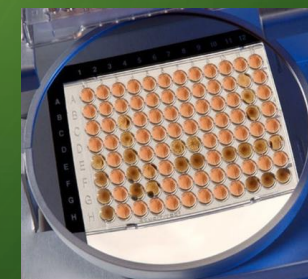


OFFICIAL SURVEILLANCE ON AMR BY CLASSICAL AND MOLECULAR METHODS IN LUXEMBOURG

M. MEO¹, D. CLAUDE², M. PERRIN¹, S. LOSCH²

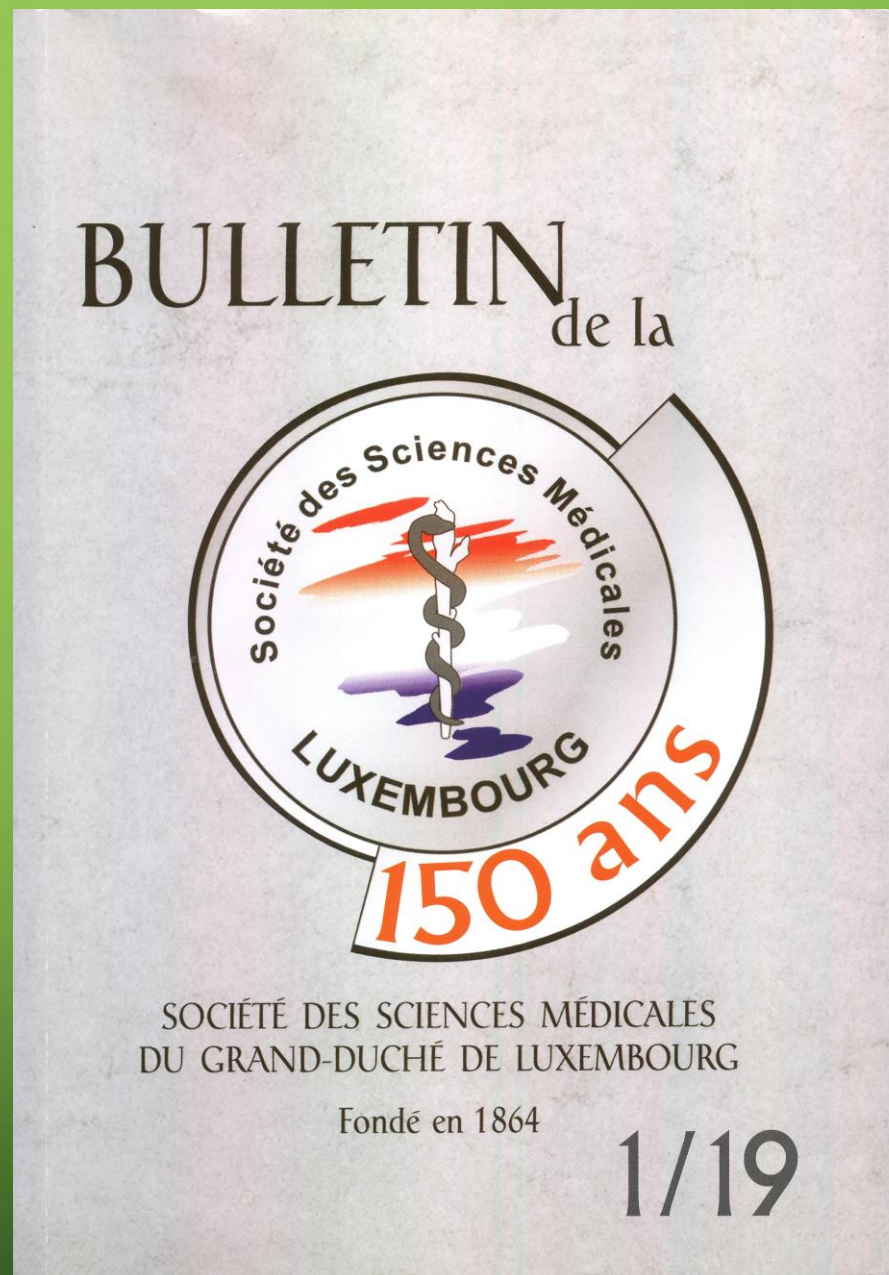
¹LABORATOIRE NATIONAL DE SANTÉ, 1 RUE LOUIS RECH, 3555 DUDELANGE

²LABORATOIRE DE MÉDECINE VÉTÉRINAIRE DE L'ÉTAT, 1 RUE LOUIS RECH, 3555 DUDELANGE



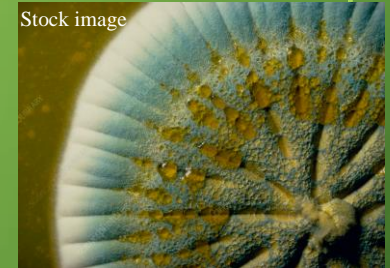
Data published:

Meo M., Claude D., Perrin M.,
Losch S.
2019, Bulletin de la Société des
Sciences médicales 1/19, p.17-38



OVERVIEW

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



MATERIAL & METHODS : SAMPLES



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

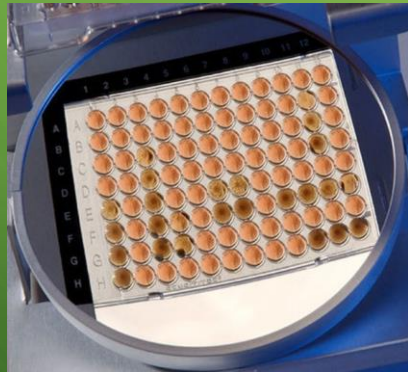


MATERIAL & METHODS

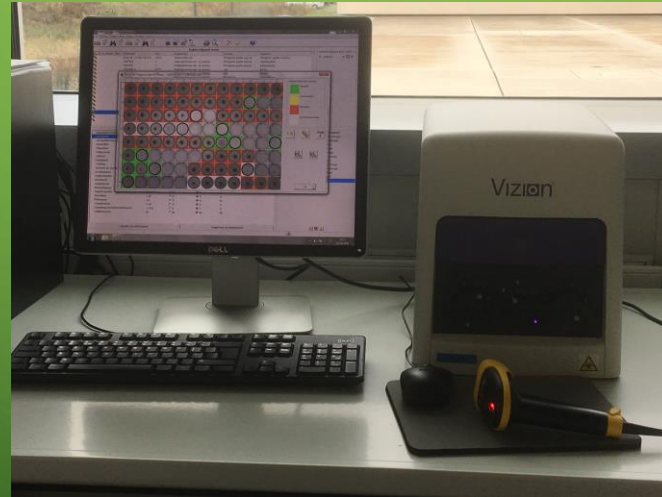


Culture → isolation → antibiogram

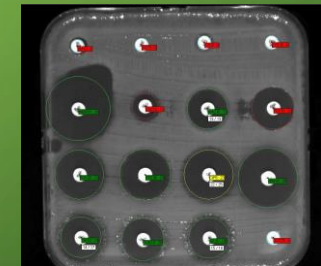
id. LMVE + ready strains → abg



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

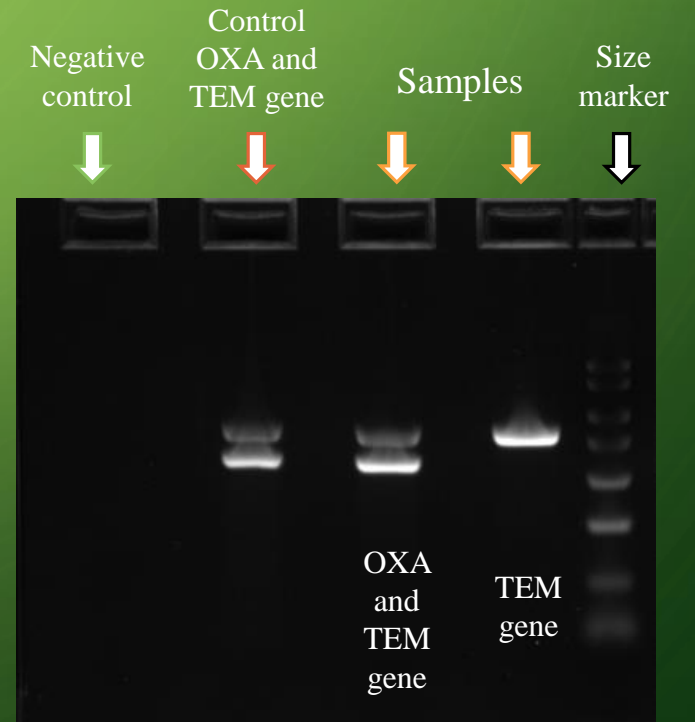
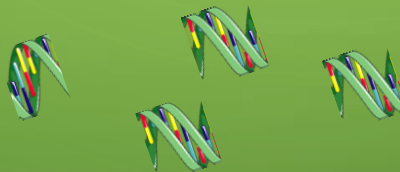


Culture



DNA
extraction

PCR : ESBL gene research
and amplification



Molecular methods for ESBL

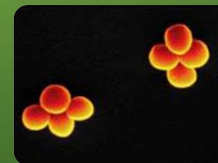
TARGET BACTERIA (NAP)



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)

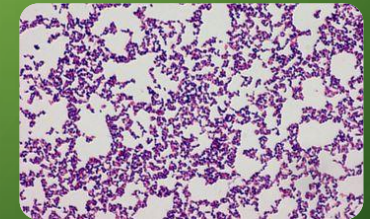


→ *comparison: human vs. non human of these zoonotic agents*
data 2016 – 2018

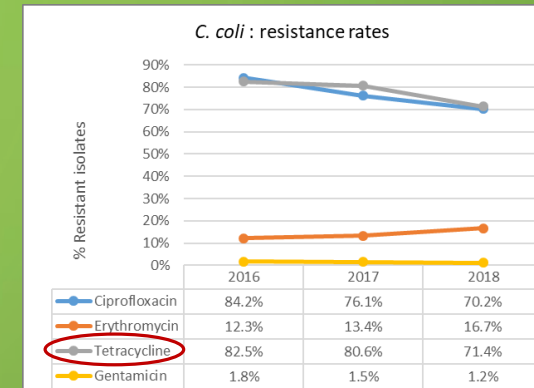
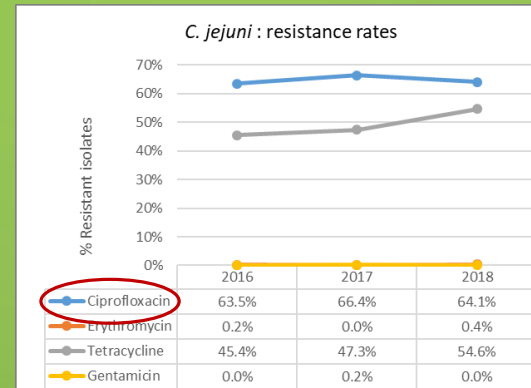
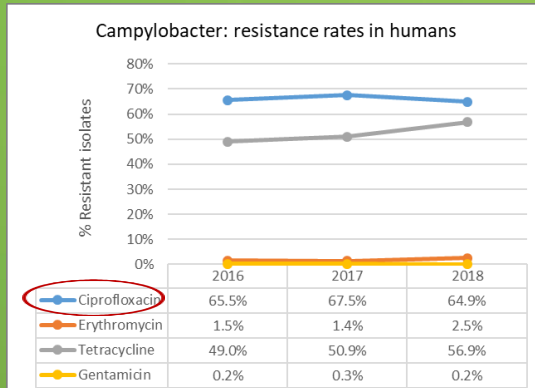
TARGET BACTERIA “BULLETIN”



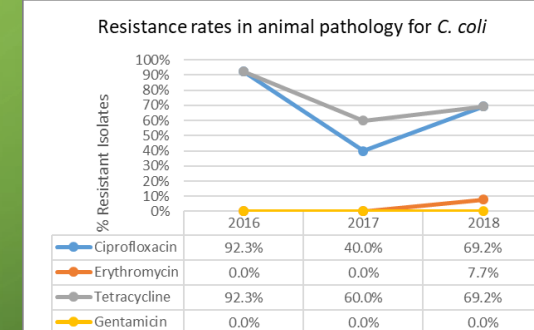
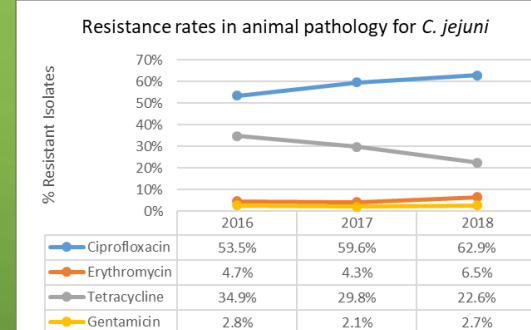
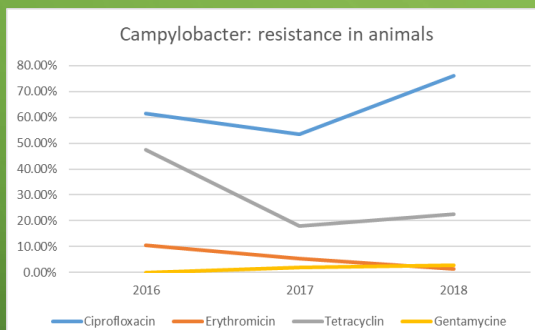
- *Pasteurella multocida*: Zoonotic agent (→ pulmonary diseases)
isolated from milk or lungs
- *Trueperella pyogenes*: → suppurative mastitis, pneumonia in
ruminants, pigs, horses, poultry and other species,
- *Campylobacter hyointestinalis* → proliferative enteritis
Isolated from bovine faeces
- *Staphylococcus aureus*: **milk vs food**
→ skin, udder or systemic diseases, food poisoning



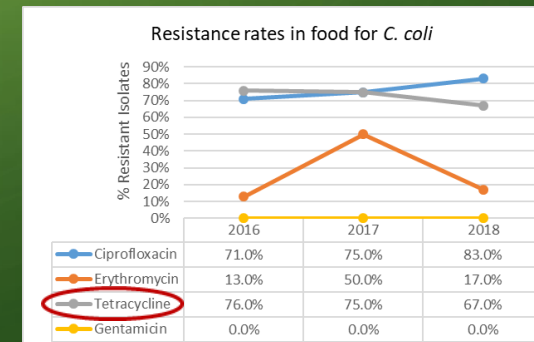
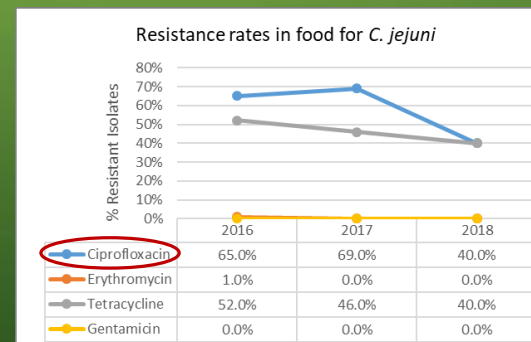
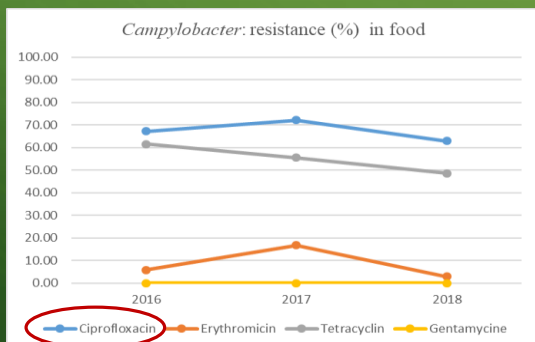
RESULTS: *CAMPYLOBACTER*



1700 strains



188 strains



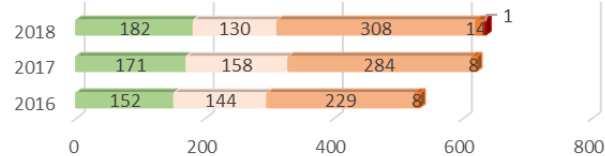
162 strains

RESULTS : *CAMPYLOBACTER*

MULTIDRUGRESISTANCE

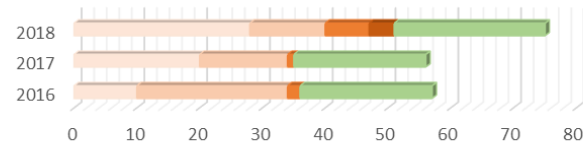


Multi Drug Resistant *Campylobacter*



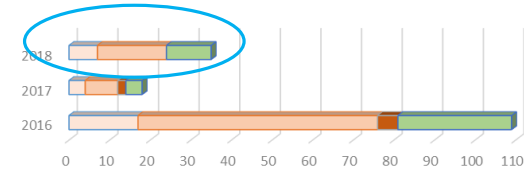
	2016	2017	2018
S	152	171	182
1 AMR	144	158	130
2 AMR	229	284	308
3 AMR	8	8	14
4 AMR			1

Campylobacter: multiresistant animal pathology strains



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

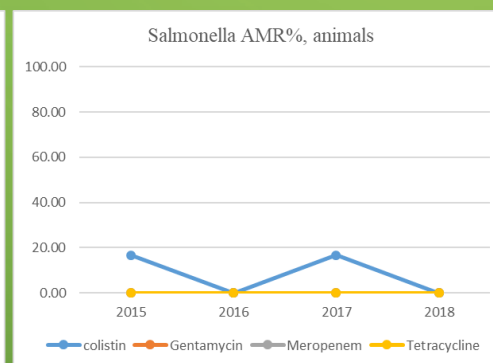
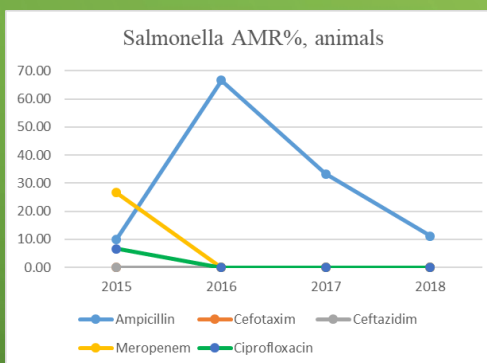
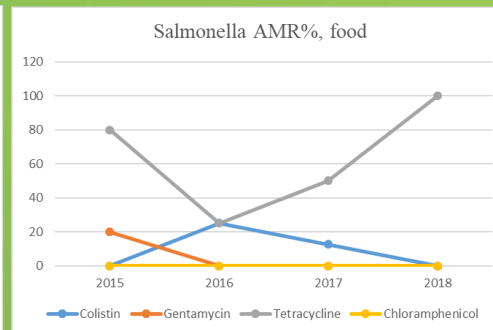
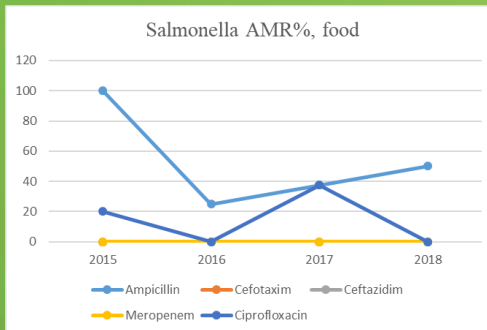
Campylobacter: multiresistant food strains



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

6 ab tested

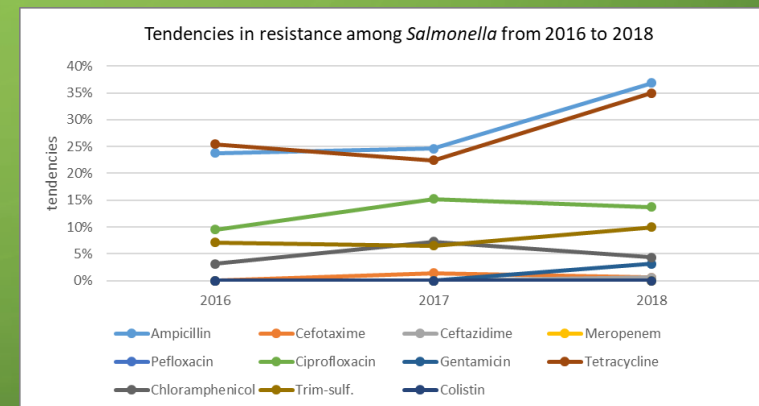
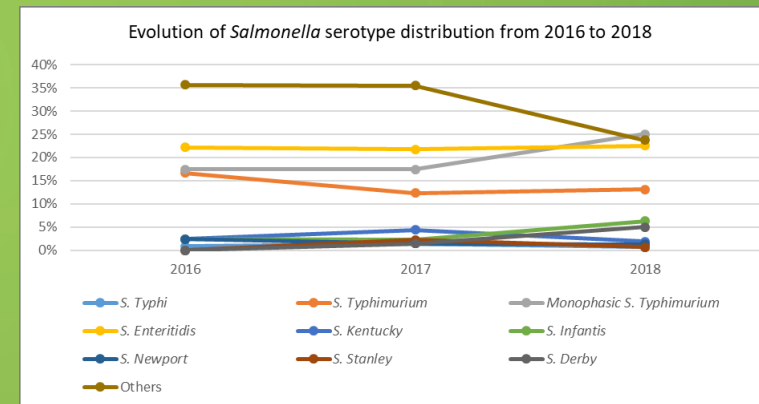
RESULTS: *SALMONELLA*



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)

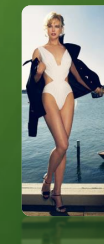
Colistin resistant *S.* in animals:
Enteritidis, Give, Livingston (2015)
Dublin (2019)



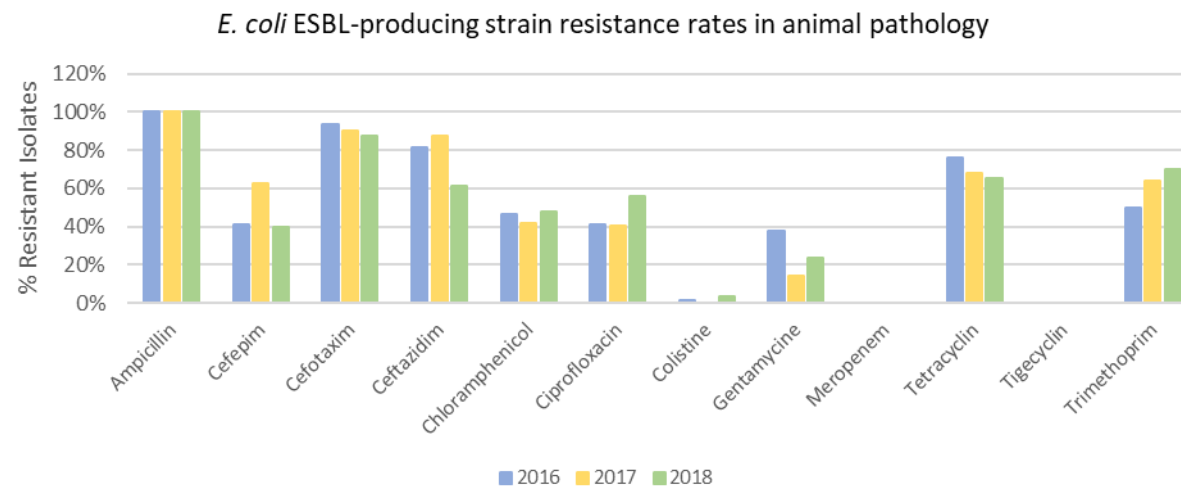
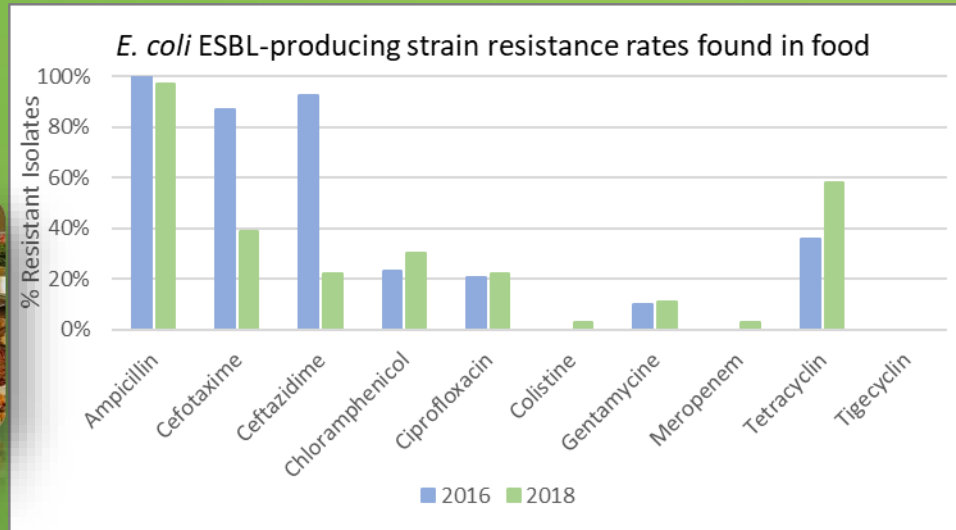
S. enteritidis, *S. Typhimurium* (bi-mono ph.) !!!

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

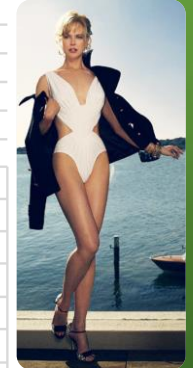
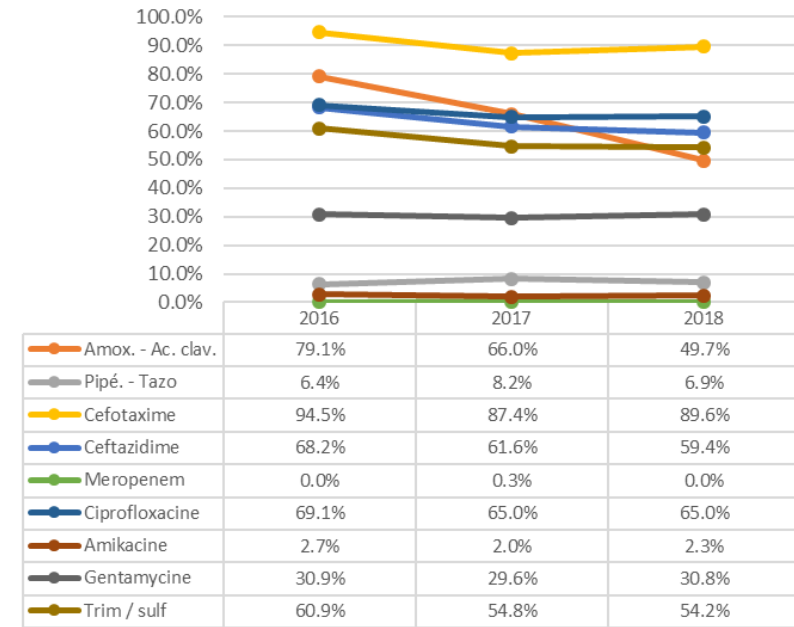
424 strains



RESULTS: *E. COLI* ESBL



E. coli ESBL : resistance rates in humans



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

2016-2018

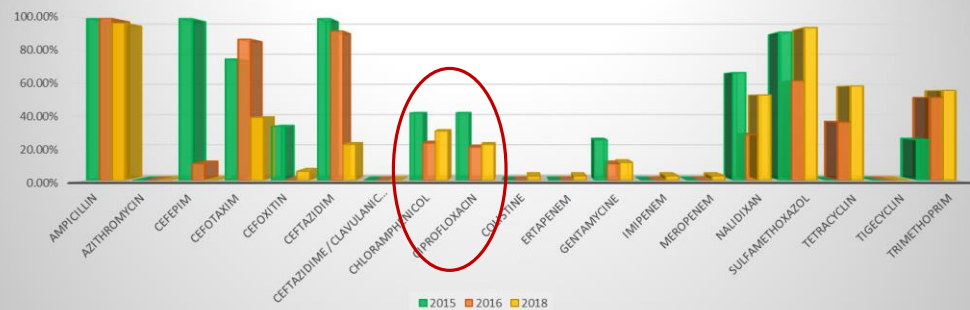


RESULTS: *E. COLI* ESBL

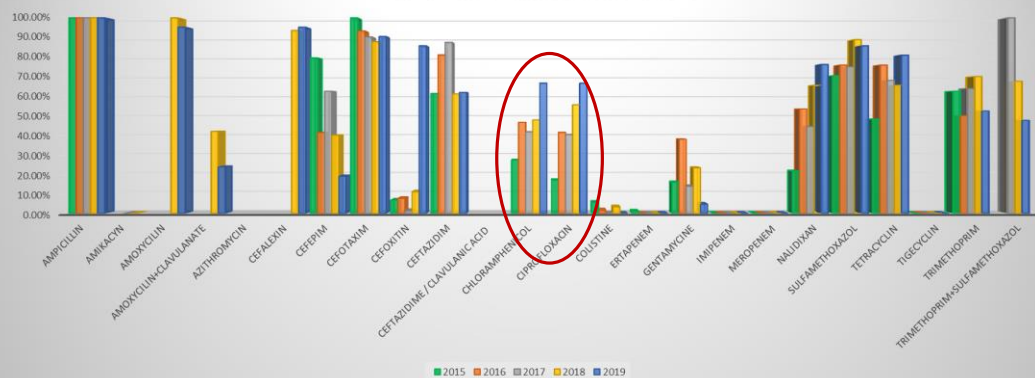
E.coli ESBL isolates tested

	2015	2016	2017	2018	2019
food	12	39	3	36	3
animals	88	58	72	90	28

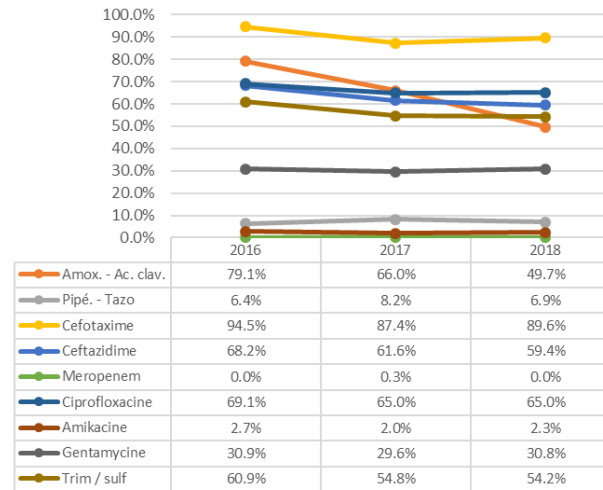
E. coli ESBL: resistance rate in food



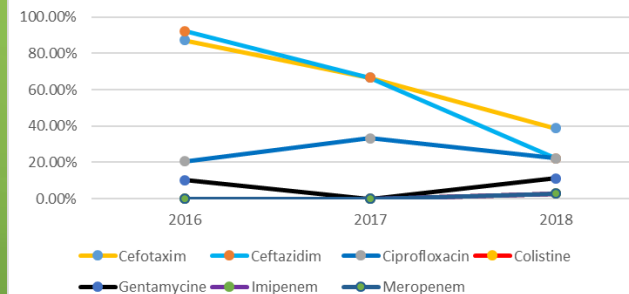
E. coli ESBL: resistance rate in animals



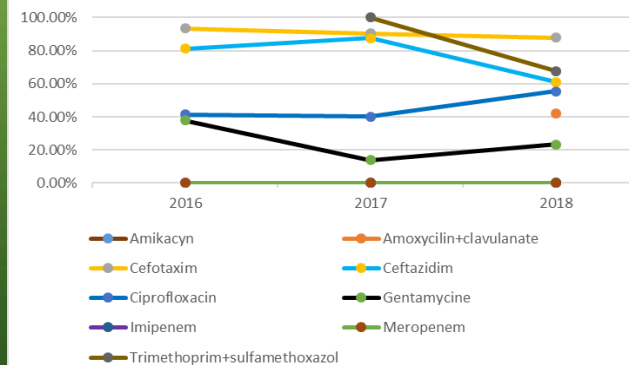
E. coli ESBL : resistance rates in humans



E. coli ESBL resistance rates in food



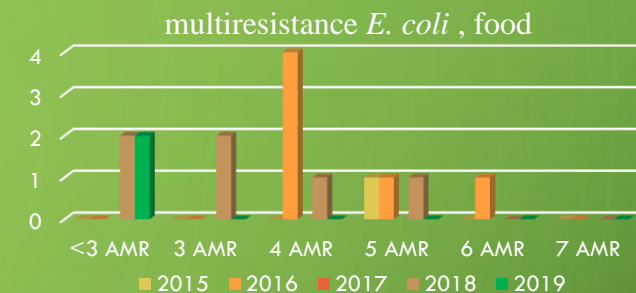
E. coli ESBL: resistance rates in animals



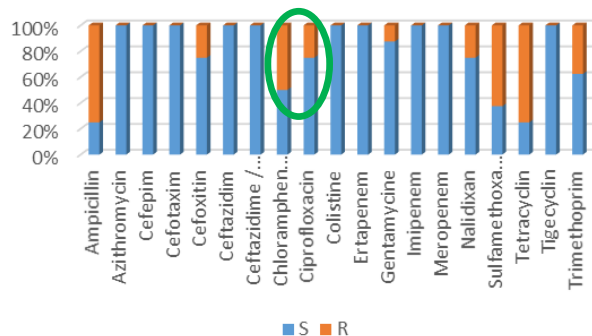
RESULTS: *E. COLI*



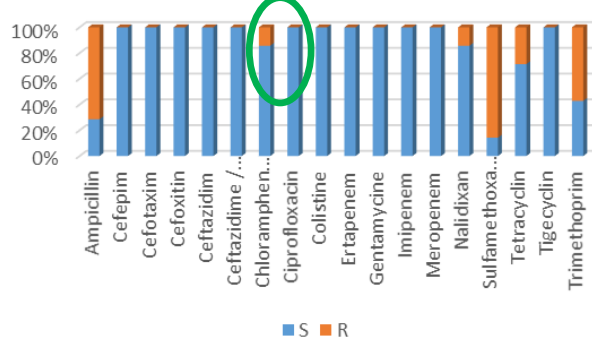
MDR



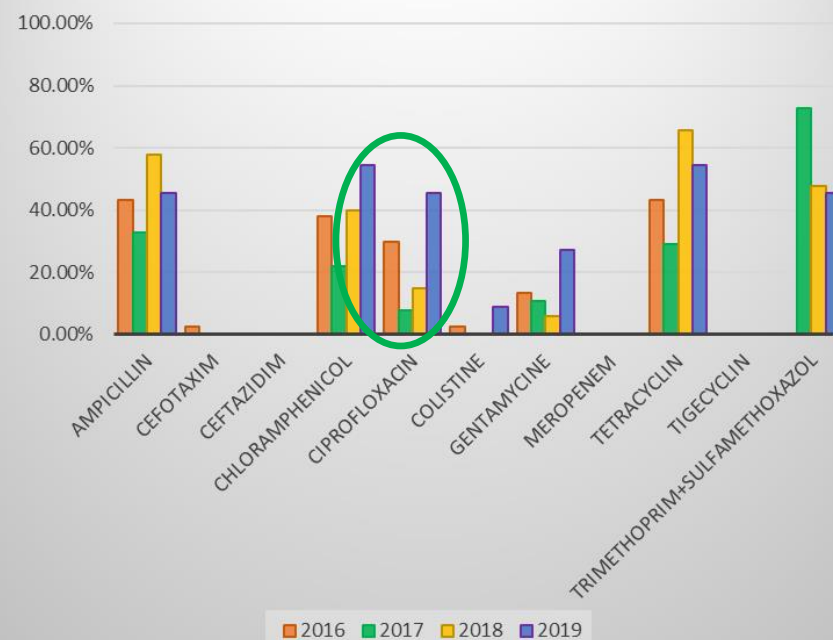
E. coli 2016, food



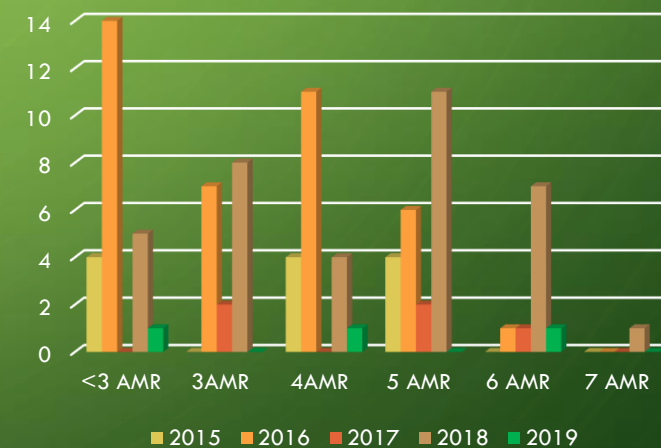
E. coli 2018, food



E. coli, animals



multiresistance *E. coli* ESBL, food

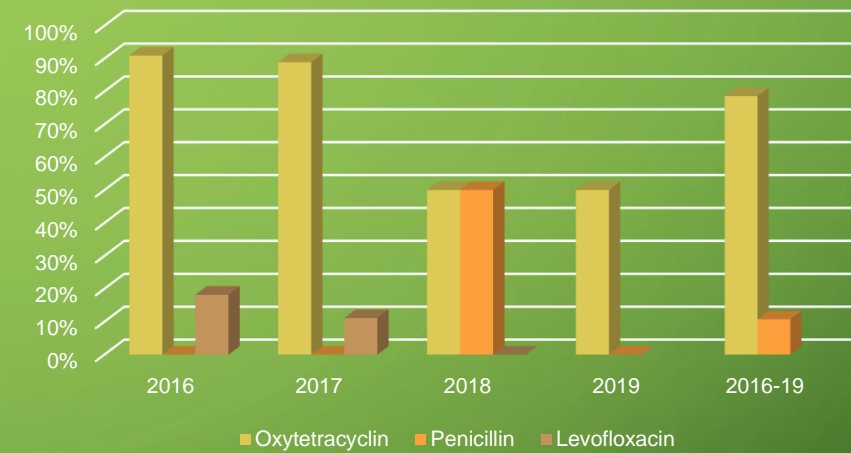


SPECIAL VETERINARY STRAINS:

AMR for *Pasteurella multocida* from 2016 to 2019

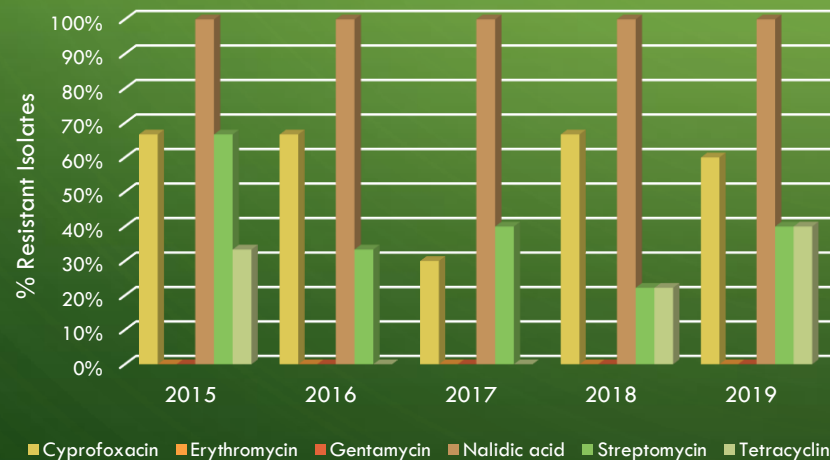


AMR for *Trueperella pyogenes* from 2016 to 2019

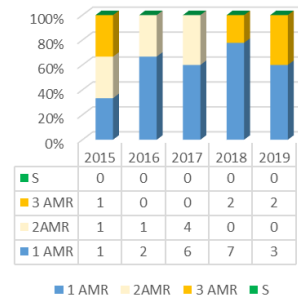


2/39 MDR

AMR for *C. hyointestinalis*



Campylobacter hyointestinalis
MDR

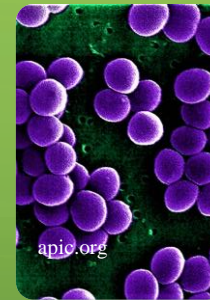
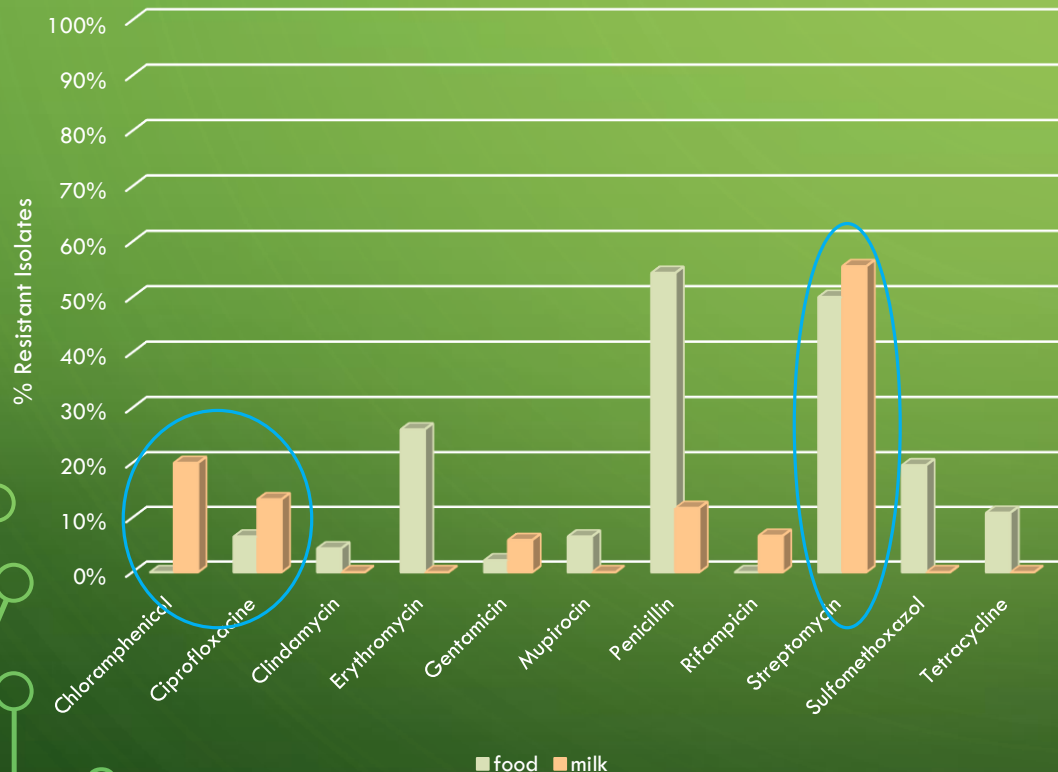


SPECIAL VETERINARY STRAINS: *STAPHYLOCOCCUS AUREUS*

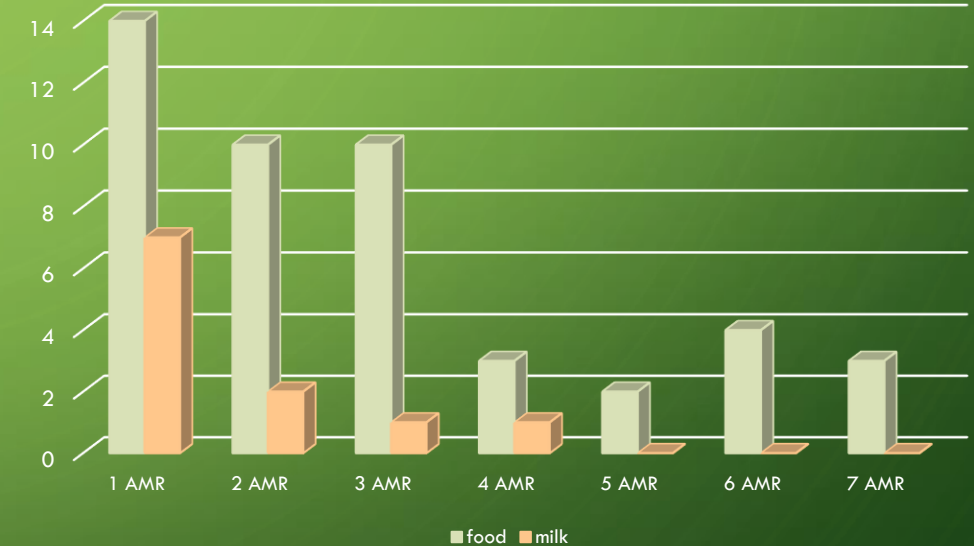
2018-2019: 6 milk/ 46 food strains



Staphylococcus aureus resistance for 2018 and 2019



MDR in *S. aureus* for 2018 and 2019



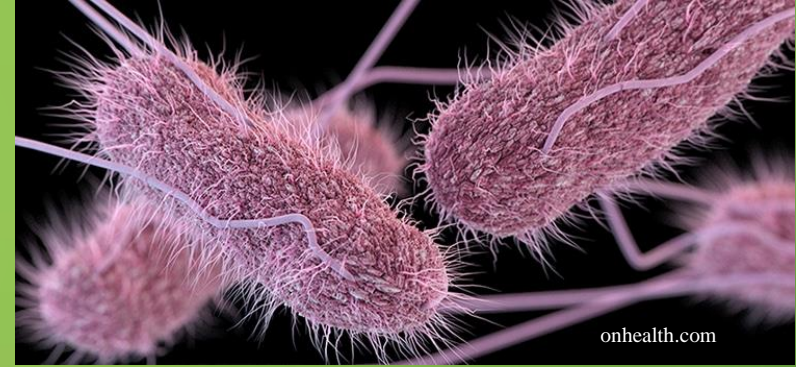
regardless of status (MRSA or not), MDR more pronounced in food strains (*S. aureus* of human origin) than in milk

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*



Hospital.vallhebron.com

E.Coli:

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

Human strains	Fluoroquinolone > cephalosporin > aminopenicillins
Food strains	aminopenicillins > sulfonamides > tetracyclines > phenicols
Animal strains	Tetracyclines > penicillins > phenicols (<i>E.coli</i>) β-lactams > sulfonamides > tetracyclines > fluoroquinolones (<i>E.coli</i> 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 β -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



THANK YOU FOR YOUR ATTENTION

Many thanks to our teams

Mitsou Adam
Dominique Claude
Anne Diederich
Esther Gasperini
Marilyne Rotondella



Jean-Christophe Even
Brigitte Martin
Marie Meo
Juliette Mirouf
Alexandre Mzabi
Anne-Marie Walisch

Serge Losch



Monique Perrin

