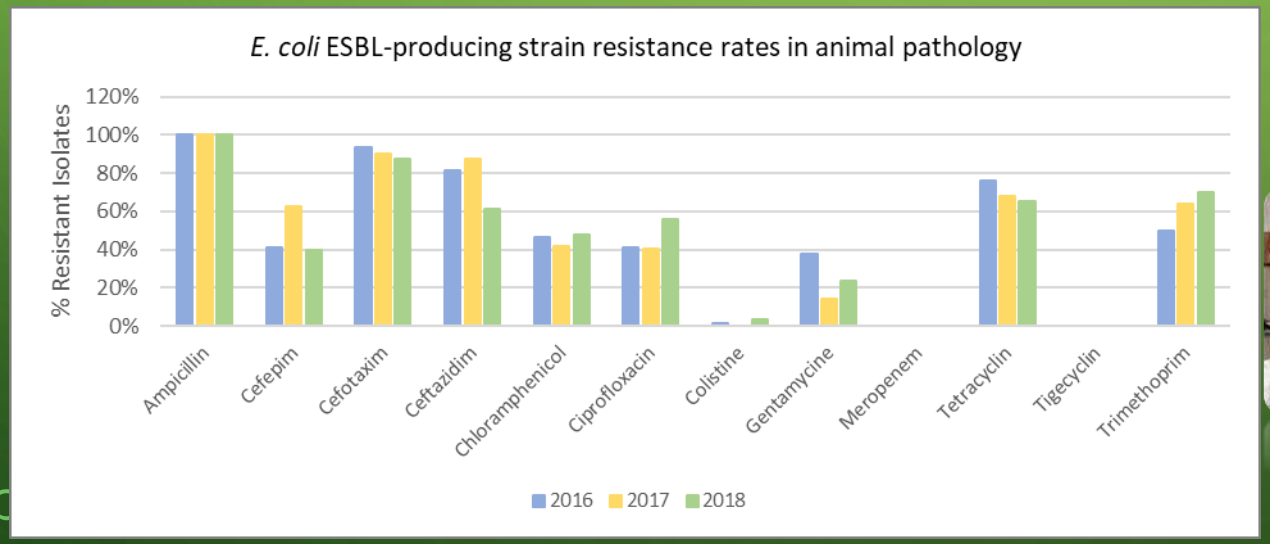
RESULTS: *E. COLI* ESBL





	2016	2017	2018
Amox. - Ac. clav.	79.1%	66.0%	49.7%
Pipé. - Tazo	6.4%	8.2%	6.9%
Cefotaxime	94.5%	87.4%	89.6%
Ceftazidime	68.2%	61.6%	59.4%
Meropenem	0.0%	0.3%	0.0%
Ciprofloxacine	69.1%	65.0%	65.0%
Amikacine	2.7%	2.0%	2.3%
Gentamycine	30.9%	29.6%	30.8%
Trim / sulf	60.9%	54.8%	54.2%





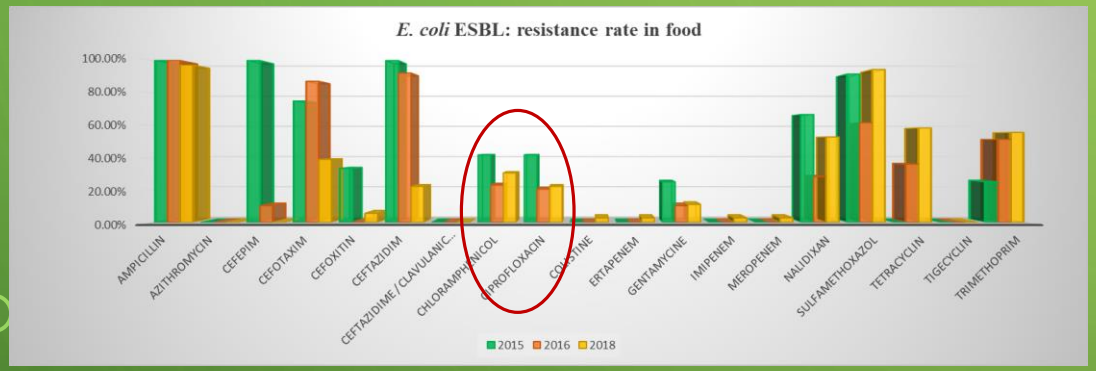
CTX-M 1,9; SHV, TEM, OXA-1 like most frequent genes

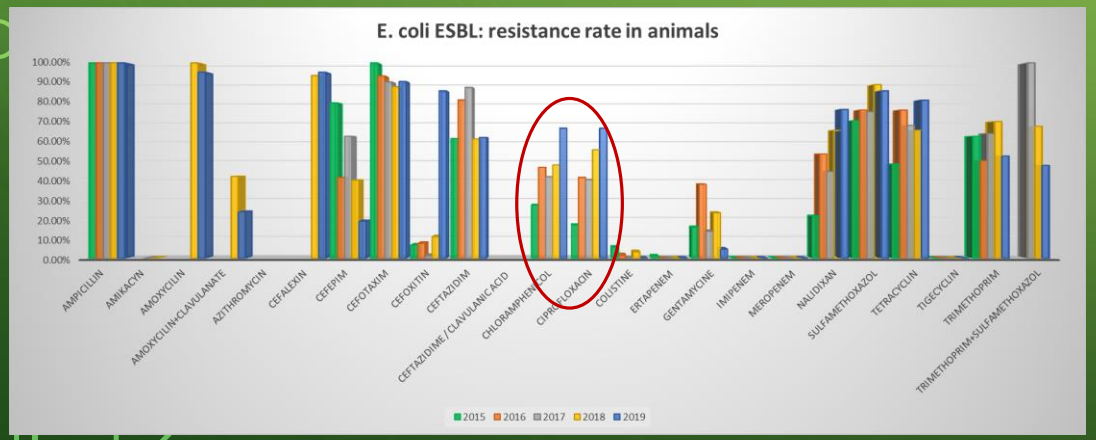
Associations → MDR

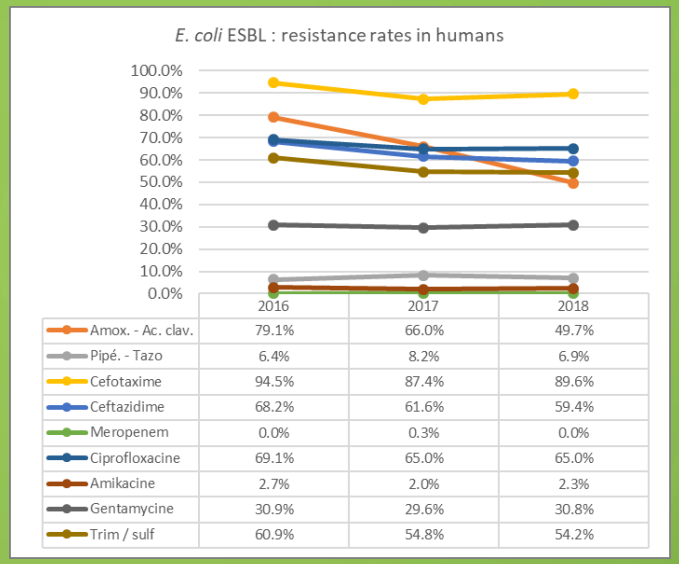
2016-2018

RESULTS: *E. COLI ESBL*

E.coli ESBL isolates tested					
	2015	2016	2017	2018	2019
food	12	39	3	36	3
animals	88	58	72	90	28







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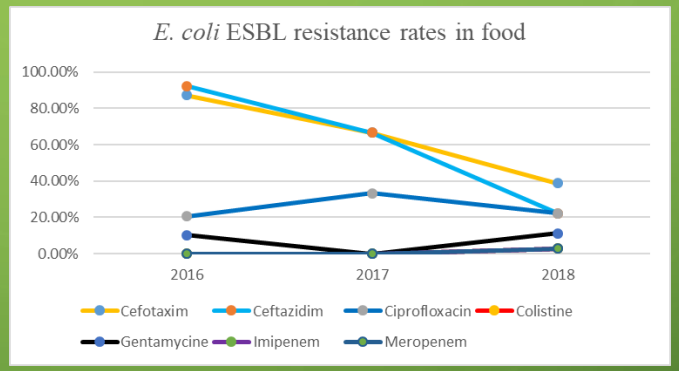
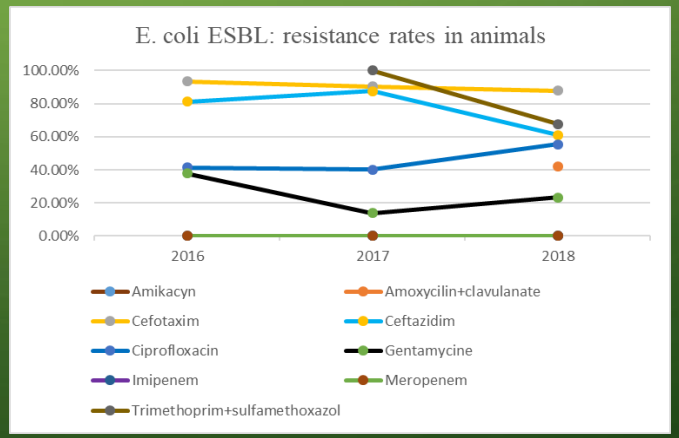
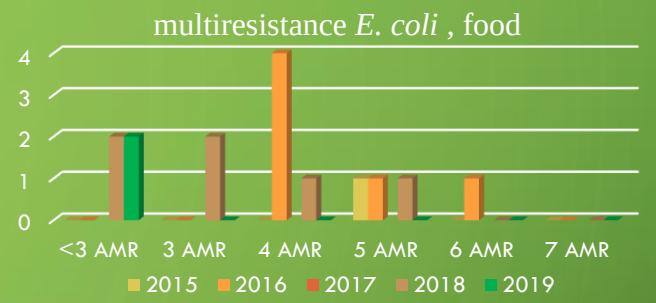
Cefotaxime	94.5%	87.4%	89.6%
Ceftazidime	68.2%	61.6%	59.4%
Meropenem	0.0%	0.3%	0.0%
Ciprofloxacine	69.1%	65.0%	65.0%
Amikacine	2.7%	2.0%	2.3%
Gentamycine	30.9%	29.6%	30.8%
Trim / sulf	60.9%	54.8%	54.2%

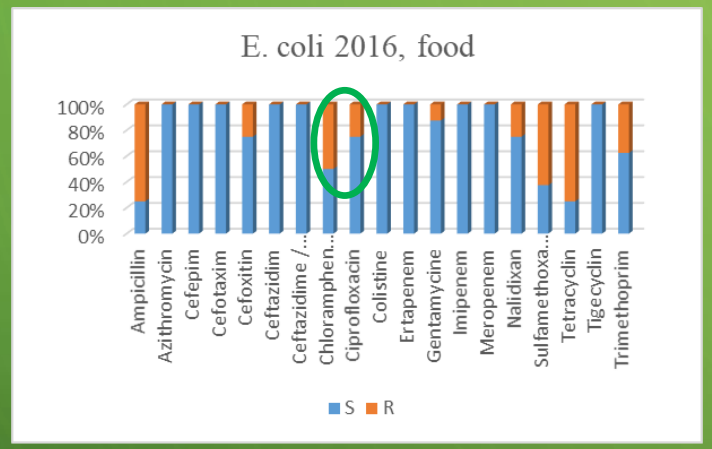


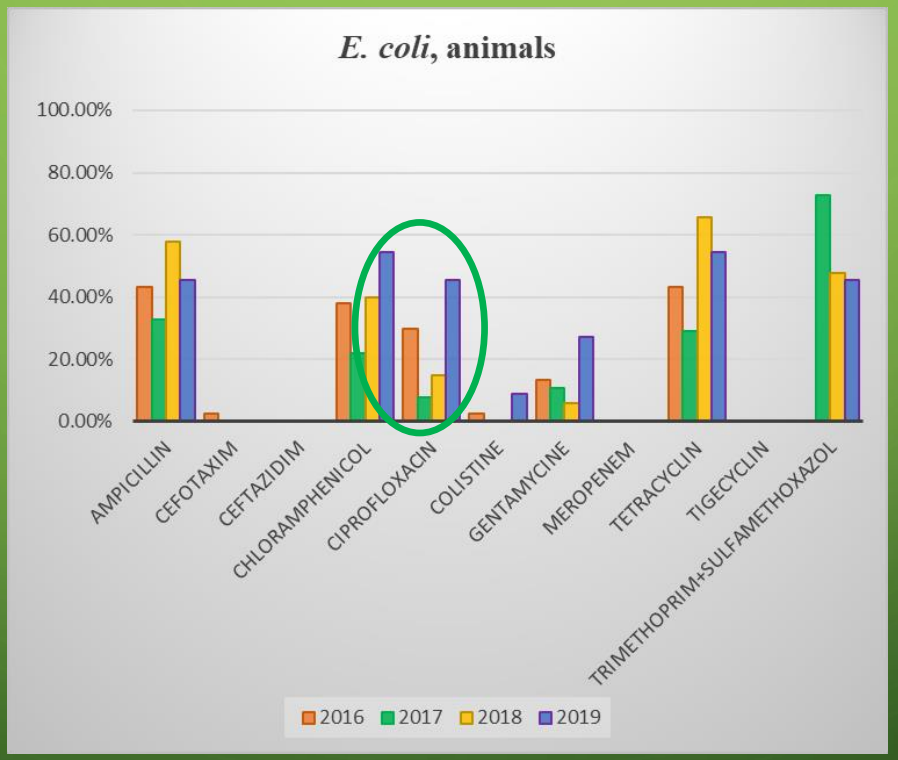


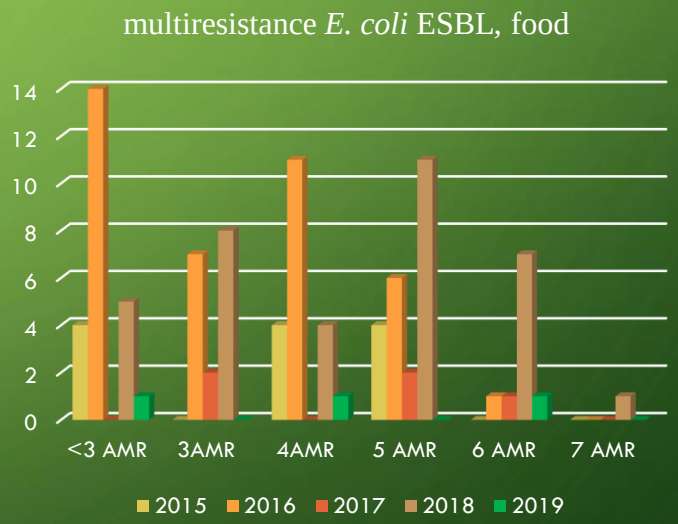
RESULTS: *E. COLI*

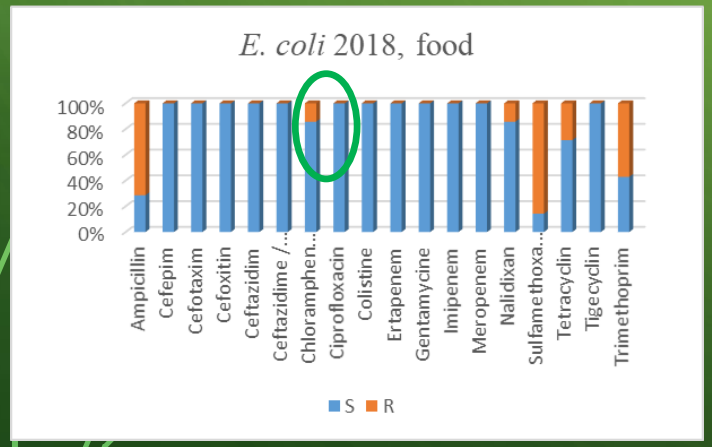
MDR





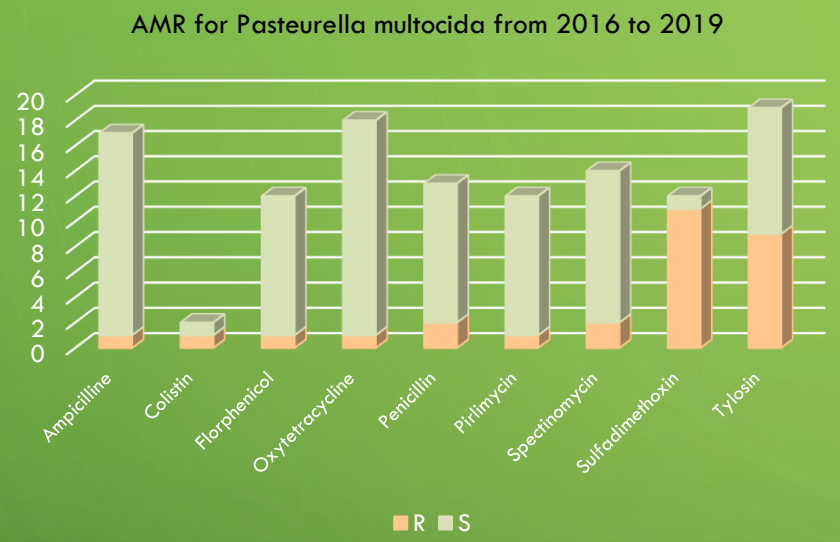


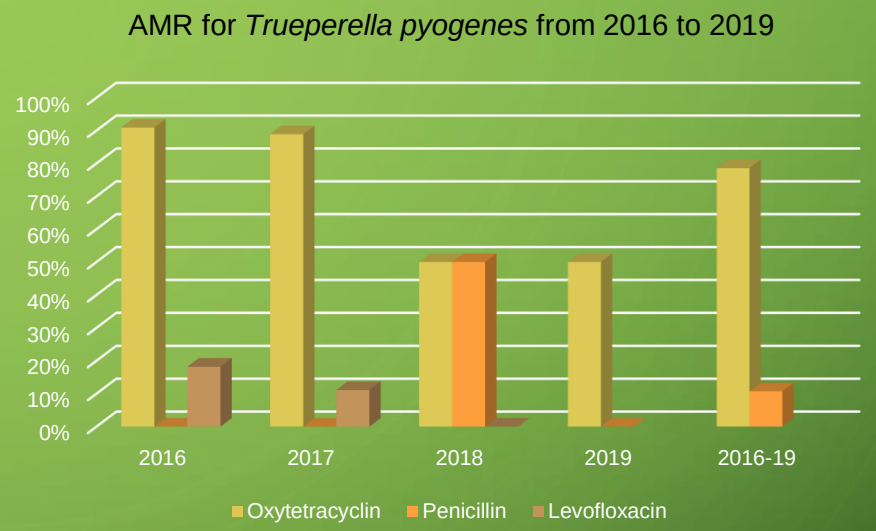




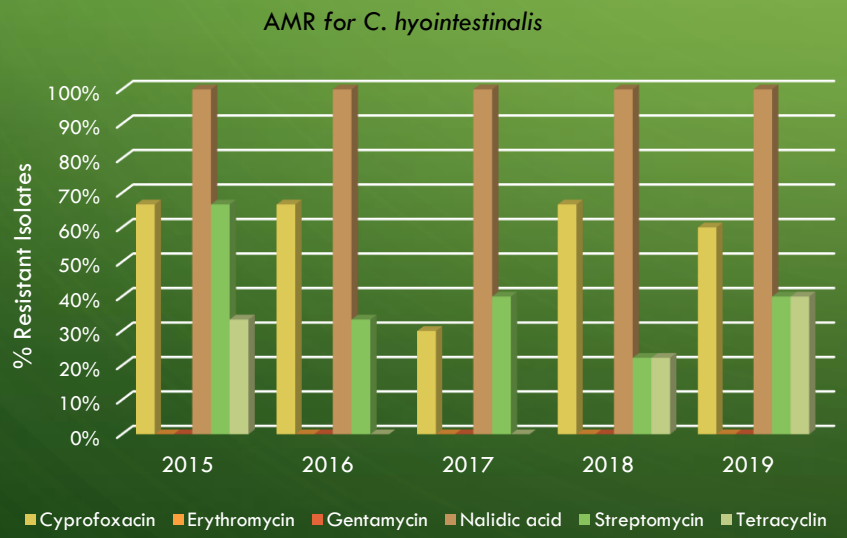
SPECIAL VETERINARY STRAINS:

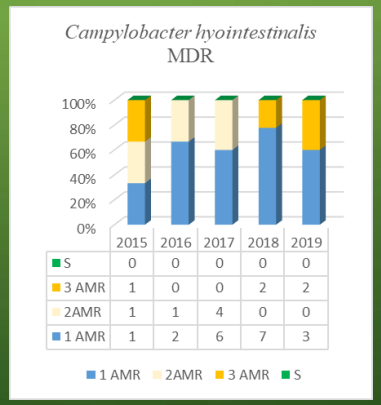
Lung>milk





2/39 MDR

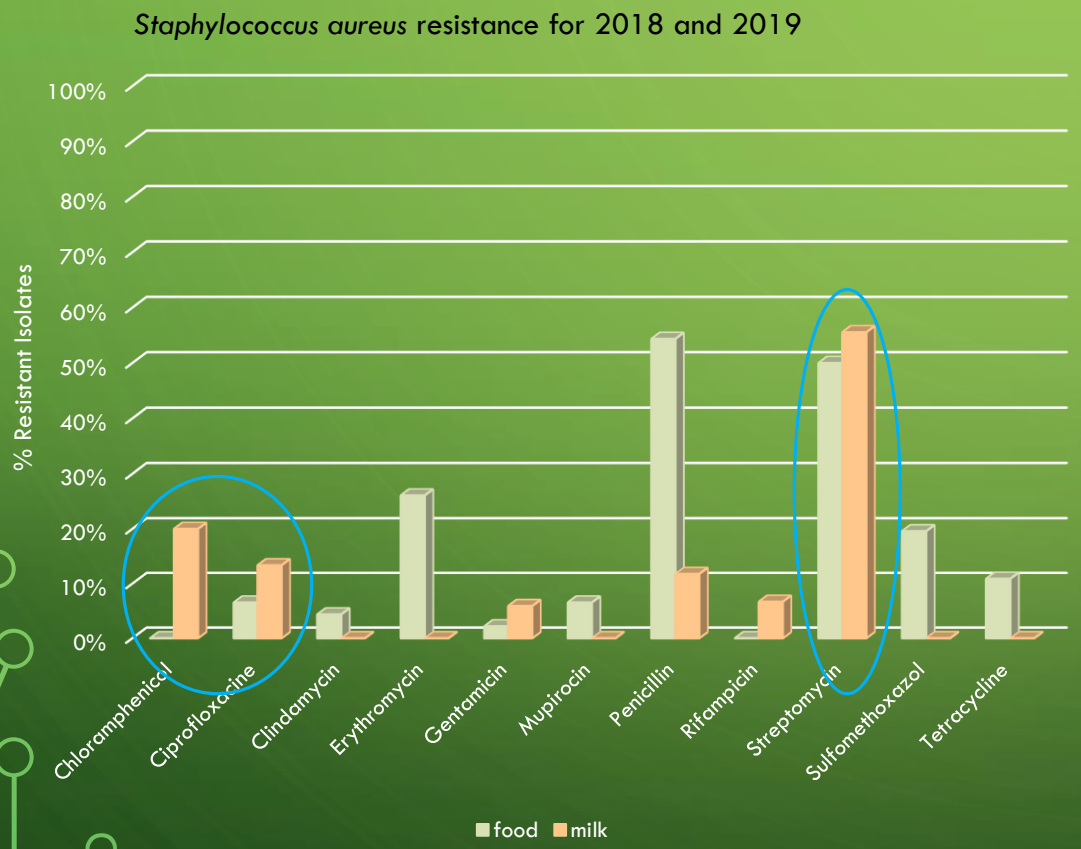


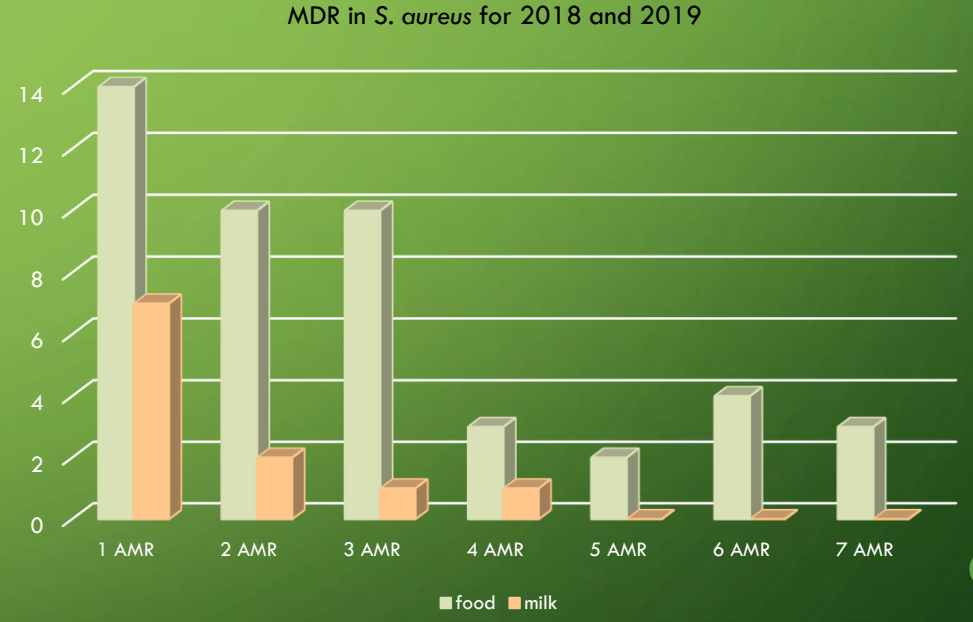


	2015	2016	2017	2018	2019
S	0	0	0	0	0
3 AMR	1	0	0	2	2
2AMR	1	1	4	0	0
1 AMR	1	2	6	7	3

SPECIAL VETERINARY STRAINS: *STAPHYLOCOCCUS AUREUS*

2018-2019: 6 milk/ 46 food strains





regardless of status (MRSA or not), MDR more pronounced in food strains (*S. aureus* of human origin) than in milk

DISCUSSION: *CAMPYLOBACTER*

- Ab treatment usually not required, but effective treatment → shorter illness
- AMR levels (70%, too high) for ciprofloxacin comparable, although tendencies differ
  → better don't use anymore in first line (human and vet medicine)
- Fluoroquinolone resistance persist even after ab pressure removed
- Macrolide resistance↘ if antibiotic pressure↘
- Reservoirs: ~61% poultry, 33% ruminants, 5% environmental in Luxembourg
  (EFSA→ EU: 80% poultry)

DISCUSSION: *SALMONELLA*

- Food samples = screening
- Animal samples: screening and clinical cases
- Human samples: clinical cases

- no epidemic diffusion of highly resistant *S. Infantis* or *S.Kentucky* in L
- *S. Kentucky* resistance to ciprofloxacin and cefotaxime of concern (invasive salmonellosis in humans)
- Colistin resistance of serious concern
- Lower levels of AMR in vet medicine (except for Ampicillin)
- no correlations detectable between human AMR strains and non-human

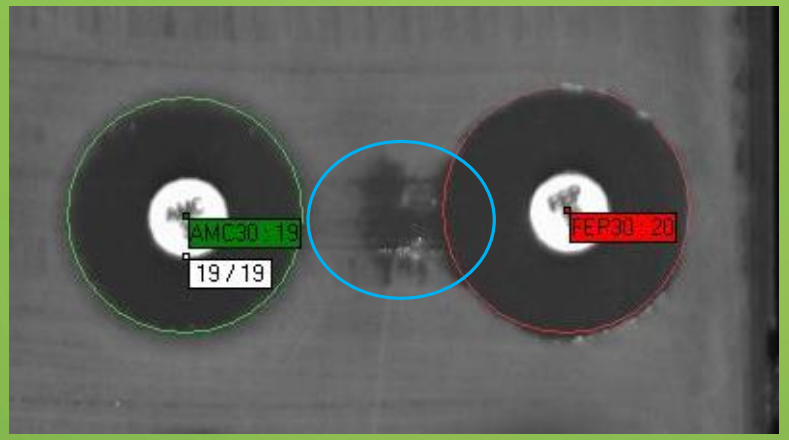
DISCUSSION: *E. COLI*

E.Coli:

	ESBL	AmpC	ESBL + Ampc	CarbAp
Cefotaxime	R (>1)	R (>1)	R (>1)	
	ou	ou	ou	
Ceftazidime	R (>1)	R (>1)	R (>1)	
Cefoxitine	S(<=8)	R (>8)	R (>8)	
Meropenem	S(<=0,12)	S(<=0,12)	S(<=0,12)	R (>0,125)
Cefotaxime/ Ac. Clavulanique	S (Pos)	R (Neg)	S (Pos)	
	et/ou	et	et/ou	
Ceftazidime/ Ac. Clavulanique	S (Pos)	R(Nég)	S (Pos)	

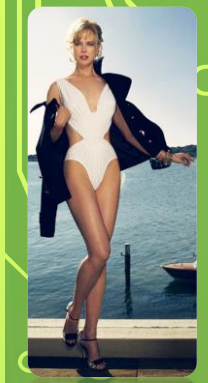
Human strains	Fluoroquinolone > cephalosporin > aminopenicillins
Food strains	aminopenicillins > sulfonamides > tetracyclines > phenicols
Animal strains	Tetracyclines > penicillins > phenicols (*E.coli*) ß-lactams > sulfonamides > tetracyclines > fluoroquinolones (*E.coli* ESBL)

- food and animal strains related
- human and non human strains not related
- no significant difference between “commensal” *E. coli* and ESBL *E. coli*.



TEM, SHV, CTX, OXA…

- Mainly ß-lactamase genes found, some times associations
  → ampicillin resistance at a rate of 95 to 100%
- cephalosporins 3rd generation always affected → difficult treatment
- TEM gene correlated with much higher rates of resistance to amoxicillin-clavulanic acid and piperacillin-tazobactam than with other genes
- Discrepancy between phenotype and genotype



CONCLUSIONS = LESSONS LEARNT

- AMR = very complex topic ☹
- don’t define too quickly couples that won’t work
- Interpretation rules change over time → interpretation difficult
- Different or lack of interpretation by matrix and/or default (vet)
- Difficult to compare food-animal-human data
- Human AMR mainly by human source
- Limit ciprofloxacin use
- MDR increasing
- data base !!!
- be careful with low amount of data
- first step taken to develop a One Health approach in Luxembourg

LNS LUXEMBOURG

LE GOUVERNEMENT DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l’Agriculture, de la Viticulture et du Développement rural
Administration des services vétérinaires
Laboratoire de Médecine Vétérinaire de l’Etat

THANK YOU FOR YOUR ATTENTION

Many thanks to our teams

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