



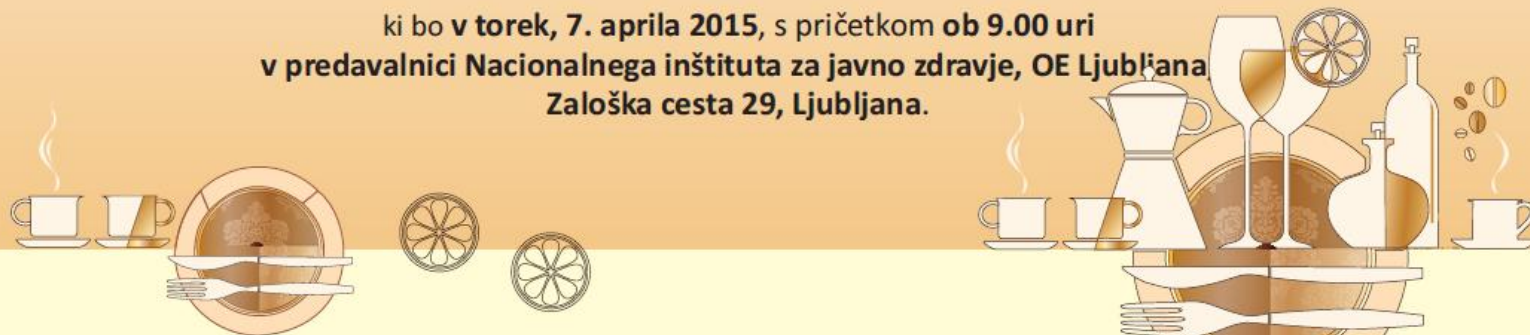
Univerza v Ljubljani  
Biotehniška fakulteta  
Oddelek za živilstvo  
Katedra za biotehnologijo, mikrobiologijo in varnost živil

# Mikrobna rezistenca v živilski verigi

**Sonja SMOLE MOŽINA**

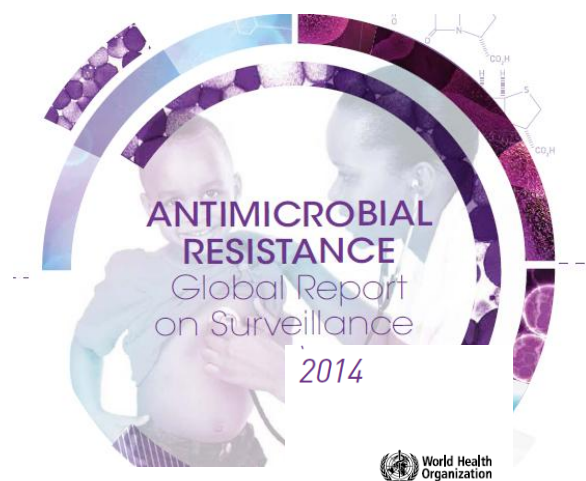
## NACIONALNI POSVET O VARNOSTI ŽIVIL

ki bo v torek, 7. aprila 2015, s pričetkom ob 9.00 uri  
v predavalnici Nacionalnega inštituta za javno zdravje, OE Ljubljana  
Zaloška cesta 29, Ljubljana.



# Vsebinski sklopi predstavitve

- Uvod – poskus ilustracije problema
- Primeri statističnih podatkov
- Primeri raziskovalnih podatkov
- Zaključki
- Nujni ukrepi



## SECTION 05

### Surveillance of antimicrobial resistance in other areas

59

5.1 Antibacterial resistance in food-producing animals and the food chain .....	59
5.1.1 Ongoing surveillance of antimicrobial resistance in food-producing animals and food.....	59
5.1.2 Integrated surveillance of antimicrobial resistance in foodborne bacteria .....	60
5.1.3 Antimicrobials of particular importance in human and veterinary medicine.....	61
5.1.4 Implications for human health from zoonotic transmission of resistant bacteria and genetic material .....	61
5.1.5 WHO-FAO-OIE tripartite intersectoral collaboration on action.....	62
5.1.6 Key messages.....	62





# ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals<sup>1</sup>

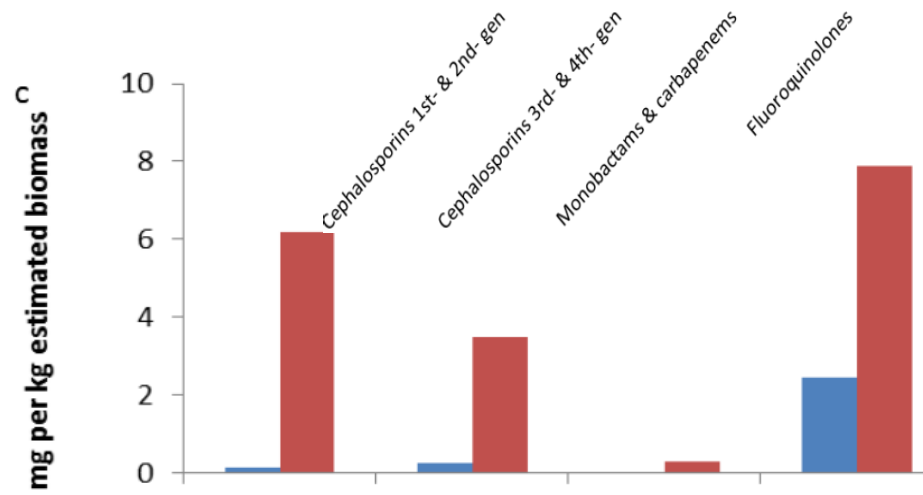
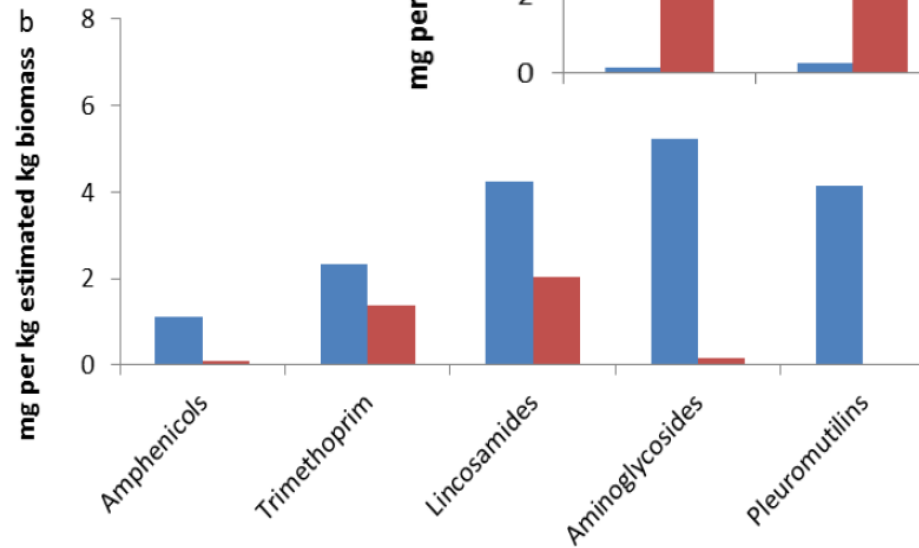
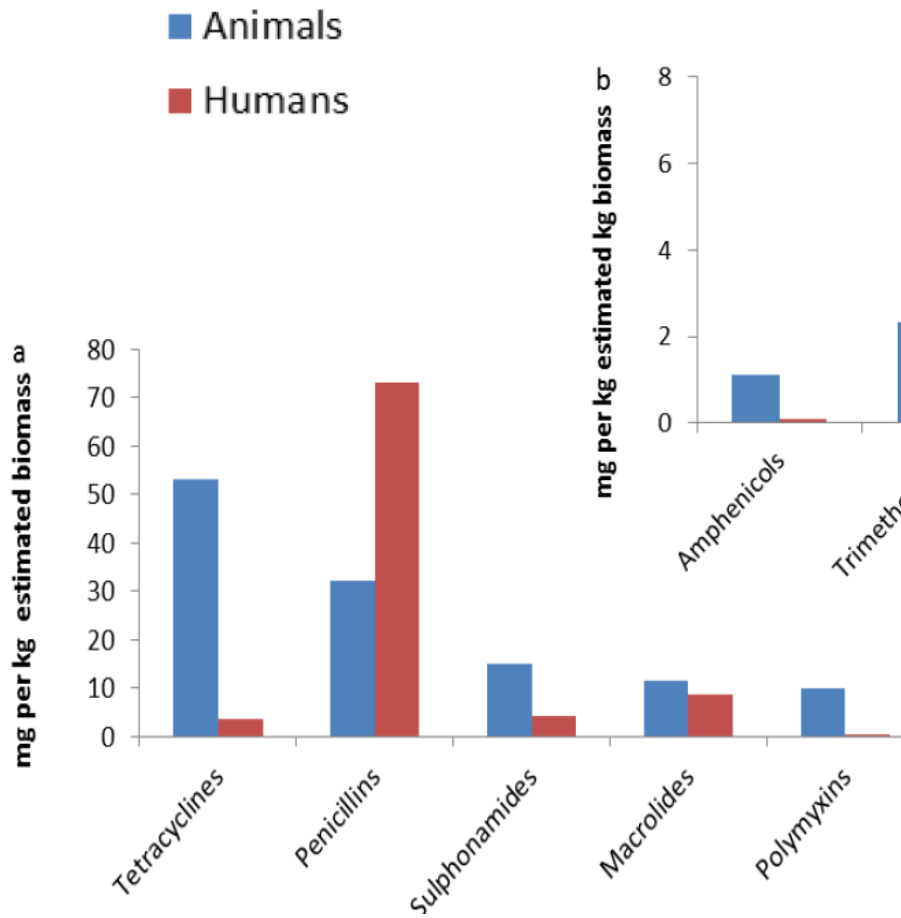
30 January 2015

**Table 1.** Harmonised set of antimicrobial substances used for the monitoring of resistance in zoonotic *Salmonella* spp. and *Campylobacter* spp. and indicator *E. coli* and enterococci isolates from food-producing animals and food over the period 2007–2013 (EFSA, 2007; 2008)

<i>Salmonella</i> spp.	<i>Campylobacter coli/C. jejuni</i>	Indicator <i>E. coli</i>	<i>Enterococcus</i> spp.
Ampicillin	Erythromycin	Ampicillin	Ampicillin
Cefotaxime	Ciprofloxacin	Cefotaxime	Chloramphenicol
Chloramphenicol	Tetracycline	Chloramphenicol	Erythromycin
Ciprofloxacin	Nalidixic acid	Ciprofloxacin	Gentamicin
Gentamicin	Gentamicin	Gentamicin	Linezolid
Nalidixic acid		Nalidixic acid	Quinupristin/dalfopristin
Sulfonamides		Sulfonamides	Streptomycin
Tetracycline		Tetracycline	Tetracycline
Trimethoprim		Trimethoprim	Vancomycin

Vir: ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals





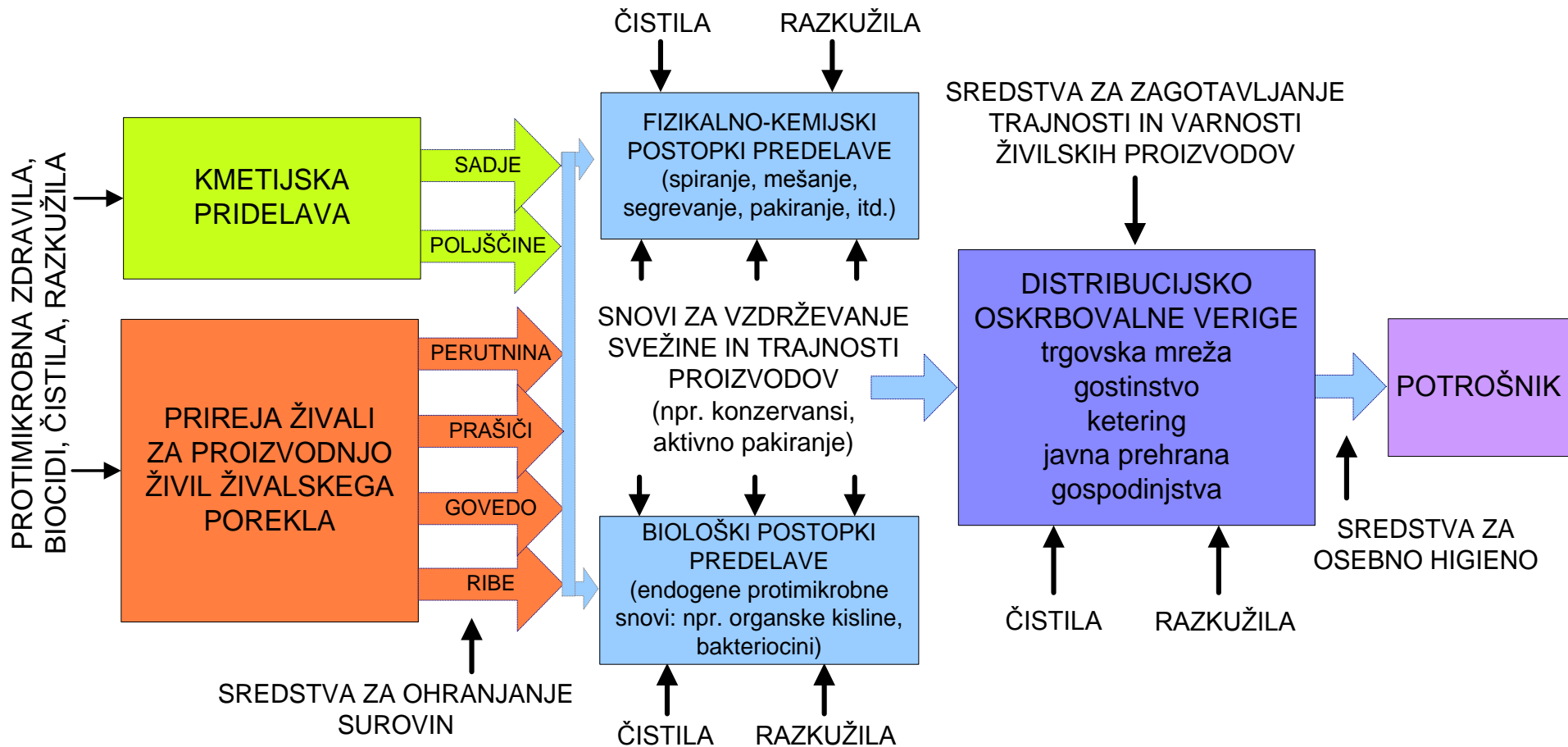
**Vir:** ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals



# Glavni poudarki poročila ECDC/EFSA/EMA

- V splošnem poraba protimikrobnih zdravil večja pri živalih;
- V večini analiziranih primerov potrjena pozitivna zveza med porabo in odpornostjo bakterij;
- Najmočnejša zveza potrjena pri indikatorskih bakterijah *E. coli*;
- Pozitivna zveza med porabo in odpornostjo potrjena tudi pri patogenih bakterijah *Salmonella* spp. in *Campylobacter* spp.

# Protimikrobne snovi v živilski verigi



# Kakšno je dejansko stanje?

---



- Pri katerih mikroorganizmih se pojavlja odpornost, kako pogosta je in na katere snovi?
- V katerih živilskih verigah oz. s katerimi izdelki se prenašajo odporni mikroorganizmi in zakaj?
- S kakšnimi metodami in sistemi se stanje spremlja in analizira... ali je primerljivo med različnimi obdobji in področji?

Zelo kompleksno področje...



## SCIENTIFIC REPORT OF EFSA AND ECDC

**The European Union summary report on trends and sources of zoonoses,  
zoonotic agents and food-borne outbreaks in 2013<sup>1</sup>**

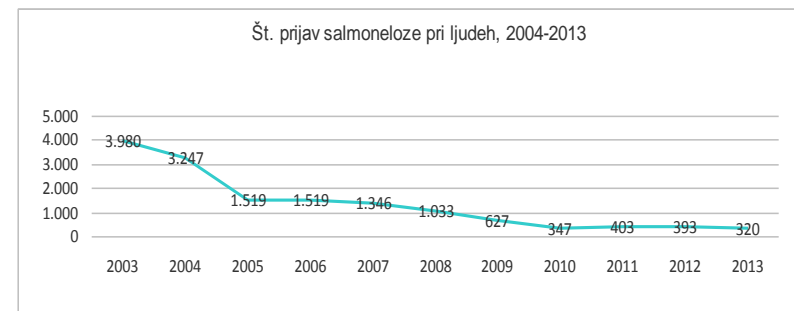
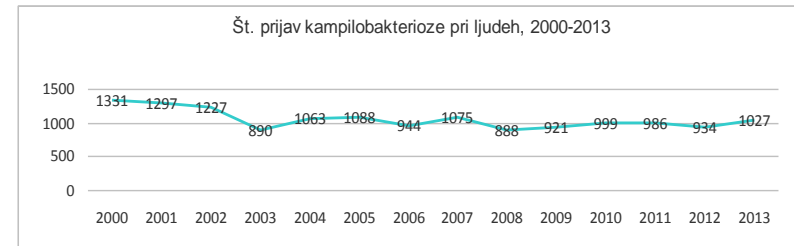
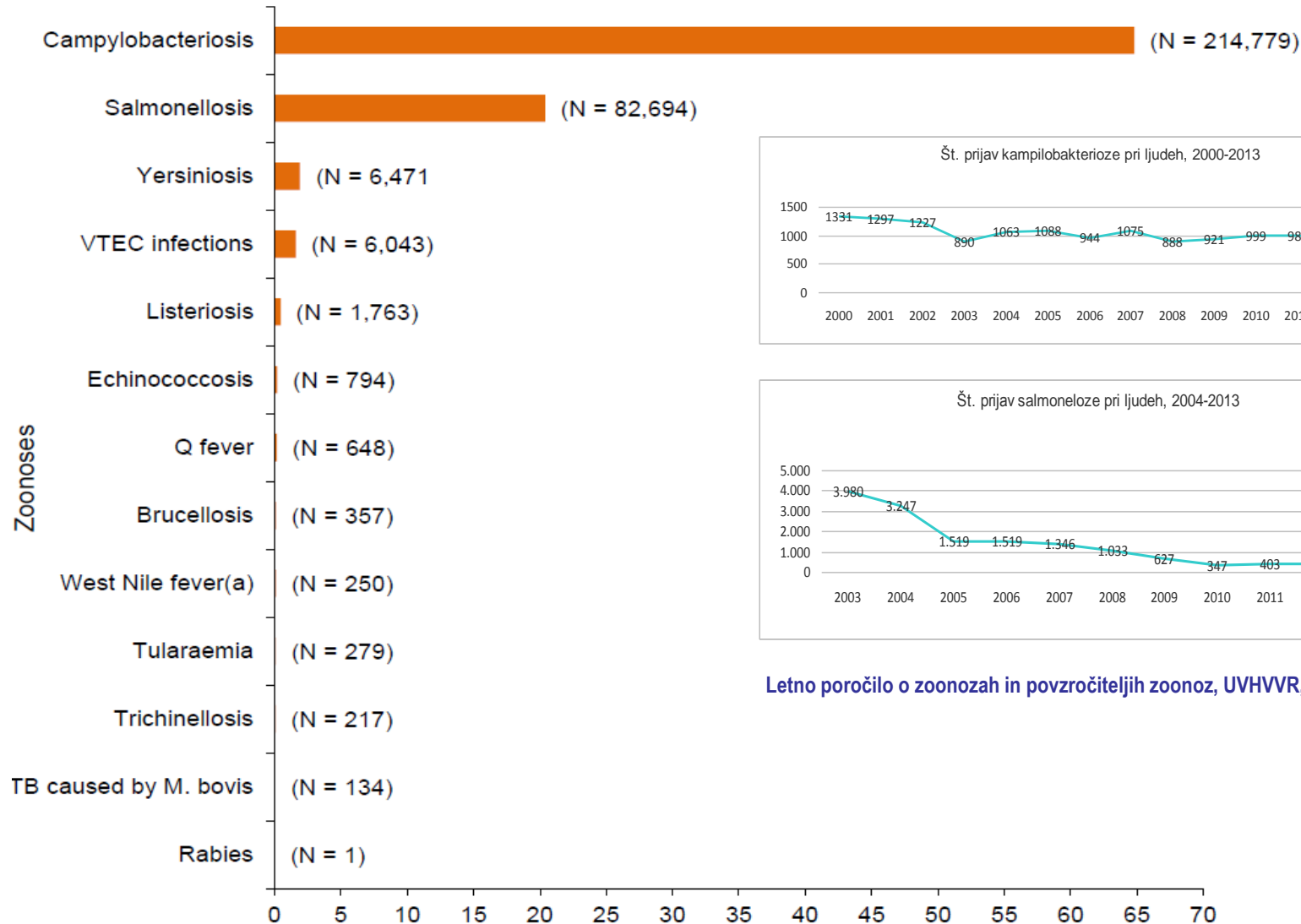
162 strani...

## SCIENTIFIC REPORT OF EFSA AND ECDC

**EU Summary Report on antimicrobial resistance in zoonotic and indicator  
bacteria from humans, animals and food in 2013<sup>1</sup>**

178 strani...

## Reported notification rates of zoonoses in confirmed human cases<sup>(b),(c)</sup> in the EU, 2013



Letno poročilo o zoonozah in povzročiteljih zoonoz, UVHVVR, 2014

# Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the EU in 2013

Figure 35. Trends in ciprofloxacin, erythromycin and nalidixic acid resistance in *Campylobacter jejuni* from *Gallus gallus* in reporting MSs and non-MSs, 2007–2013, quantitative data

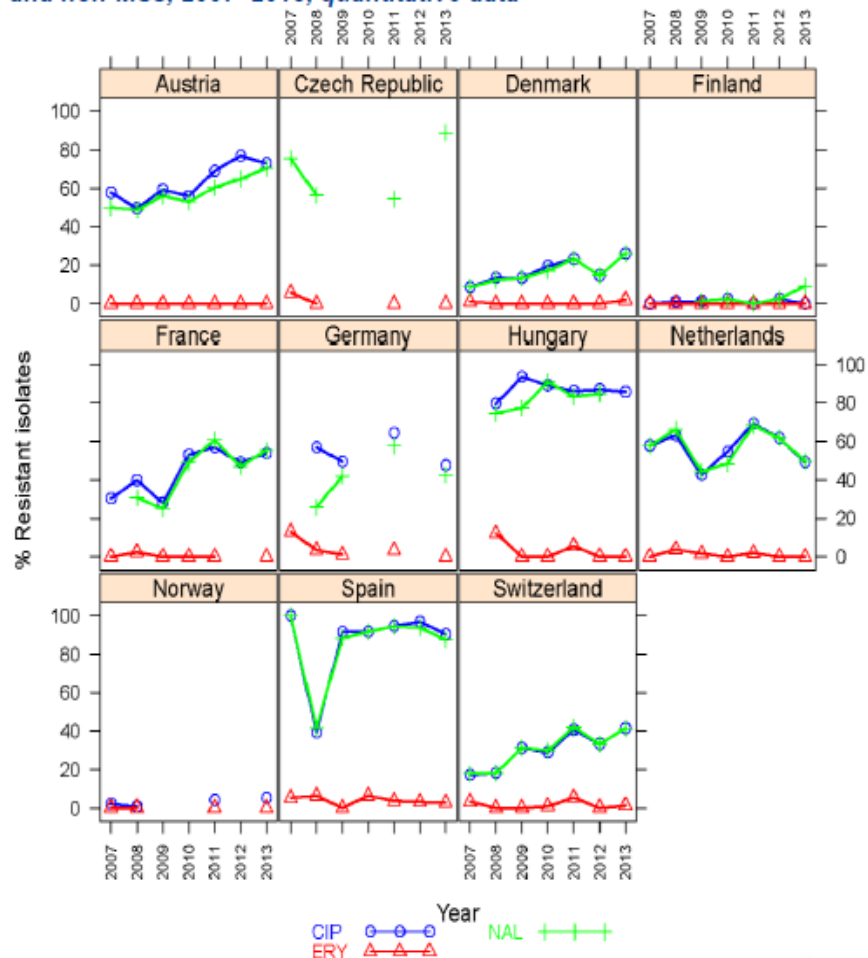
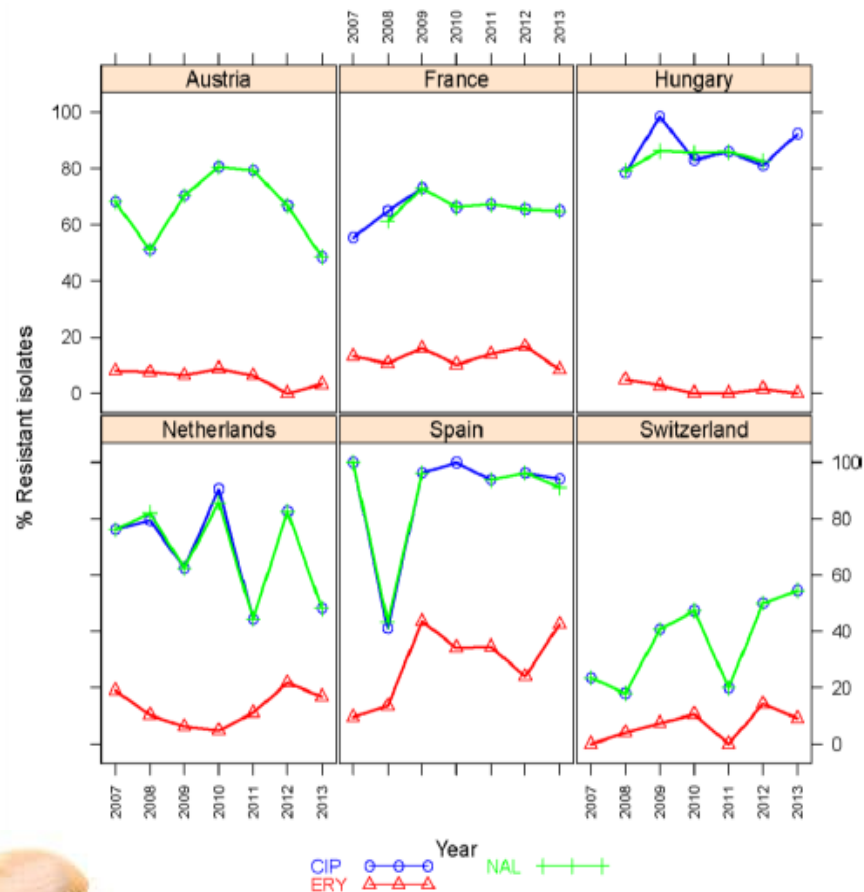


Figure 36. Trends in ciprofloxacin, erythromycin and nalidixic acid resistance in *Campylobacter coli* from *Gallus gallus* in reporting MSs and one non-MS, 2007–2013, quantitative data



# Problem mnogokratne odpornosti

Figure 39. Frequency distribution of *Campylobacter jejuni* isolates completely susceptible and resistant to one to five antimicrobials in broilers in MSs and non-MSs reporting isolate-based data, 2013

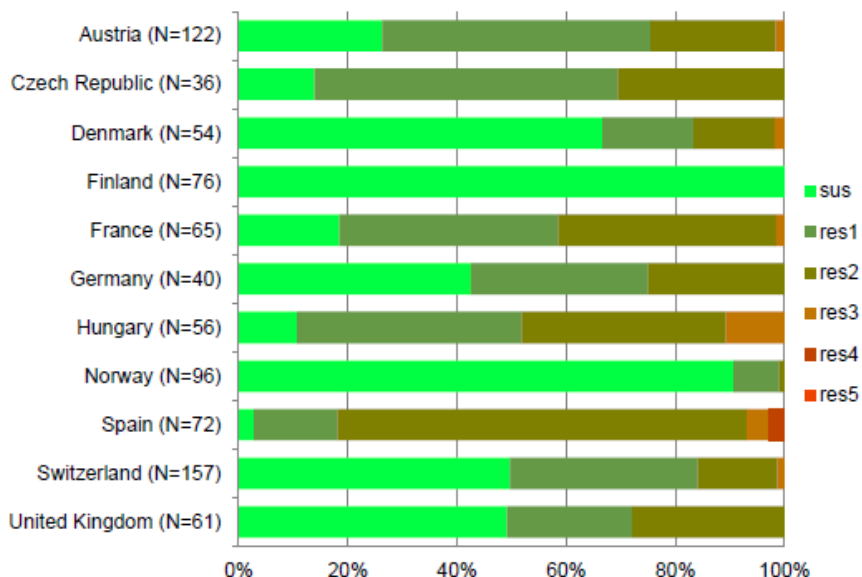


Figure 40. Frequency distribution of *Campylobacter coli* isolates completely susceptible and resistant to one to five antimicrobials, in broilers in MSs and one non-MS reporting isolate-based data, 2013

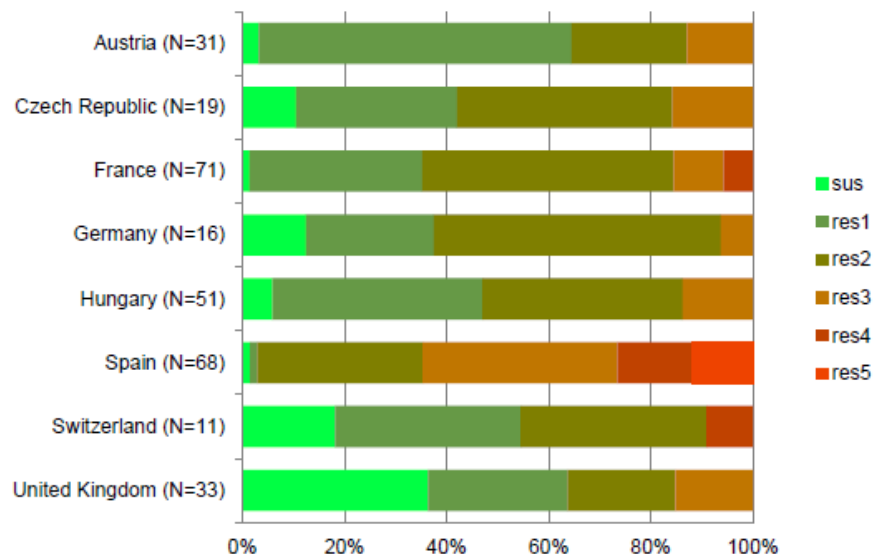
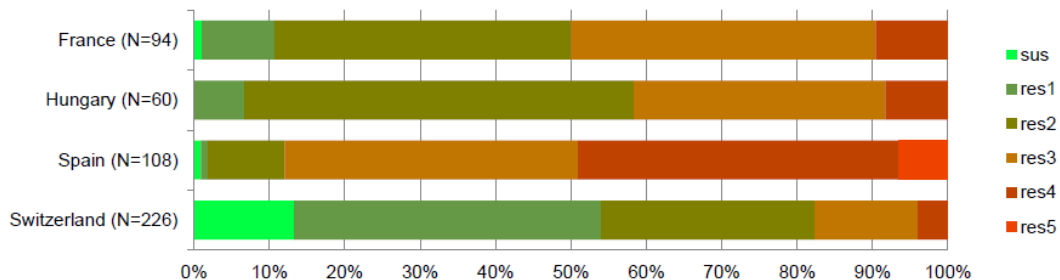


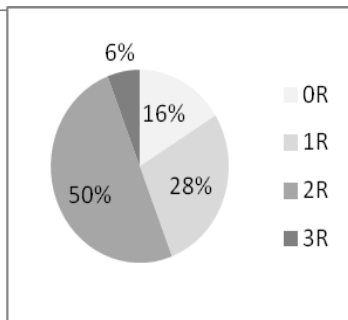
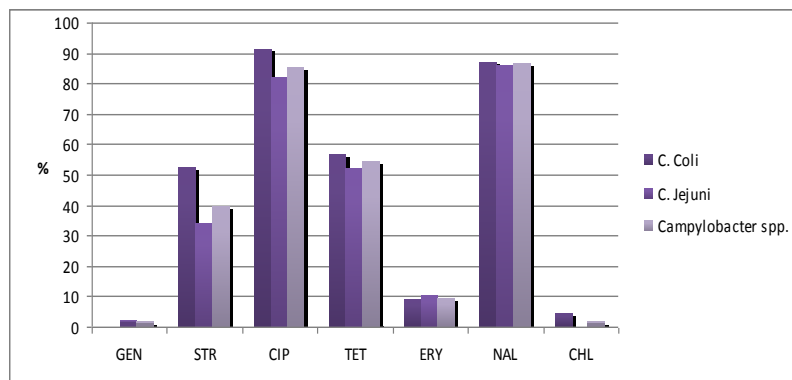
Figure 42. Frequency distribution of *Campylobacter coli* isolates completely susceptible and resistant to one to five antimicrobials, in fattening pigs in MSs and one non-MS reporting isolate-based data, 2013



# Pa v Sloveniji?

2009

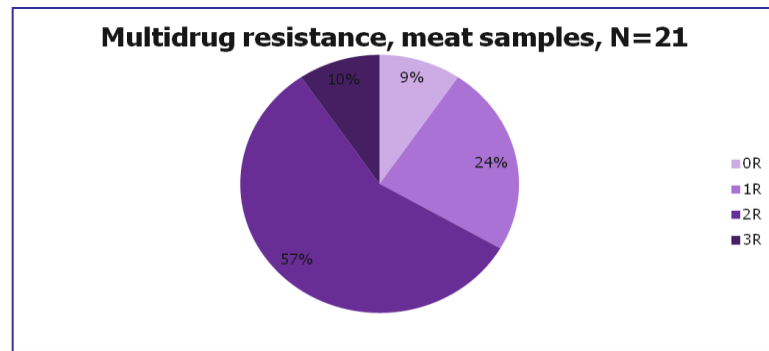
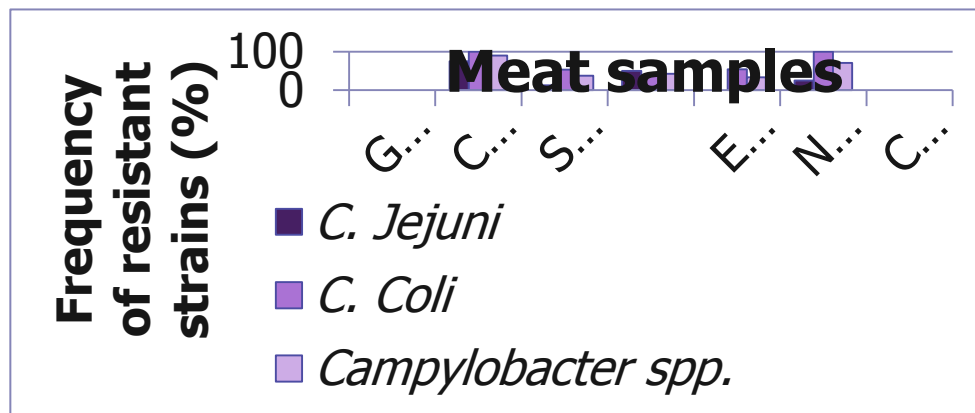
Tip živila	Št. vzorcev	Št. <i>Campy</i> poz. vzorcev
Sveže pišč. meso	187	139 (74.3 %)
Marin. pišč meso	25	12 (48.0 %)
Zel. list. zel.	50	0
Solate s pišč. M.	50	0



S. Smole Možina: Mikrobna rezistenca v živilski verigi

2011

Tip živila	Št. vzorcev	Št. <i>Campy</i> poz. vzorcev
Sveže pišč. meso	28	21 (75 %)
Površinske vode	90	19 (21.1 %)

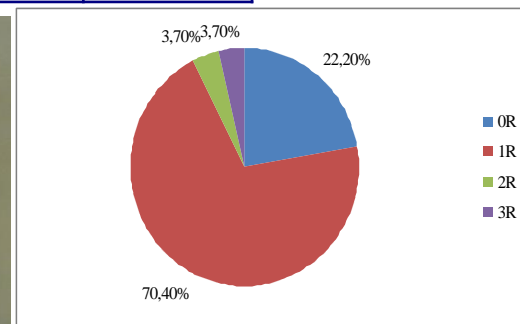
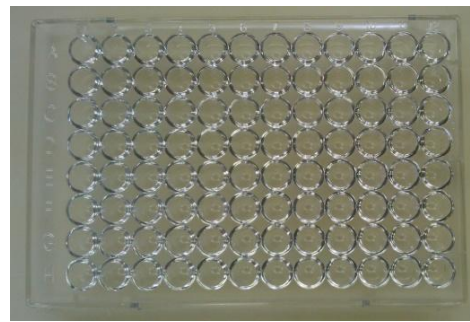
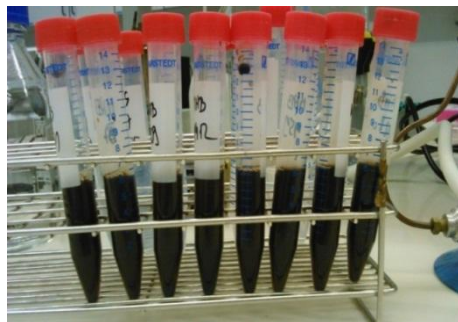
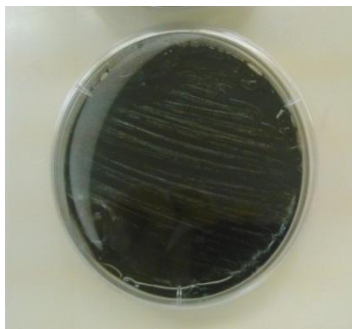


# Pa v Sloveniji?

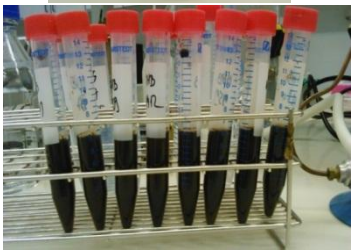
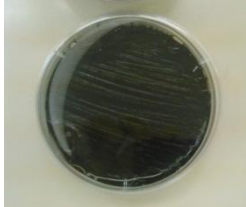
2013

Preglednica števila odpornih (R) in števila občutljivih (S) izolatov *Campylobacter jejuni* iz svežega piščančjega mesa v prodaji I. 2013 na posamezne testirane antibiotike (CRP V4-1110)

	GEN	CIP	TET	ERY	NAL	CHL	STR
R	1	20	2	1	17	1	1
S	26	7	25	26	10	26	26
R (%)	3,7	74	7,4	3,7	63	3,7	3,7
S (%)	96,3	26	92,6	96,3	37	96,3	96,3



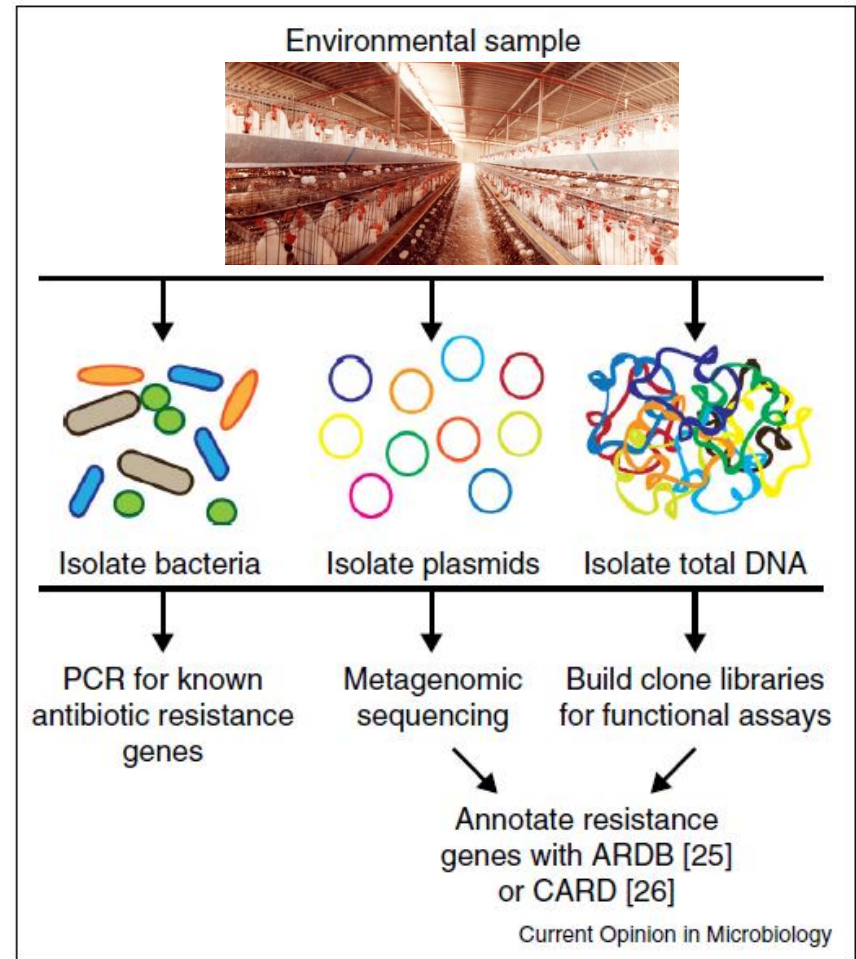
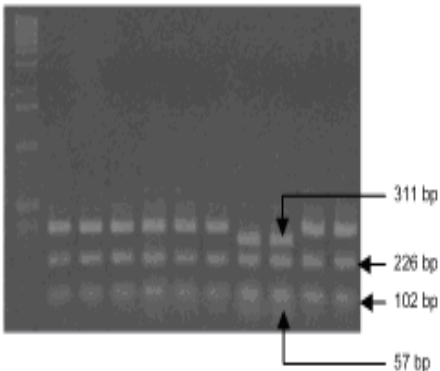
# Kaj pa metode ugotavljanja odpornosti?



Iščemo, kar poznamo..



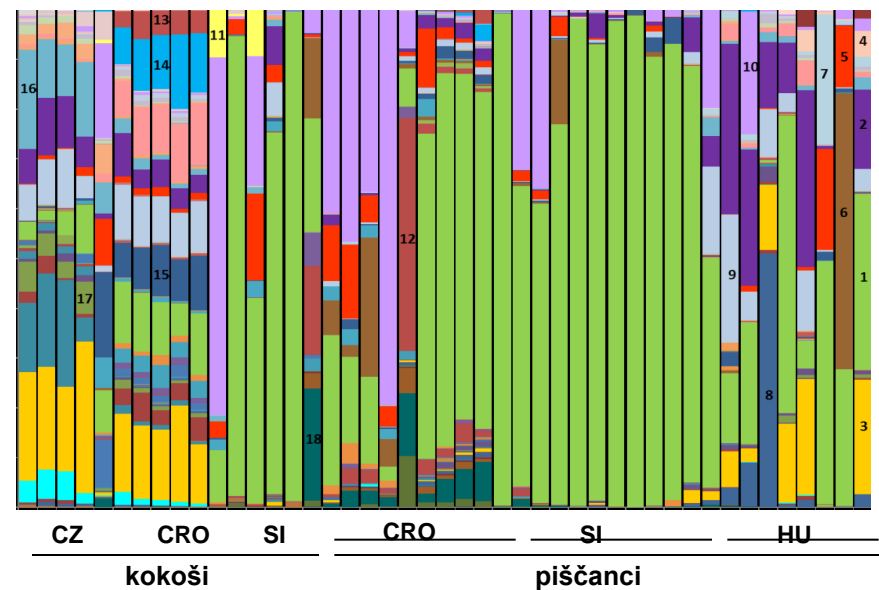
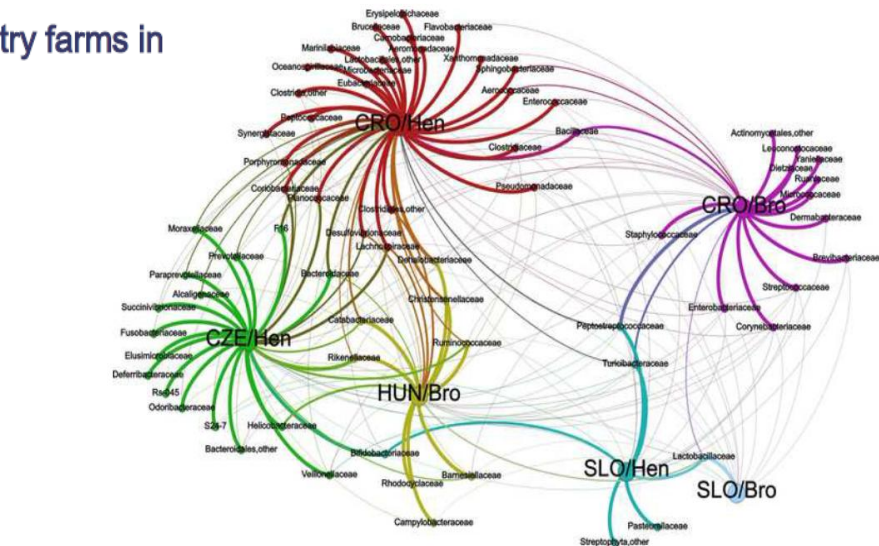
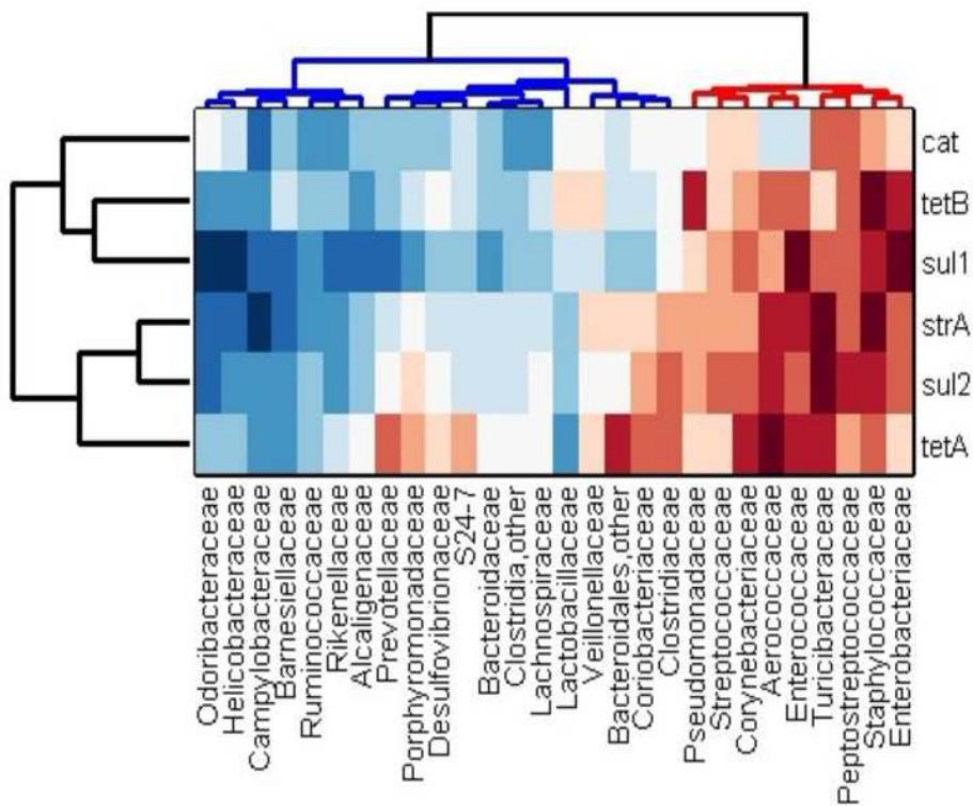
Strain	MIC (µg/mL)	Nucleotide position 2075
ATCC 33559	2	CAAGACGGAA AGACC
VC 110722	8	CAAGACGGAA AGACC
VC 110725	8	CAAGACGGAA AGACC
M37	512	CAAGACGGAG AGACC
VC 11076	>512	CAAGACGGAG AGACC
137	>512	CAAGACGGAG AGACC
140	>512	CAAGACGGAG AGACC



Antibiotic resistance gene discovery in food-producing animals

Current Opinion in Microbiology 2014, 19:25–29

# Characterisation of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia



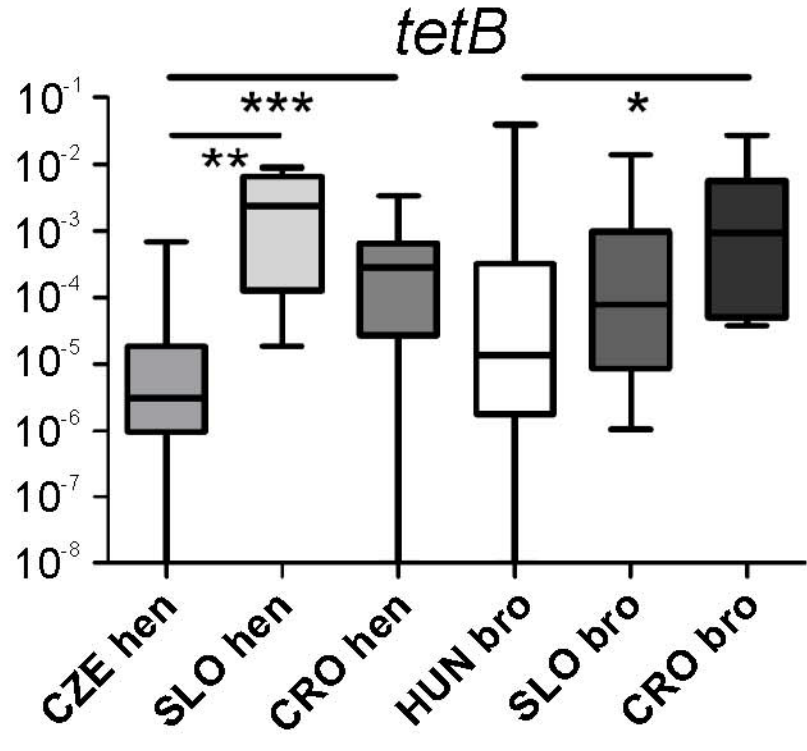
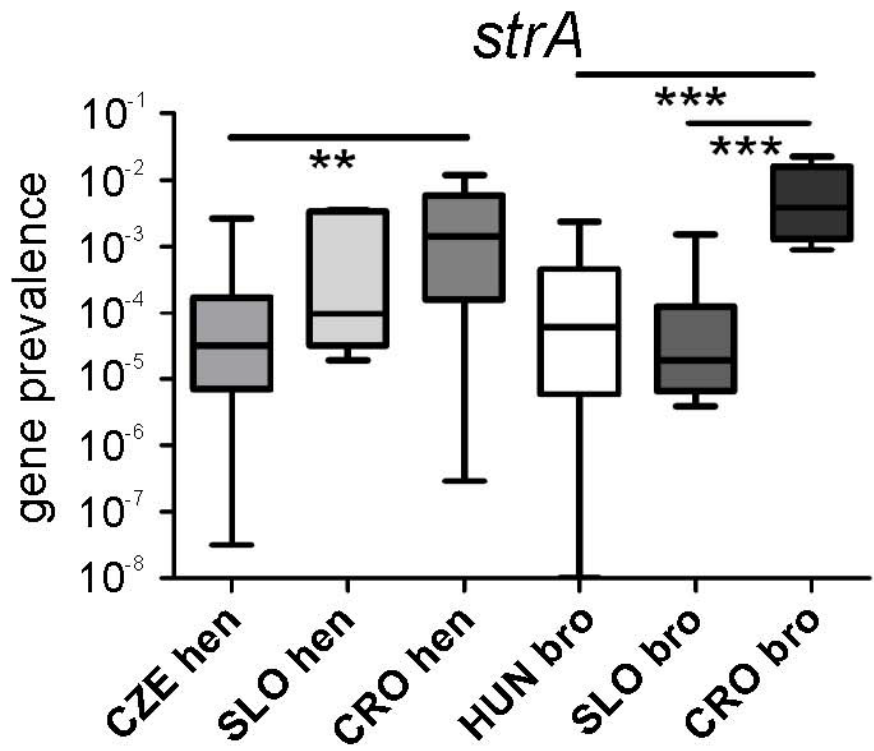
Petra Videnska<sup>1</sup>, Md. Masudur Rahman<sup>1</sup>, Marcela Faldynova<sup>1</sup>, Vladimir Babak<sup>1</sup>, Marta Elsheimer Matulova<sup>1</sup>, Estella Prukner-Radovic<sup>2</sup>, Ivan Krizek<sup>2</sup>, Sonja Smole-Mozina<sup>3</sup>, Jasna Kovac<sup>3</sup>, Ama Szmolka<sup>4</sup>, Bela Nagy<sup>4</sup>, Karel Sedlar<sup>5</sup>, Darina Cejkova<sup>1</sup>, Ivan Rychlik<sup>1\*</sup>



# Primer uporabe metagenomike za ugotavljanje rezistenčnih genov...

## PLOS ONE

### Characterisation of egg laying hen and broiler fecal microbiota in poultry farms in Croatia, Czech Republic, Hungary and Slovenia

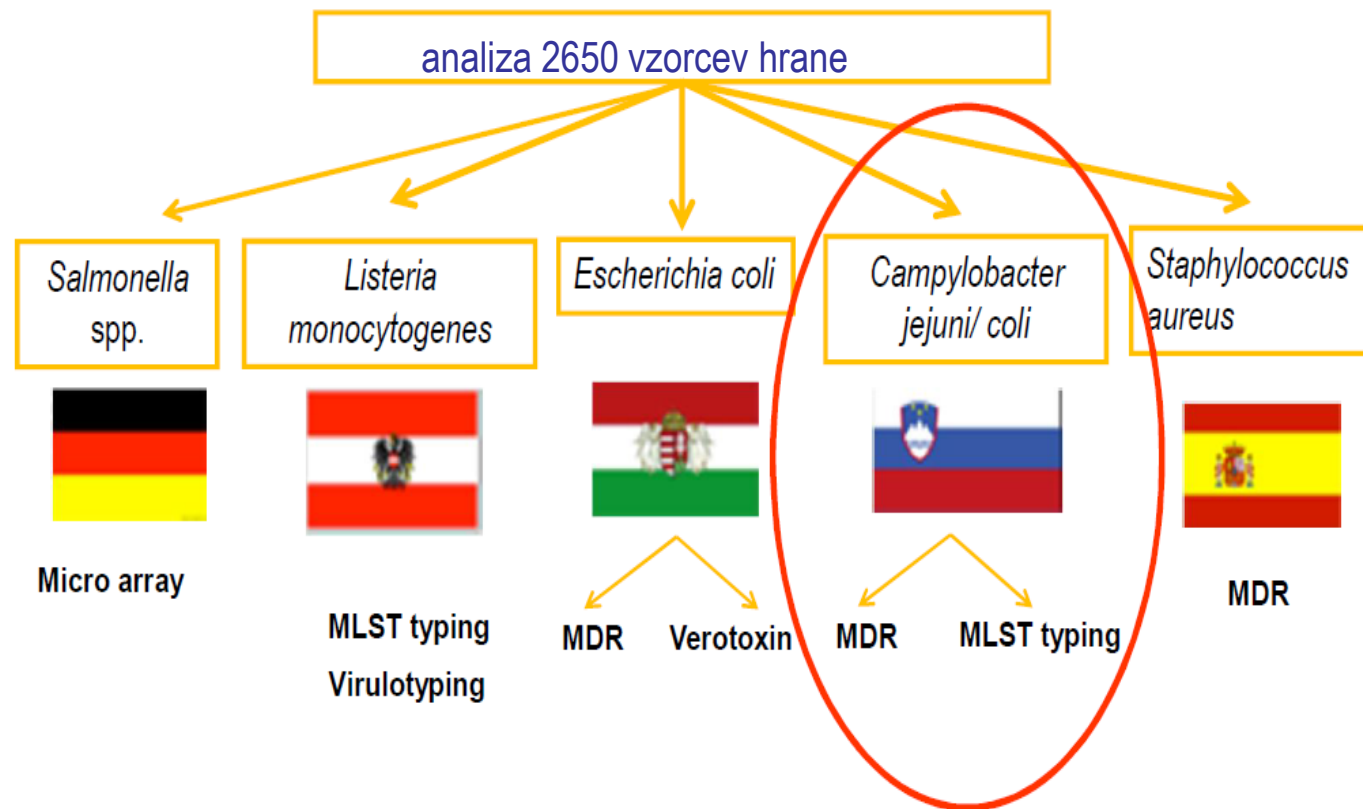


# ZAŠČITA POTROŠNIKOV Z ZMANJŠANJEM MIKROBIOLOŠKEGA TVEGANJA S HRANO – PROJEKT “PROMISE” IN PRISPEVEK SLOVENSКИH SODELAVCEV

»PROMISE« (PROtection of consumers by Microbial mitigation through combating SEgregation of expertise) je raziskovalni projekt, ki ga v 7. OP financira EU z namenom boljšega povezovanja strokovnjakov za učinkovito reševanje problemov, povezanih z varnostjo hrane. Projekt vključuje raziskovalne in druge skupine, kot so zavodi in uradi za varno hrano v starih in novih članicah ter kandidatkah za EU.

## DS 1: Nadzor spregledanih virov prenosa patogenov (nelegalen vnos hrane v EU)

- Cilj – zbrati 2500 vzorcev zasežene hrane
- Odkrivanje prisotnosti bakterij:
  - *E. coli* (verotoksigene)
  - *Campylobacter* (termotolerantni)
  - *Salmonella*
  - *Staphylococcus aureus*
  - *Listeria monocytogenes*
- Analiza odpornosti proti protimikrobnim zdravilom in virulenčnih lastnosti ter genotipizacija

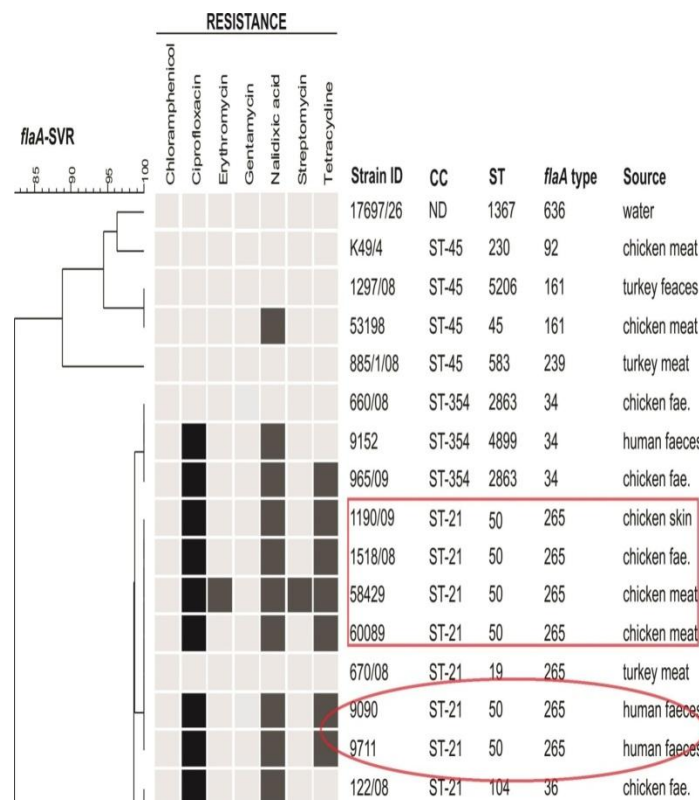
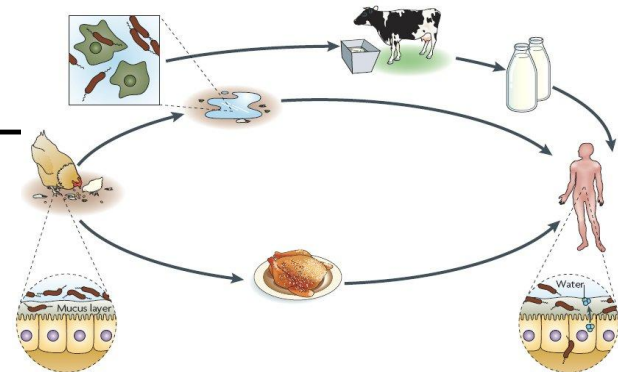


# The evidence for clonal spreading of quinolone resistance with a particular clonal complex of *Campylobacter jejuni*

Table 1. Prevalence of *Campylobacter jejuni* isolates from different sources in identified clonal complexes in this study

CC	No. of STs	Source (no. of isolates)				Total
		Human	Animal	Meat	Water	
21	4	4	10 (53%)	5		19
45	5		1	6 (75%)	1	8
48	3	1	1	1		3
206	1		1			1
353	2	3		3		6
354	3	1	3	1		5
403	1		1			1
464	2		1	1		2
607	1	1				1
658	2				2	2
Not defined	4		1		3	4
<b>Total</b>	<b>28</b>	<b>10</b>	<b>9</b>	<b>11</b>	<b>6</b>	<b>52</b>

CC, Clonal complex; STs, sequence types.



## The evidence for clonal spreading of quinolone resistance with a particular clonal complex of *Campylobacter jejuni*

Table 2. Distribution of antibiotic resistant strains in clonal complexes

CC	No. of isolates	STR	CIP	TET	ERY	NAL	MDR
21	19	1	18 (95%)	7 (37%)	1	18 (95%)	1
45	8		1	1		2	
353	6		4 (67%)			4 (67%)	
354	5		3 (60%)	2		2 (40%)	
206	1		1	1		1	
403	1			1			
464	2		1				
48	3	1	1	1	1	1	1
607	1						
658	2		1			1	
Other	4		2			1	
Total	52	2	7	6	2	6	2

CC, Clonal complex; STR, streptomycin; CIP, ciprofloxacin; TET, tetracycline; ERY, erythromycin; NAL, nalidixic acid; MDR, multidrug-resistant strain (resistant against  $\geq 3$  unrelated antibiotics).

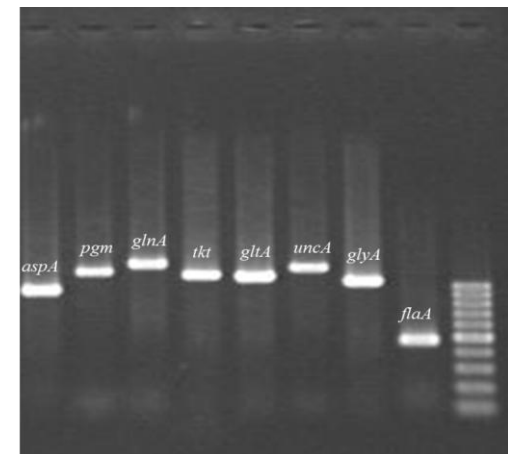
J. KOVAČ<sup>1</sup>, N. ČADEŽ<sup>1</sup>, M. LUŠICKY<sup>2</sup>, E. MØLLER NIELSEN<sup>3</sup>, M. OCEPEK<sup>4</sup>, P. RASPOR<sup>1</sup> AND S. SMOLE MOŽINA<sup>1\*</sup>



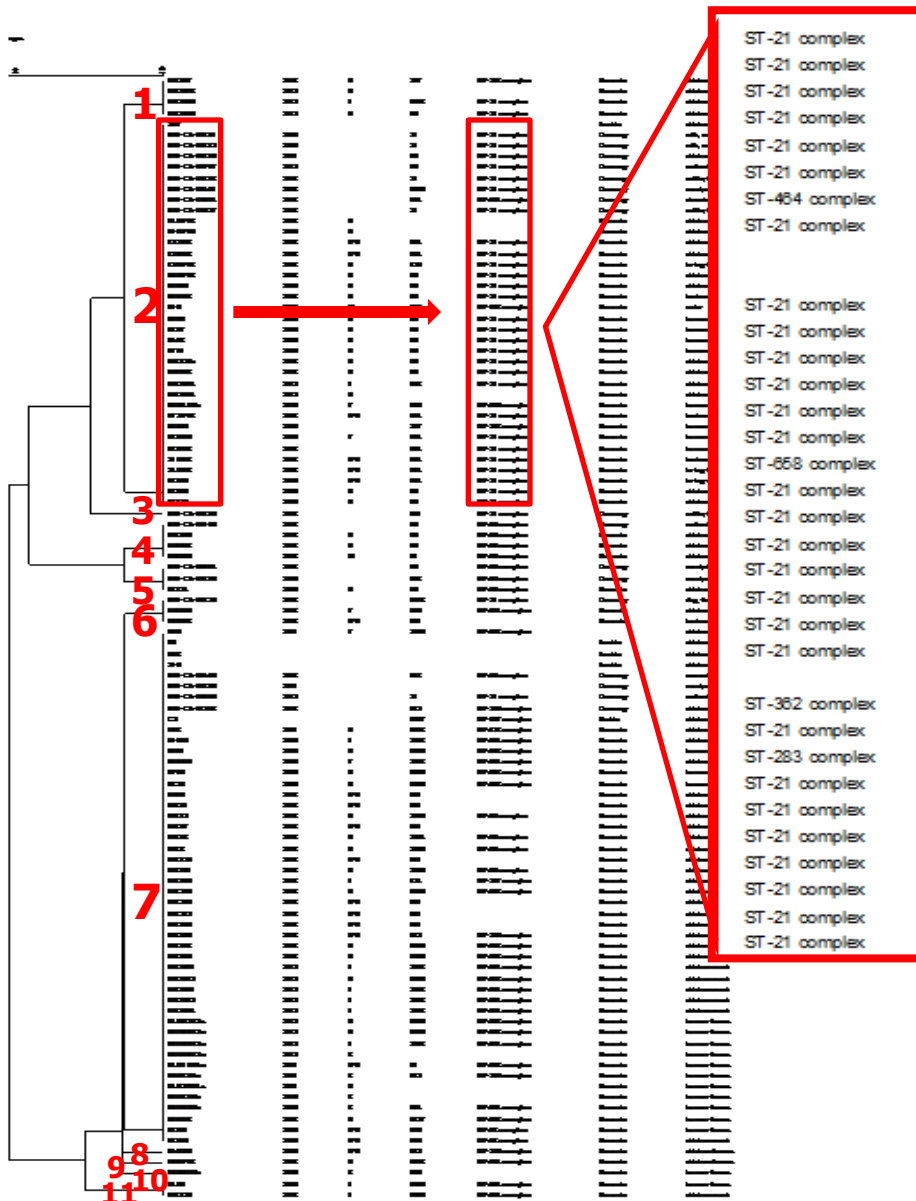
# Je klonsko širjenje rezistence lokalno omejeno?

- **Večja raziskava 185 sevov CIP-R *C. jejuni*:**
  - iz različnih geografskih regij (60 % iz Slovenije, 23 % Avstrije, 15% Nemčije, ostalo iz Balkanskih držav)
  - različni izvori: živali in meso (71%), humani (23%), okoljski (voda, divje živali (6%))

- **MLST, *flaA* , PFGE genotipizacija**



# Molekularna epidemiologija CIP-R *C. jejuni*



Širjenje antibiotične rezistence (CIP-R)

Širjenje odpornih klonov

CIP-R sevi imajo točkasto mutacijo v QRDR regiji gena *gyrA*

Ugotavljanje genetske podobnosti na osnovi sekvence QRDR in tipa MLST



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 265877.



# Kaj lahko storimo?

RESEARCH ARTICLE

## Antibiotic Resistance Modulation and Modes of Action of (-)- $\alpha$ -Pinene in *Campylobacter jejuni*

Jasna Kovač<sup>1</sup>, Katarina Šimunović<sup>1</sup>, Zuowei Wu<sup>2</sup>, Anja Klančnik<sup>1</sup>, Franz Bucar<sup>3</sup>, Qijing Zhang<sup>2</sup>, Sonja Smole Možina<sup>1\*</sup>

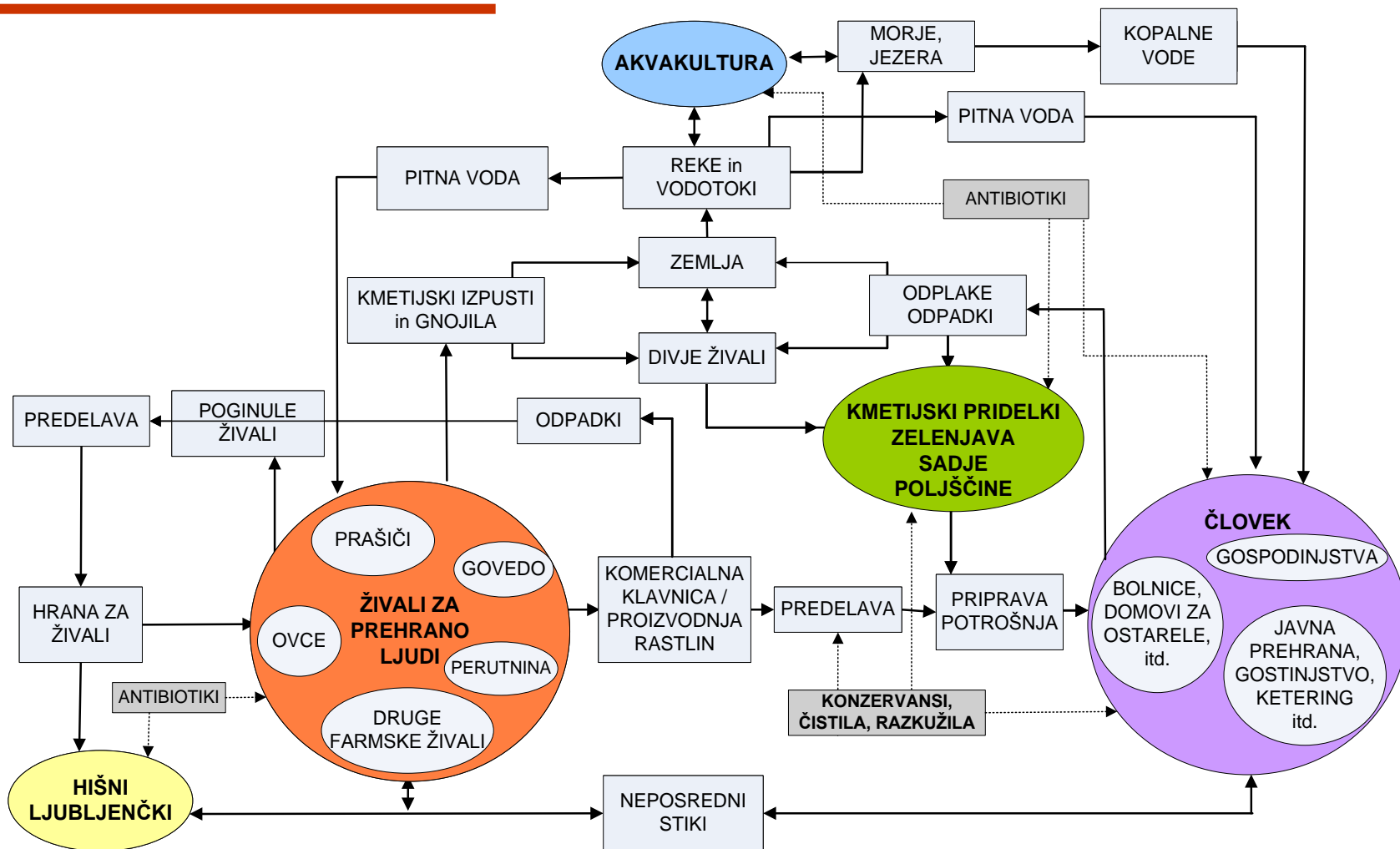
PLOS ONE | DOI:10.1371/journal.pone.0122871 April 1, 2015

1 / 14

## EPIDEMIOLOŠKE ZNAČILNOSTI ANTIBIOTSKE ODPORNOSTI TER PROTIMIKROBNO IN MODULATORNO DELOVANJE IZBRANIH FITOKEMIKALIJ NA BAKTERIJE *Campylobacter jejuni*

**Jasna Kovač, BF, 16.4.2015**  
DOKTORSKA DISERTACIJA

# Povzetek o razvoju odpornosti na protimikrobne snovi v živilski verigi





# Zaključki

---

- Tveganje za razvoj mikrobne odpornosti je veliko:  
(po mnenju mnogih zapostavljeno)
  - ker se v kmetijstvu porabi največ protimikrobnih snovi;
  - ker se uporabljajo v subterapevtskih koncentracijah;
  - ker se uporabljajo klinično pomembne ali njim zelo sorodne skupine učinkovin;
  - ker smo širjenju odpornih sevov izpostavljeni z uživanjem živilskih proizvodov in s širjenjem v okolje (preko tal, vode, zraka...)

# Nujni ukrepi

---

- Racionalna uporaba protimikrobnih snovi  
(strokovna in odgovorna- razum in etični odnos pred profitno logiko!
- Javno dostopni rezultati sistemov spremljanja rezistence in porabe antibiotikov!
- Podpora raziskavam
- Podpora splošnemu izobraževanju

**HVALA ZA POZORNOST!**