

Genetic relatedness of quinolone resistant *Campylobacter jejuni* in central-European countries

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

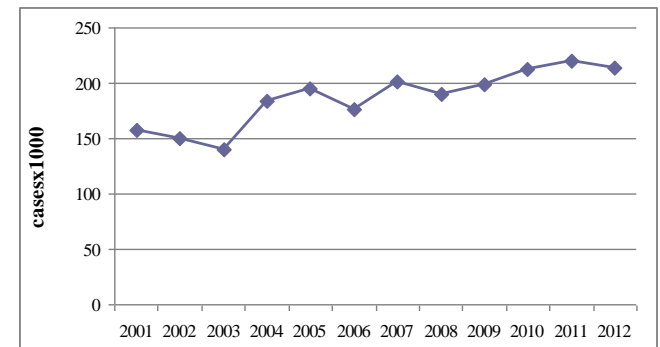
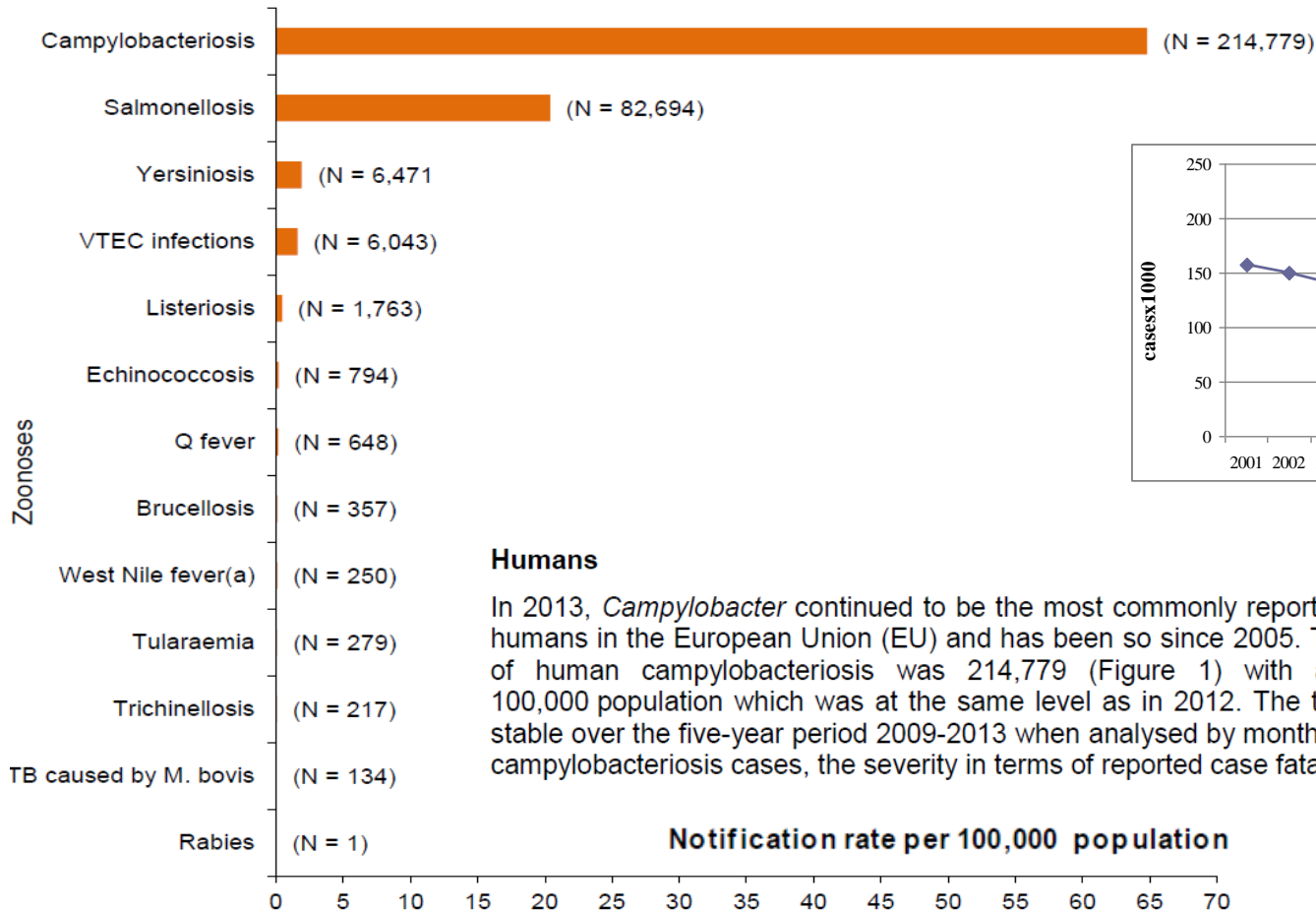
promise

Outlines of presentation

- ***Campylobacter* in EU prevalence and resistance reports .. and in PROMISE project...**
- **Materials and methods in experimental work with quinolone resistance and its spreading**
- **Results**
- **Conclusions**

Pathogenic *Campylobacter* in EU....

Reported notification rates of zoonoses in confirmed human cases^{(b),(c)} in the EU, 2013



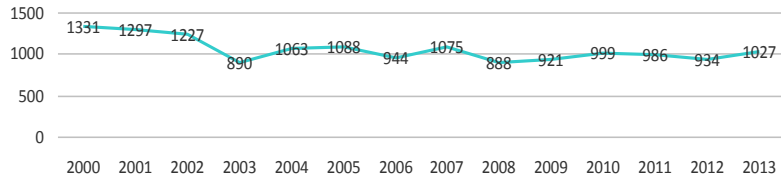
Humans

In 2013, *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) and has been so since 2005. The number of reported confirmed cases of human campylobacteriosis was 214,779 (Figure 1) with an EU notification rate of 64.8 per 100,000 population which was at the same level as in 2012. The twelve-month moving average was fairly stable over the five-year period 2009-2013 when analysed by month. Considering the high number of human campylobacteriosis cases, the severity in terms of reported case fatality was low (0.05 %) (Table 1).

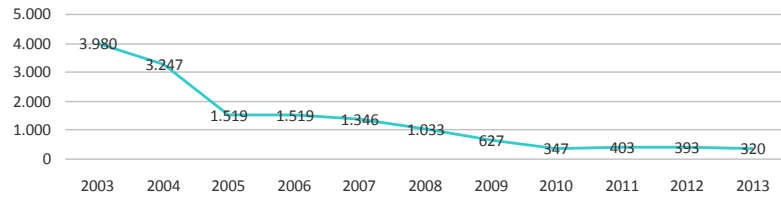
Notification rate per 100,000 population

...and some national reports

Human campylobacterioses 2000-2013, Slovenia

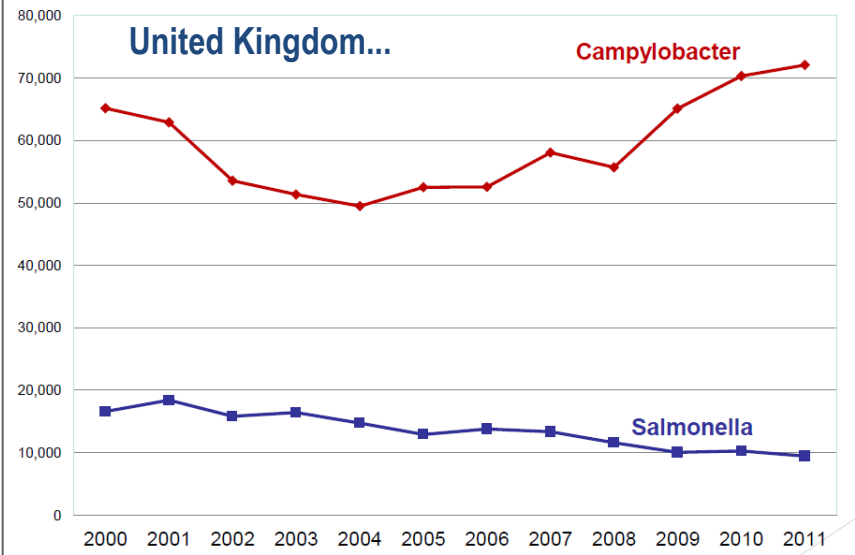


Human salmonellos 2004-2013, Slovenia



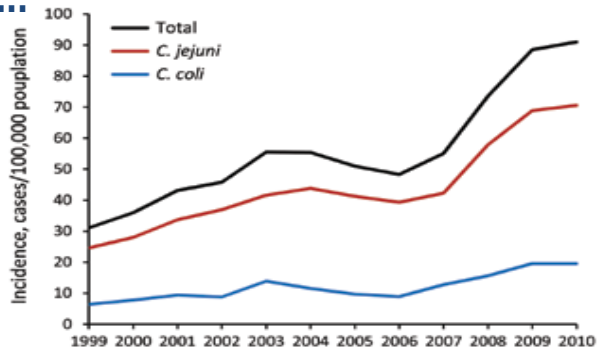
United Kingdom...

Campylobacter

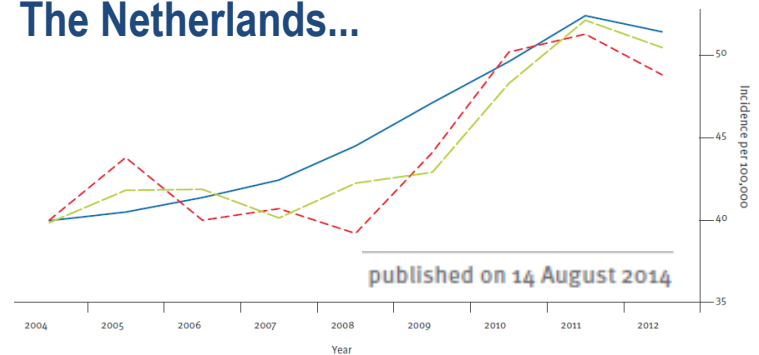


Israel...

www.cdc.gov/eid • Vol. 19, No. 11, November 2013



The Netherlands...



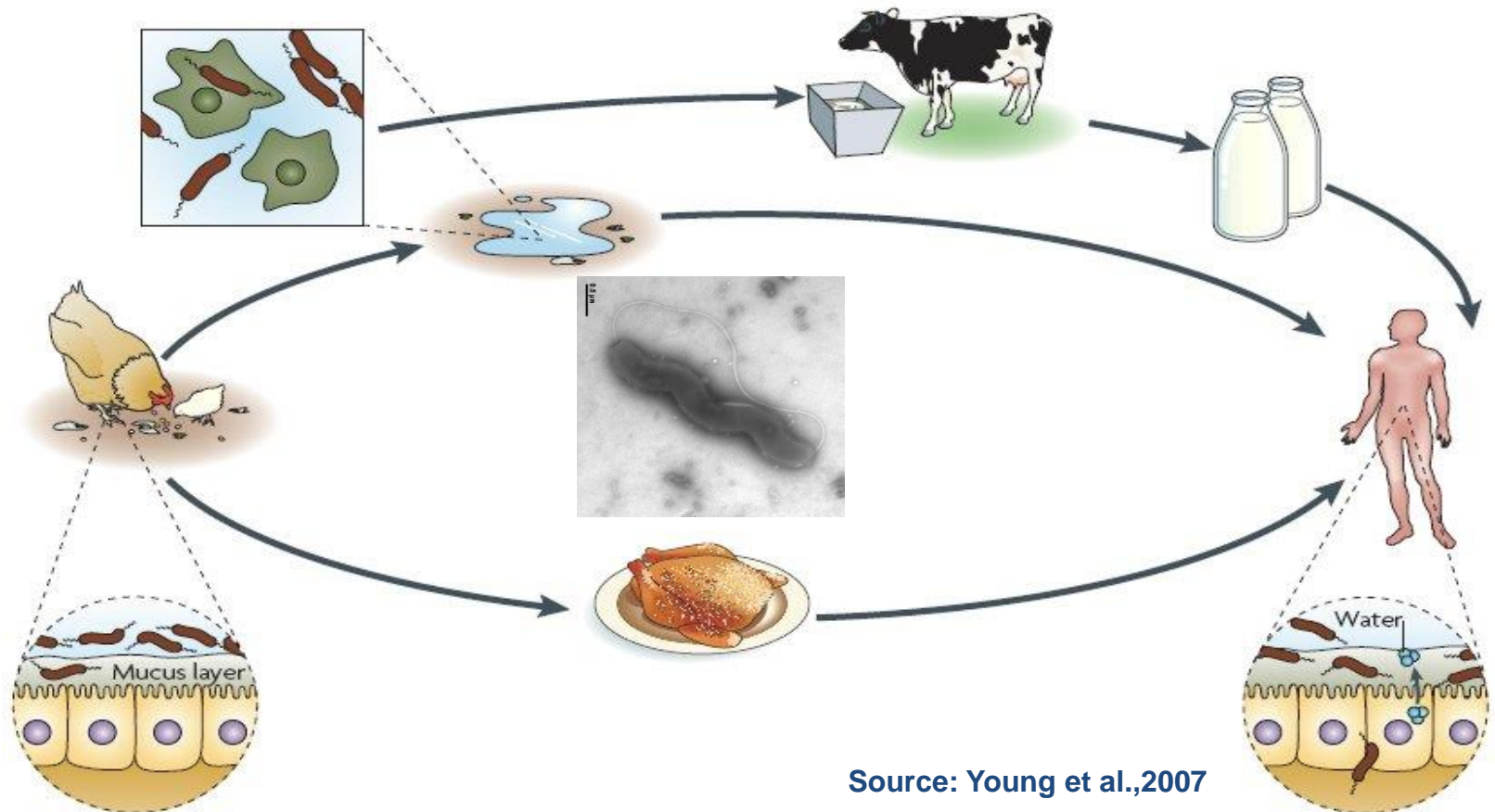
published on 14 August 2014



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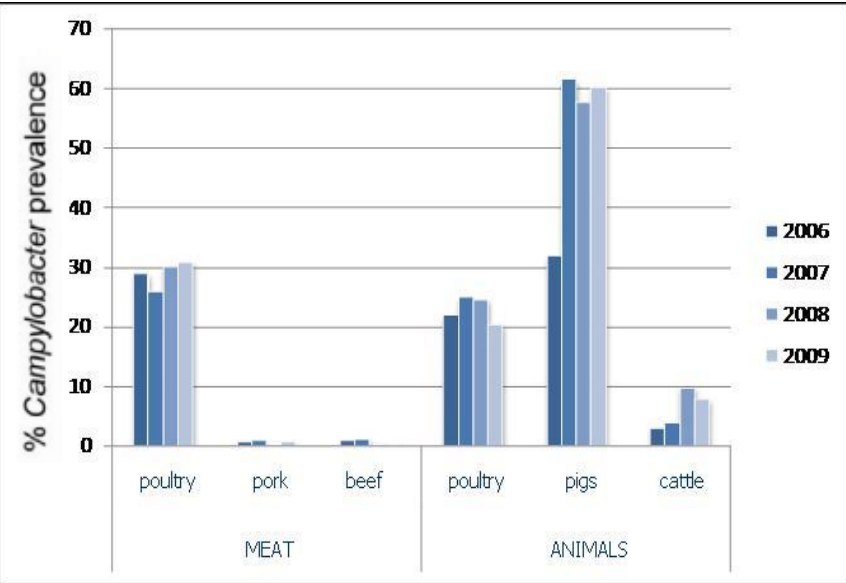
Epidemiology of *C. jejuni*



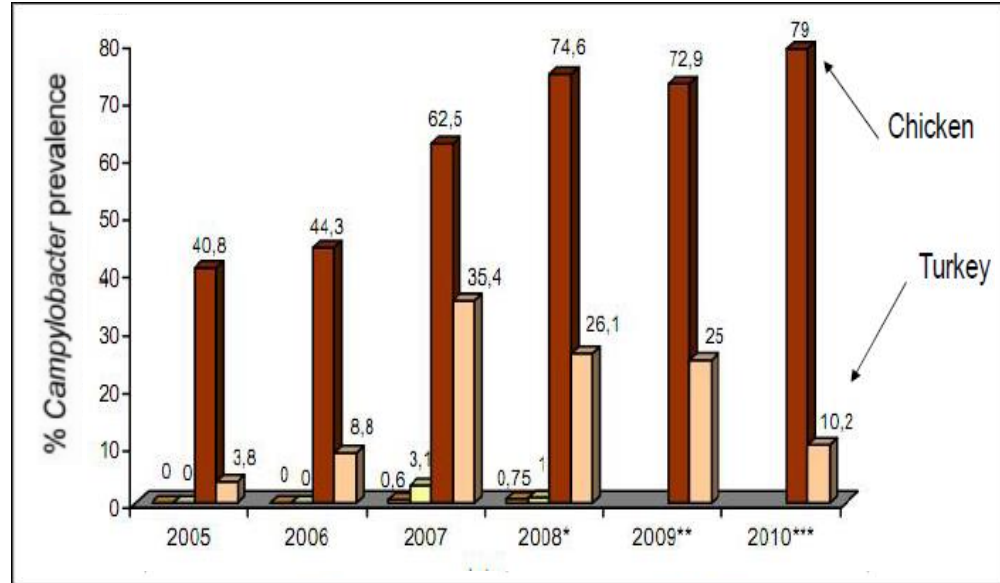
Prevalence of *Campylobacter* in farm animals and retail meat in EU...



...and some national reports (SI)



Smole Možina et al.:TIFS, 2011



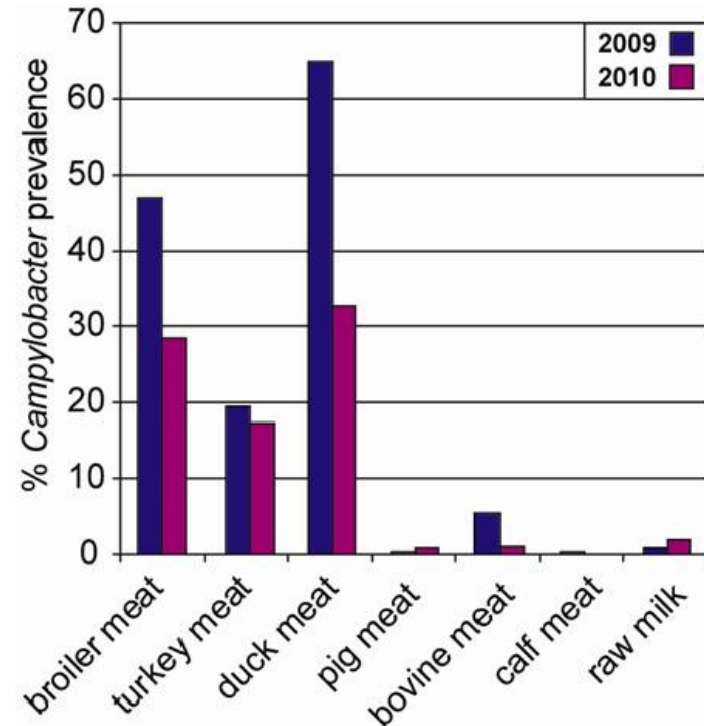
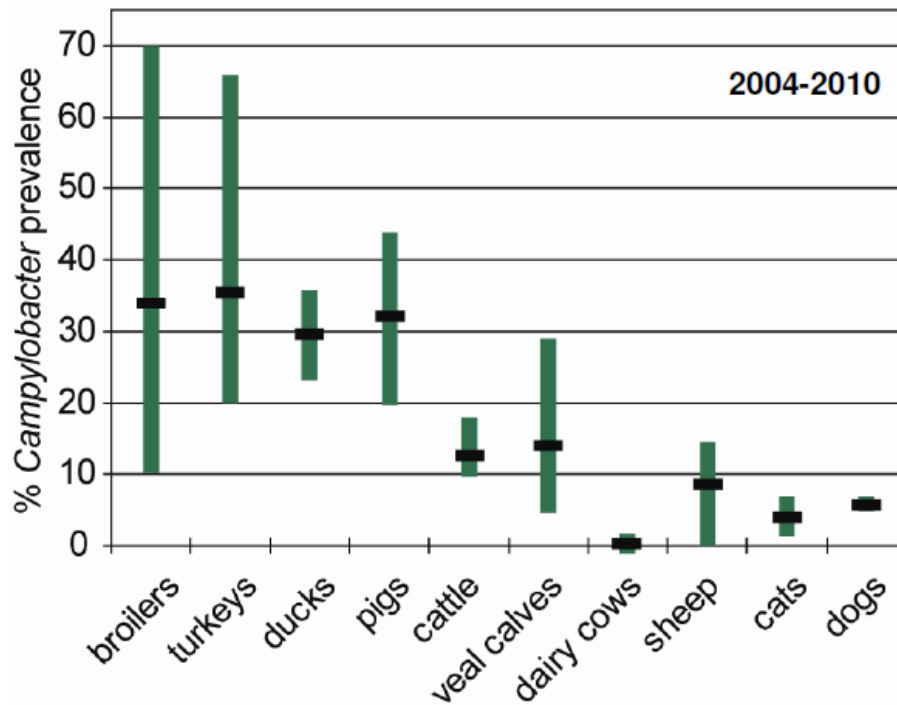
VURS, SI, 2012



...and in some national reports (Germany)

Prevalence of *Campylobacter* in German livestock and food

Stingl et al., Eur. J. Microbiol. Immunol., 2012, Vol.2:88-96



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

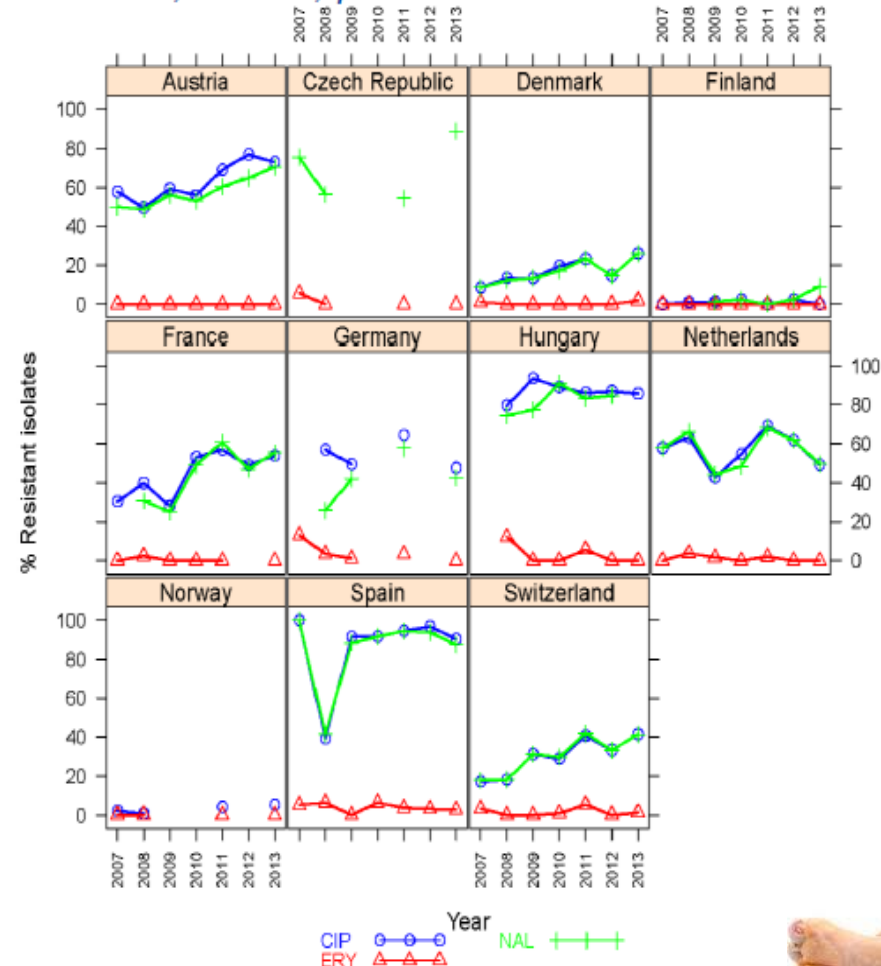
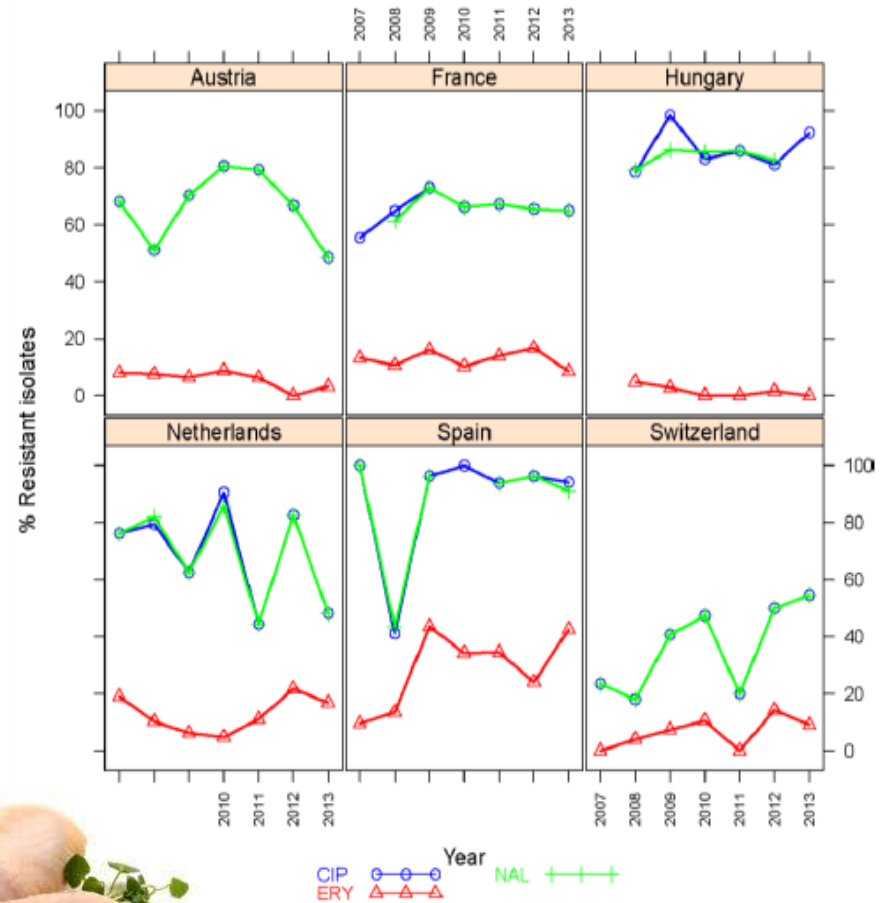


Figure 36. Trends in ciprofloxacin, erythromycin and nalidixic acid resistance in Campylobacter coli from Gallus gallus in reporting MSs and one non-MS, 2007–2013, quantitative data

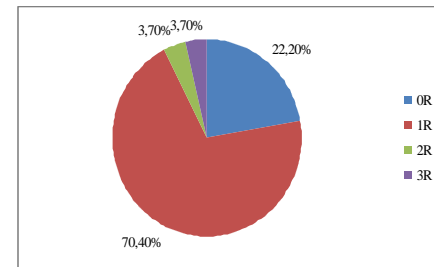
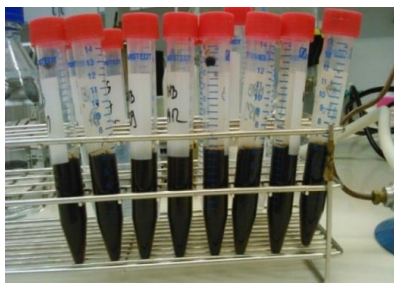
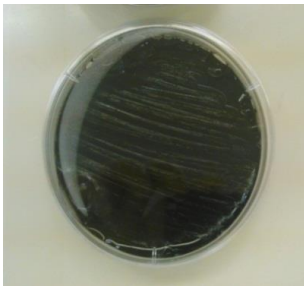


...and in Slovenia: 2012-2013 (sampling Nov 12- Jun 13)

111 samples of retail fresh chicken meat; ISO 10272 : 56% *Campy* pos., ~10-100 CFU/g, 9 PFGE types, 11 MLST types (most frequent CC-21, CC-353, CC-354).

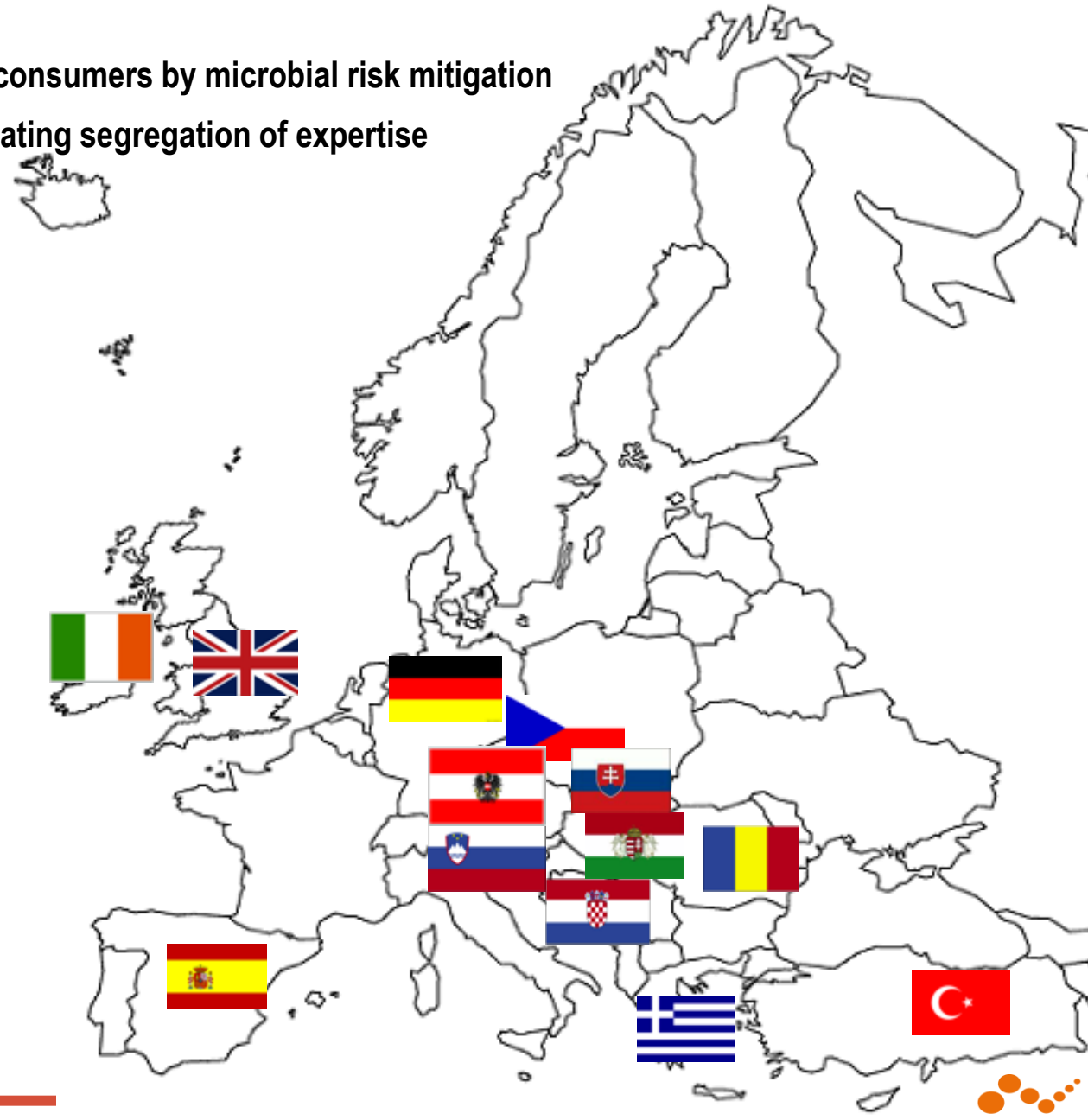
Antibiotic resistance profile of *C. jejuni* isolates (Source: CRP V4-1110)

| | GEN | CIP | TET | ERY | NAL | CHL | STR |
|--------------|-----|-----------|------------|------------|-----------|----------|------------|
| R | 0 | 27 | 2 | 1 | 21 | 0 | 1 |
| S | 35 | 8 | 33 | 34 | 14 | 35 | 34 |
| R (%) | 0 | 77 | 5.7 | 2.8 | 60 | 0 | 2.8 |
| S (%) | 100 | 23 | 94.3 | 97.2 | 40 | 100 | 97.2 |



PROMISE: Protection of consumers by microbial risk mitigation through combating segregation of expertise

2012-2014



WP 1 Neglected exogenous routes of transmission of foodborne pathogens

2650 foods items to be tested agreed

Salmonella
spp.



Micro array

Listeria monocytogenes



MLST typing
Virulotyping

Escherichia coli



MDR Verotoxin

Campylobacter jejuni/ coli



MDR MLST typing

Staphylococcus aureus



MDR

WP 1 Neglected exogenous routes of transmission of foodborne pathogens : Example – Frankfurt airport



August 2012
December 2012
April 2013



February-May 2013



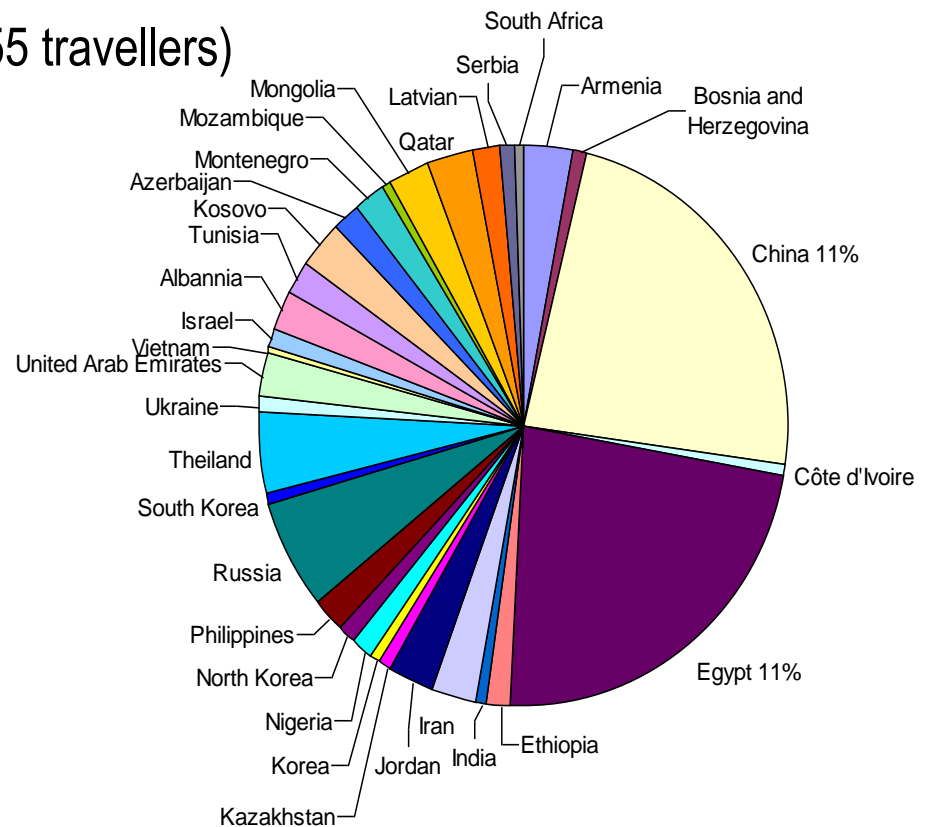
Frankfurt Airport

approx. 20 M passengers/ year (from third party countries)
2009 - 2011 ~0.3 % passengers (~ 50000/ year) inspected
approx. 7 % food items confiscated
approx. 2 kg food items per passenger
~ 2800 tons of food/ year

Source: B. Appel, IAFP Promise meeting, 2014,
published in Beutlich et al., 2014

WP 1 Neglected exogenous routes of transmission of foodborne pathogens : Example – Vienna airport

- Vienna International Airport
- August 2012 – March 2013
- 1473 products of animal origin found (6229 kg)
- 600 samples tested for pathogens and hygiene indicators
- 240 flights from 33 countries covered (61355 travellers)



Source: M. Wagner, IAFP Promise meeting, 2014

Poultry less frequently found, if yes, mostly processed...



Campylobacter very rarely identified...

We got more than 120 presumptive samples, but just a few strains confirmed as *C. jejuni*



C. jejuni isolates from PROMISE were all isolated from fresh poultry samples from Balkan countries

ANTIBIOTIC RESISTANCE

MDR *C. jejuni* strains:

- resistant against STR, CIP, TET, ERY, NAL; Thr86Ala mutation, *tetO* present
- resistant against STR, CIP, TET, NAL; Thr86Ala mutation; without ERY R conferring mutation in 23S rRNA, *tetO* present



GENOTYPING METHODS

MLST (UL), *flaA* (UL), Binary multiplex PCR (mPCR) typing (UL), PFGE (VUW)

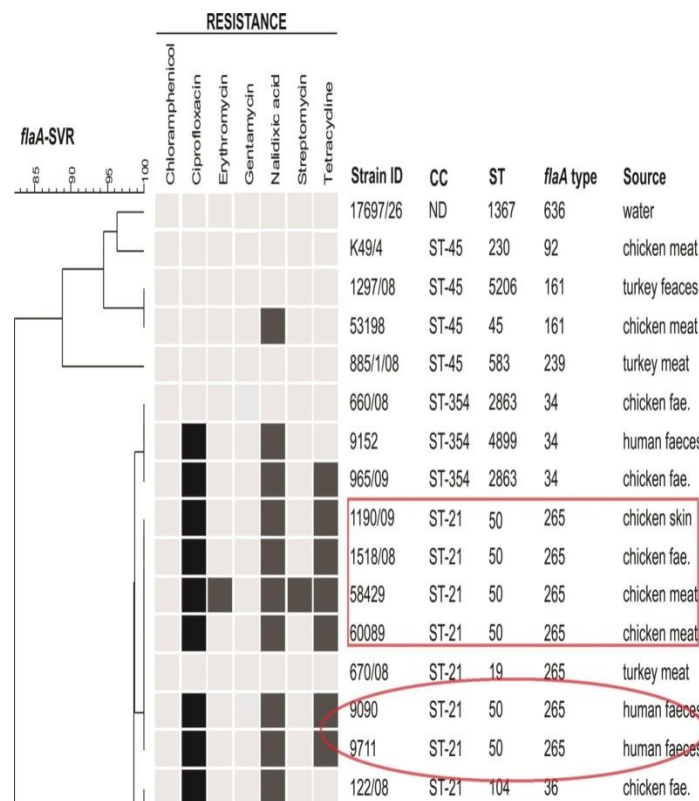
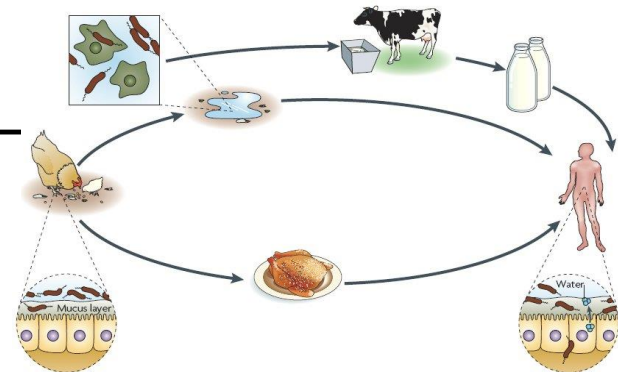
- **C2:** ST 1707, CC ST-607, *flaA* type14, mPCR clade C9ii
→ uncommon MLST sequence type and mPCR profile typical for wildlife environment
- **C33:** ST 400, CC ST-353, *flaA* 67, mPCR clade 5
→ Common MLST clonal complex and multiplex PCR profile, typical for farm animals

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 |
| Total | 28 | 10 | 9 | 11 | 6 | 52 |

CC, Clonal complex; STs, sequence types.



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

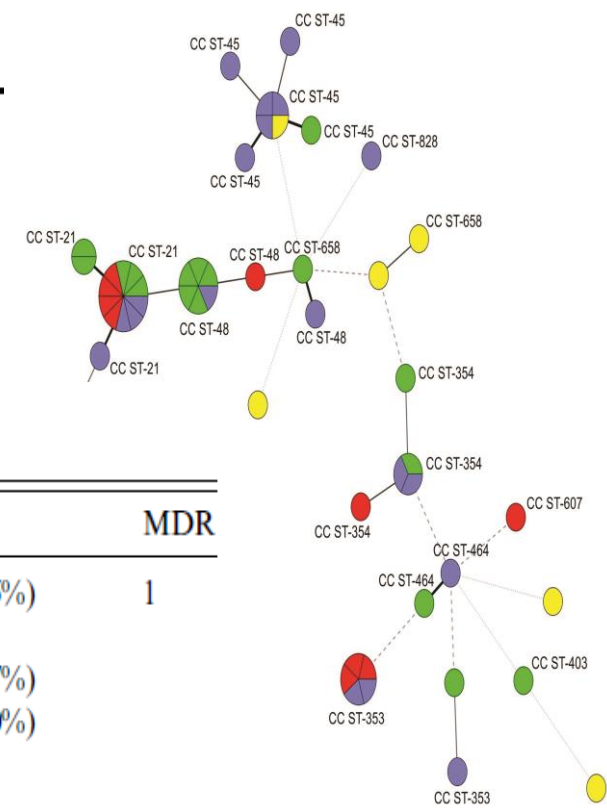
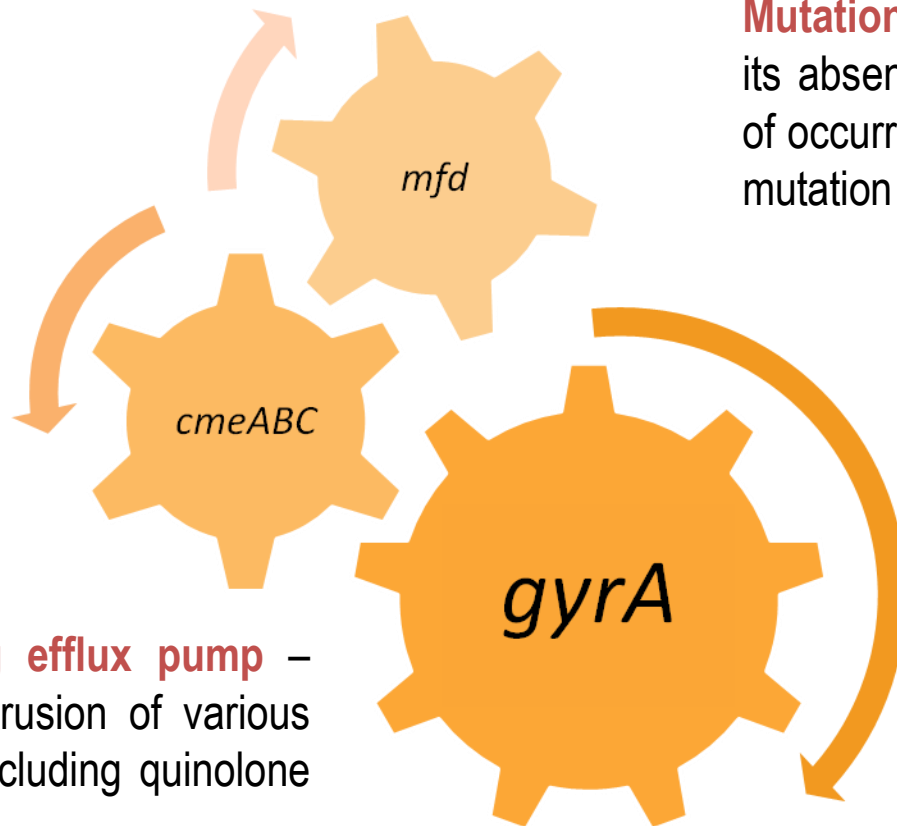


Table 2. Distribution of antibiotic resistant strains in clonal complexes

| 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 ≥ 3 unrelated antibiotics).

Mechanisms of quinolone resistance in *Campylobacter*



Multidrug efflux pump – active extrusion of various drugs, including quinolone antibiotics

Mutation frequency decline gene – its absence decreases the frequency of occurrence of resistance conferring mutation in *gyrA*

POINT MUTATIONS in quinolone resistance determining region (**QRDR**) of the DNA gyrase subunit A gene (*gyrA*)

Main mutation: **Thre86Ile**

Why does the CIP-R prevalence increase so rapidly?

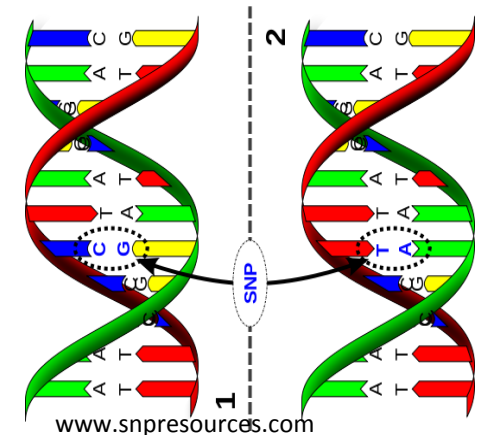
- a) Is it because of the high rate of independently generated resistance mutations?
- b) due to the rapid expansion of a resistant clone?



www.galen.ru

How to investigate this?

1. Screening for phenotypical quinolone resistance
2. General genetic characterization of isolates (MLST)
3. Genetic characterization of quinolone resistance determinant (single nucleotide polymorphism analysis – SNP of QRDR sequences)

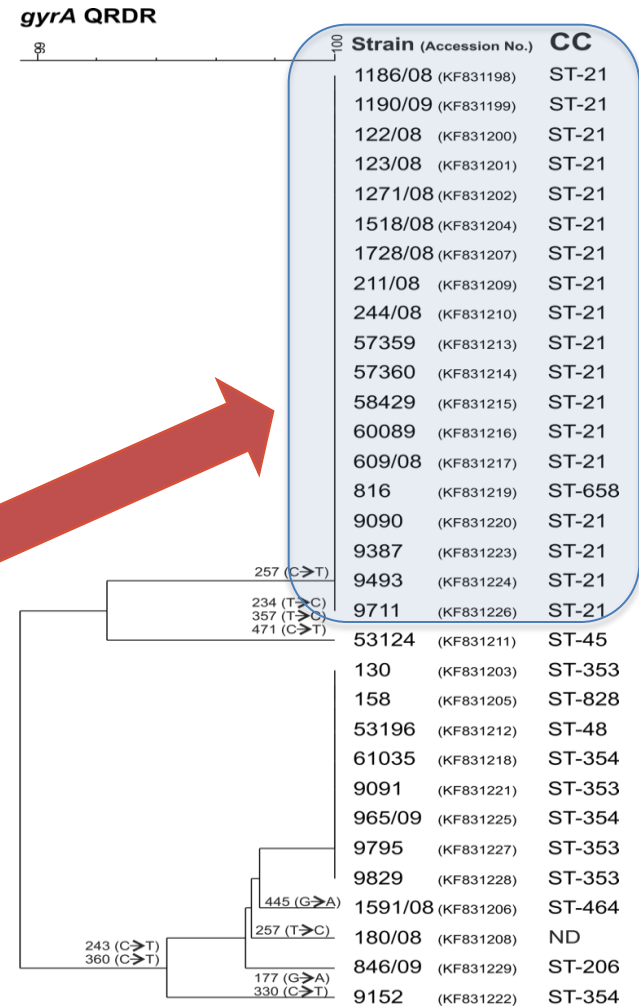


Confirming CIP-R relatedness of *C. jejuni* in Slovenia

- 61% of isolates (n=32/52) resistant to ciprofloxacin
- It turns out ciprofloxacin resistance very likely does spread clonally!

EVIDENCE:

- 95% of isolates in MLST CC-ST21 are ciprofloxacin resistant
- 56% of all ciprofloxacin resistant (n=18/32) isolates cluster in clonal complex 21 **AND they all have the same QRDR sequence type**



Is clonal spreading only local phenomenon?

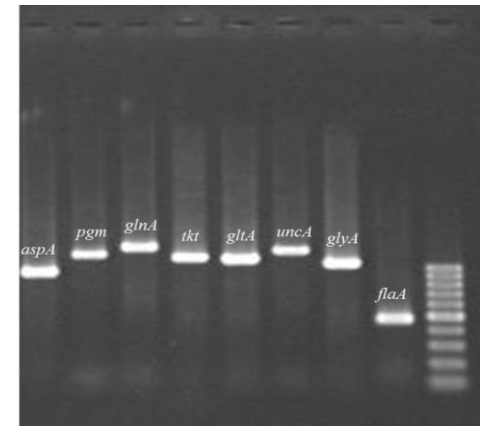
- **Larger screening with 178 strains of CIP-R *C. jejuni* was designed:**

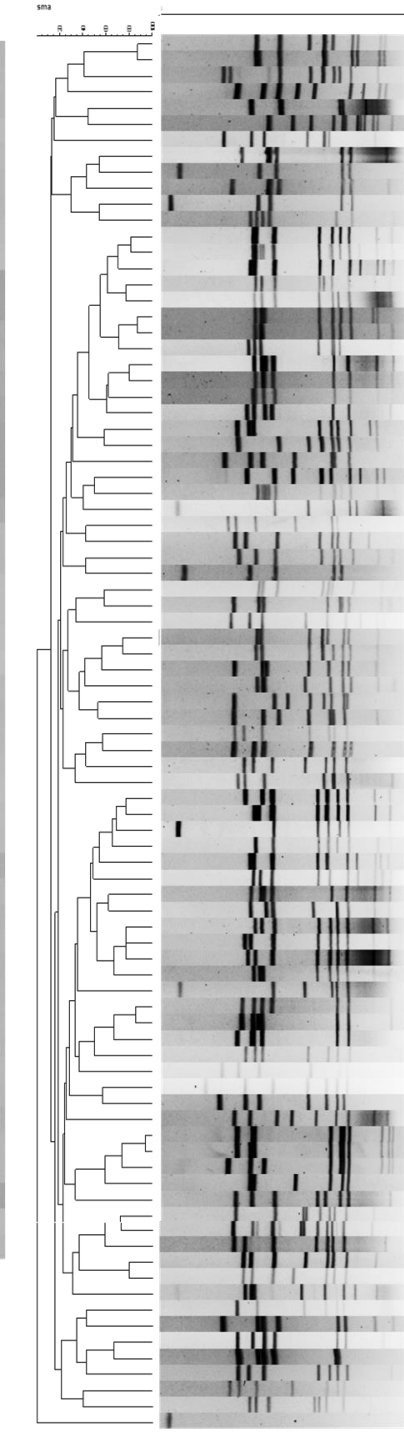
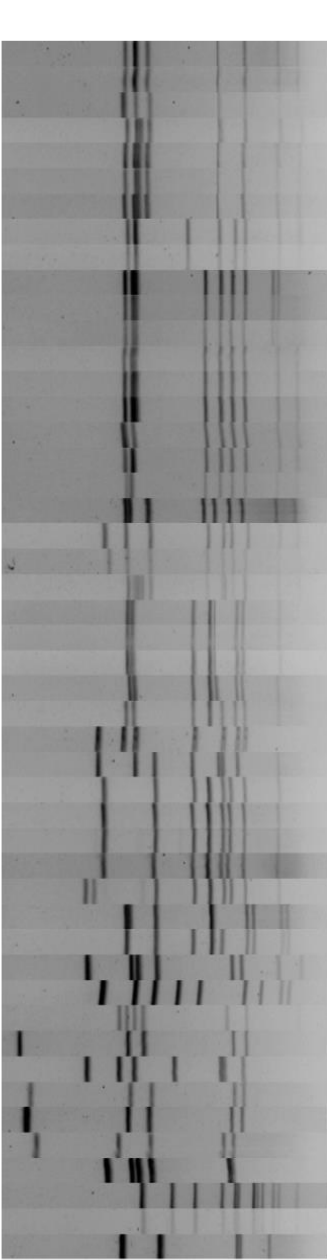
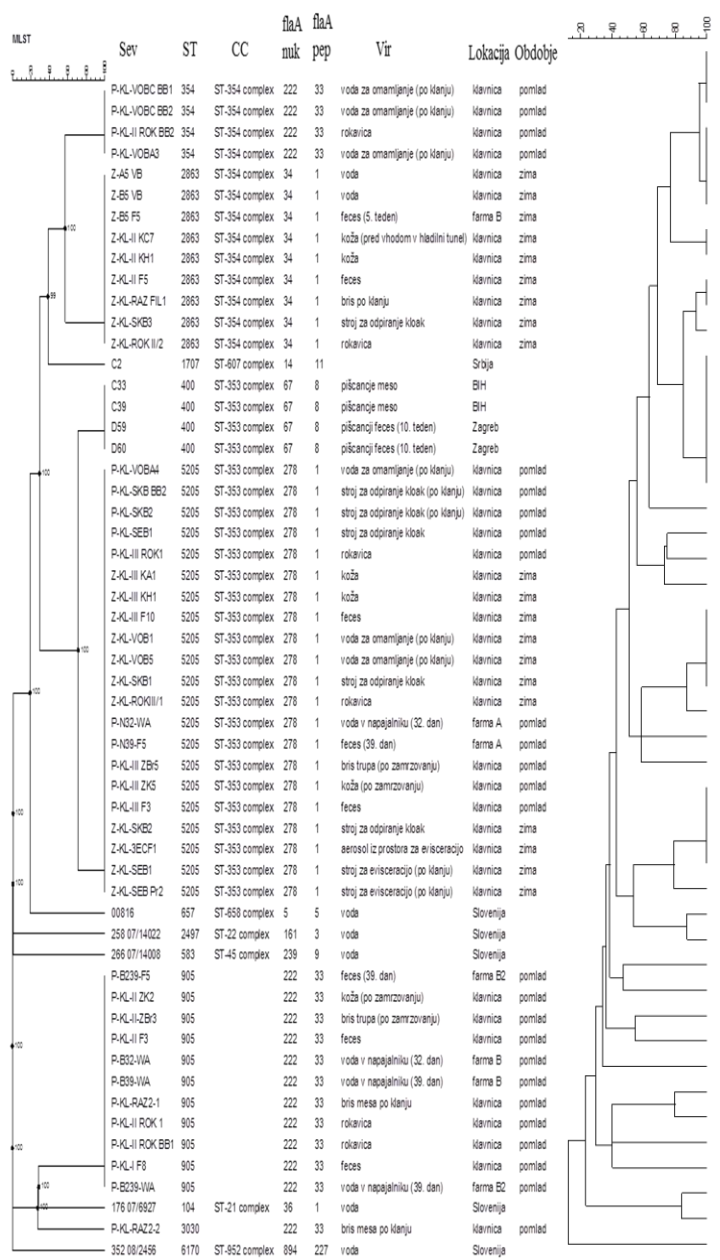
- different geographical regions (60 % from Slovenia, 23 % Austria, 15% Germany, the rest from other Balkan countries)
- different sources: animal and meat (71%), human (23%), environmental (water, wild animals (6%))

Multilocus sequence typing (MLST)

Pulsed field gel electrophoresis (PFGE)

Source discriminatory multiplex PCR





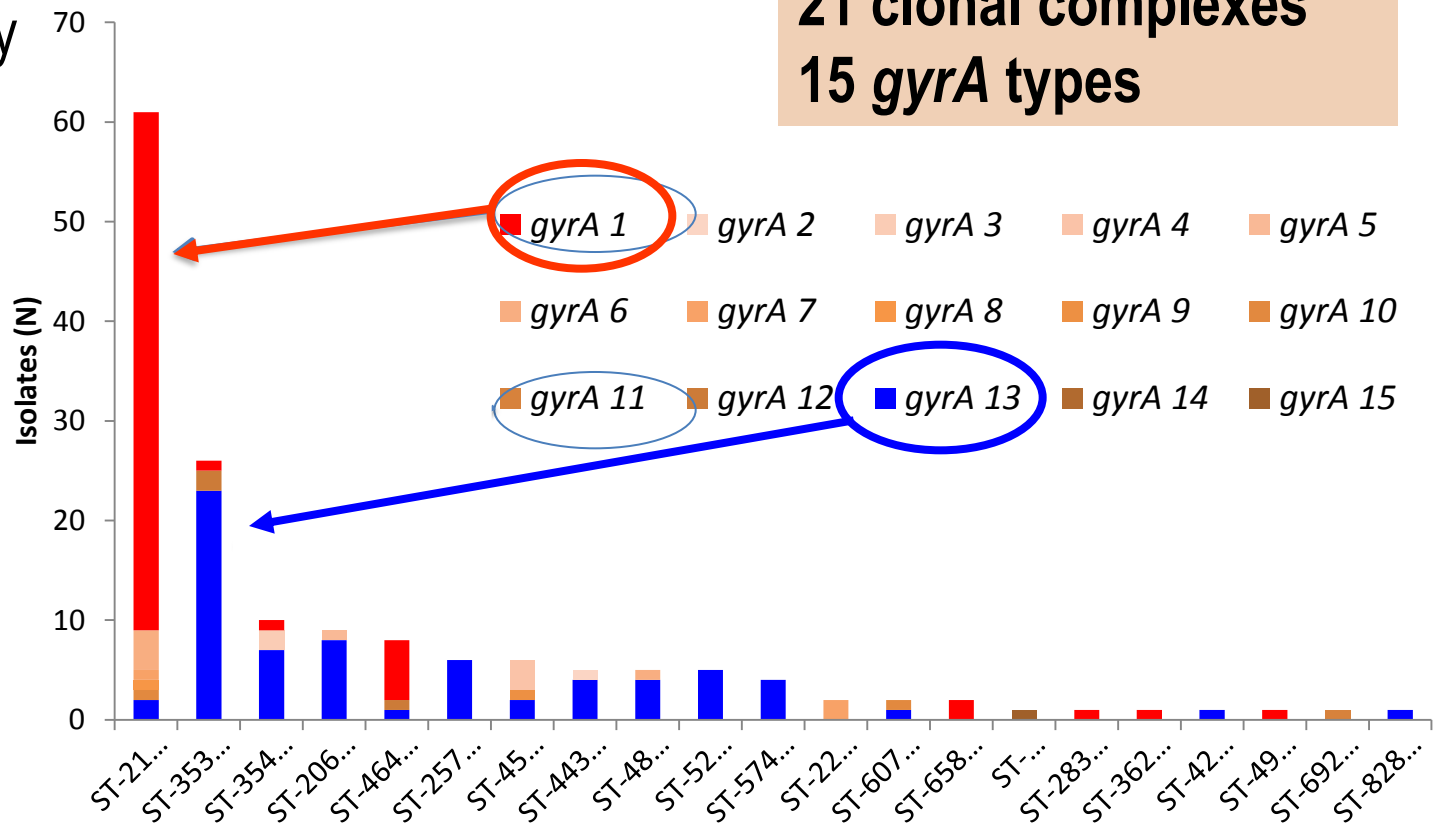
| Strain ID | PFGE-Profile |
|--|--------------|
| 1604 | 1 |
| 816 | 1Subtype |
| 60057 | 2 |
| 132 | 3 |
| 1603 | 4 |
| 128_130 | 5 |
| 02/90/09c | 6 |
| 02/64/10/c | 7 |
| 885/2/08, BFR-CA-11192 | 8 |
| 07/14022 | 9 |
| 07/807 | 10 |
| 846/09 | 11 |
| BFR-CA-07401 | 12 |
| BFR-CA-10767 | 13 |
| BFR-CA-11317 | 14 |
| BFR-CA-07731, 08/000314, MRC-11/01076 | 15 |
| 01/669/10c | 16 |
| 0990_57360_60089 | 17 |
| 9493_9711_58429_1190/09_1518/08_1728/08 | 18 |
| MRC-10/00019 | 19 |
| 375/06_122/08_123/08_57359_211/08 | 20 |
| 1297/08_1292/08_53191 | 21 |
| 9581 | 22 |
| 02/77/11c_01/711/11c | 23 |
| 193 | 24 |
| 9091_02/13/10c_654/08_9795_9829_57357 | 25 |
| 9544 | 26 |
| 196 | 27 |
| 609/08 | 28 |
| BFR-CA-06894 | 29 |
| BFR-CA-06345 | 30 |
| C2 | 31 |
| 180/08_670/08 | 32 |
| 53124 | 33 |
| 186 | 34 |
| 216 | 35 |
| 9N | 36 |
| 61035_02/154/12c_244/08_965/09_660/08 | 37 |
| 9152 | 38 |
| 71 | 39 |
| 1186/08_1271/08 | 40 |
| 70 | 41 |
| 1591/08 | 42 |
| MRC-12/00289 | 43 |
| 2252/09 | 44 |
| MRC-10/00171 | 45 |
| 01/427/11c | 46 |
| BFR-CA-08284 | 47 |
| BFR-CA-06829_9387 | 48 |
| 02/81/09c | 49 |
| MRC-09/00028 | 50 |
| BFR-CA-07345 | 51 |
| 08/000368 | 52 |
| CFA12, BFR-CA-11327 | 53 |
| BFR-CA-11386 | 54 |
| BFR-CA-11627, BFR-CA-06399 | 55 |
| BFR-CA-07255 | 56 |
| BFR-CA-07507, CFA5 | 57 |
| 08/000256 | 58 |
| BFR-CA-06896 | 59 |
| H1 | 60 |
| 02/136/09c | 61 |
| 02/84/10c | 62 |
| 01/302/12c | 63 |
| 02/321/12c | 64 |
| 4N | 65 |
| C33 | 66 |
| MRC-13/01900 | 67 |
| 179 | 68 |
| 59 | 69 |
| 183 | 70 |
| 154 | 71 |
| MRC-12/00284 | 72 |
| 02/195/12c | 73 |
| 02/35/10c | 74 |
| MRC-11/00015 | 75 |
| 161 | 76 |
| MRC-13/00006 | 77 |
| 02/109/12c | 78 |
| 02/16/09c | 79 |
| ATCC 33560 | 80 |
| H7 | 81 |
| 17697/26 | 82 |
| 267 | 83 |
| MRC-10/00032 | 84 |
| BFR-CA-07871 | 85 |
| 07/14008_02/55/11c_885/2/08_11192_07/14022 | 86 |

International clonal relationship of CIP-R in *C. jejuni*

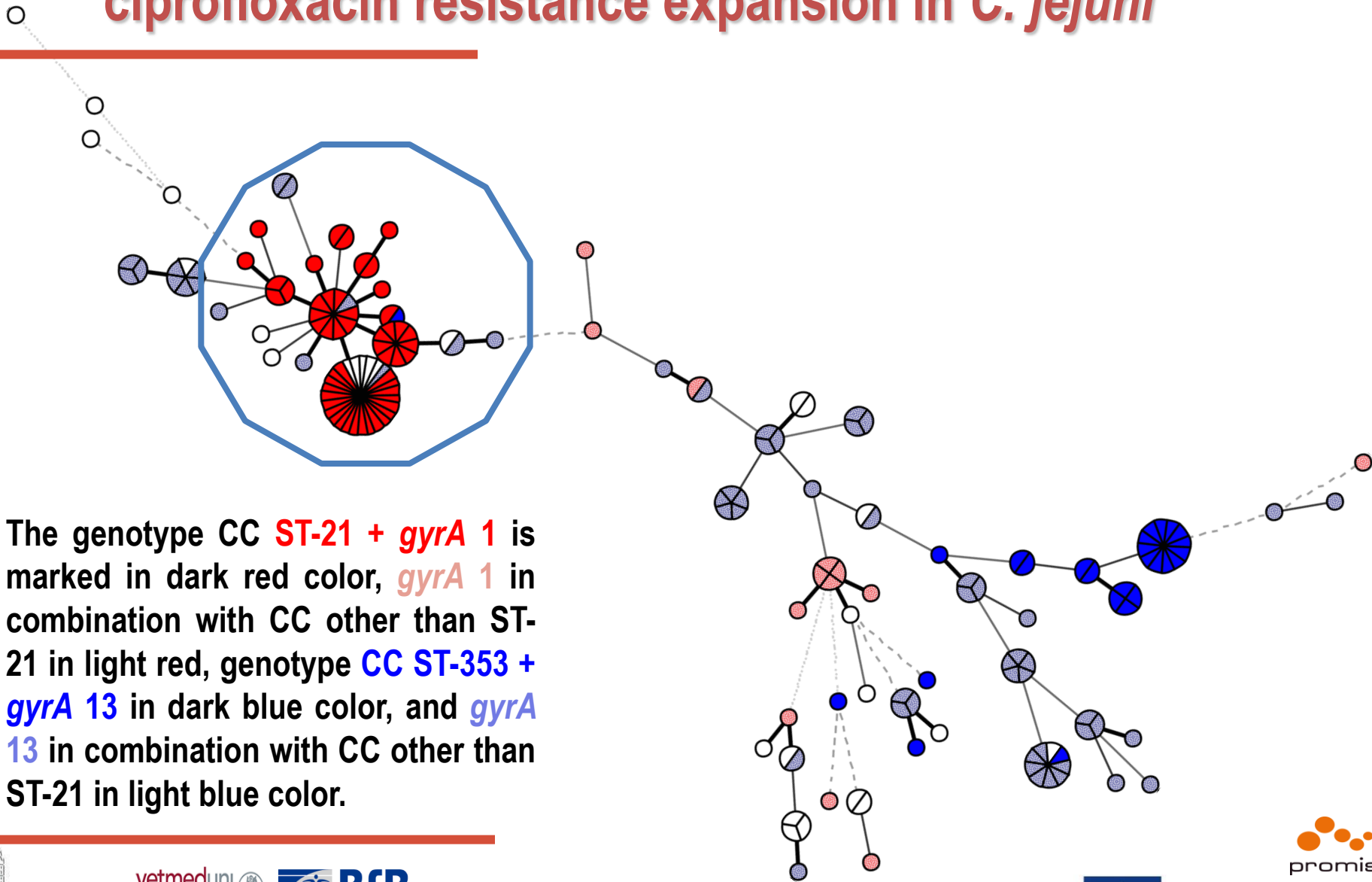
Further investigation of CIP resistant isolates from

- Slovenia
- Austria
- Germany

67 STs
21 clonal complexes
15 *gyrA* types



Confirmed indications of international clonal ciprofloxacin resistance expansion in *C. jejuni*



Conclusions

- Risk for antibiotic resistance development and spreading is still high!
- Antibiotic resistance of *C. jejuni/coli* is frequent, especially against CIP in fresh chicken meat – it is still increasing in central and south EU countries.
- CIP resistance was more commonly observed in isolates with genotypes belonging to MLST clonal complex 21, 353 and 354, which were also most frequent (32%, 19% and 12%, respectively) and in CC 464 (less frequent type, 2%).
- Clonal expansion of ciprofloxacin resistant strains was confirmed on the MLST clonality and genetic similarity of QRDR of *gyrA* gene in the CC ST-21.
- Probably underestimated driving force for the spread of antibiotic resistance!
Support for further research is needed.
- Support to general education of consumers, producers and other food-chain stakeholders is needed too!



Acknowledgement



To the congress organizers for the opportunity of this presentation!



To all of you for attention!



University of Ljubljana
Biotechnical faculty



Risiken erkennen - Gesundheit schützen



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