



TYKS-SAPA-LIIKELAITOS
Varsinais-Suomen sairaanhoitopiiri

Susceptibility testing of Salmonella and Campylobacter

Antti Hakanen

ÅUCS Mikrobiologi och genetik

Nordic AST workshop

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FiRe

- Established in 1991, all major Finnish clinical microbiology laboratories involved (24 laboratories)
 - 1996 CLSI (NCCLS) was taken as the susceptibility testing standard
 - 2011 EUCAST was taken as the susceptibility testing standard

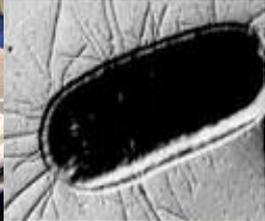
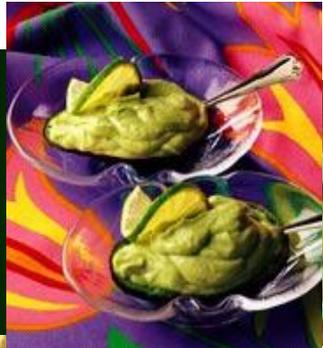
Finres report

- Coverage: >95 % of all susceptibility tests in Finland
- National antimicrobial resistance map
- Finres report in figures
 - 15 major clinical bacterial pathogens
 - One isolate / patient / specimen type / follow-up period
 - > 400.000 isolates / year
 - 3 million antimicrobial susceptibility results / year
- www.finres.fi



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Do you find the Salmonella?



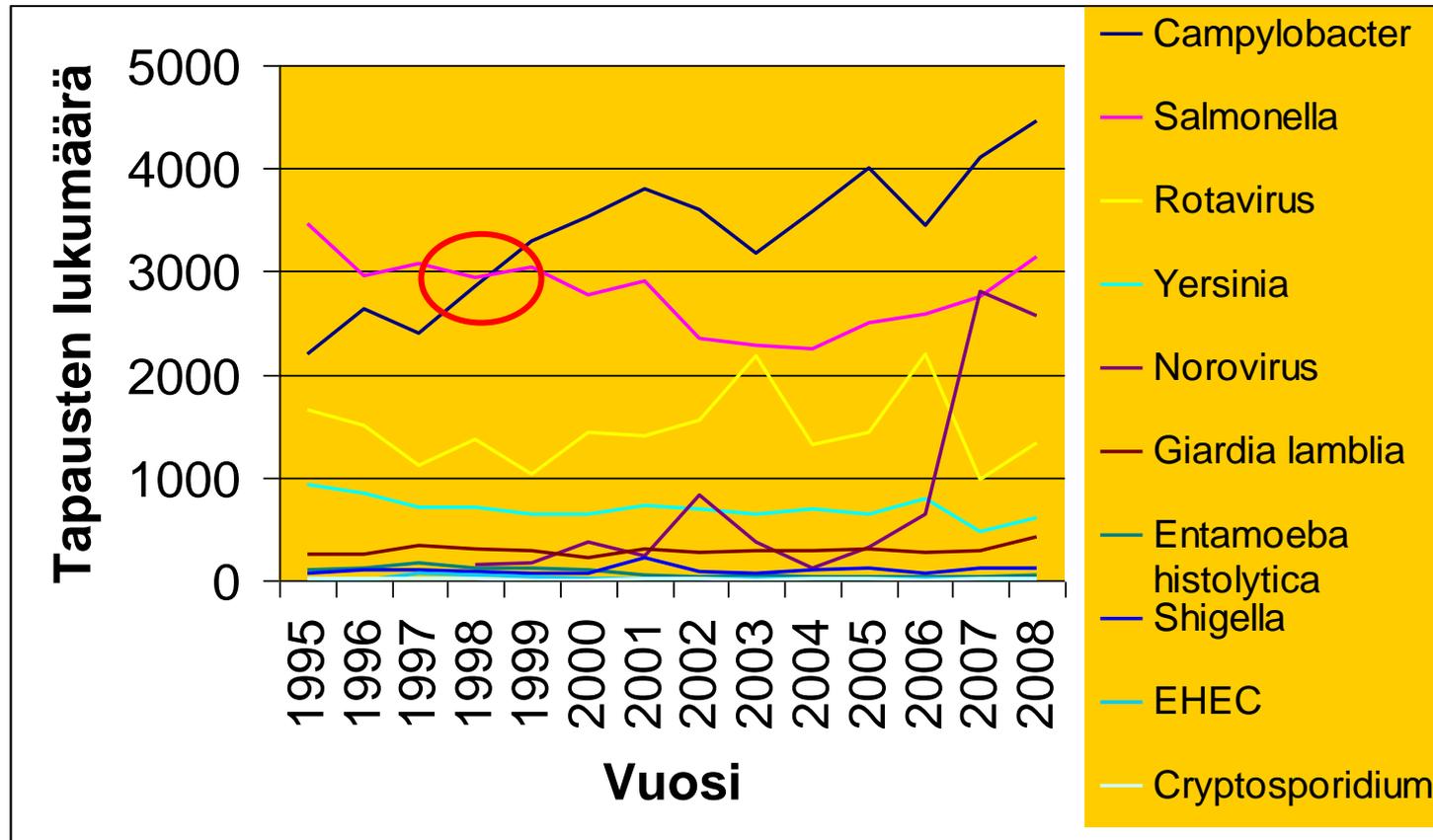


Diarrheal pathogens – US

- **United States – salmonellosis**
 - 1.4 million persons/year will be infected (10% of foodborne infections)
 - Appr. 600 persons/year die (30% of foodborne deaths)
- **United States – campylobacteriosis**
 - 2.5 million persons/year will be infected
 - 120 persons/year die



Ripulin aiheuttajat – Suomen tilanne

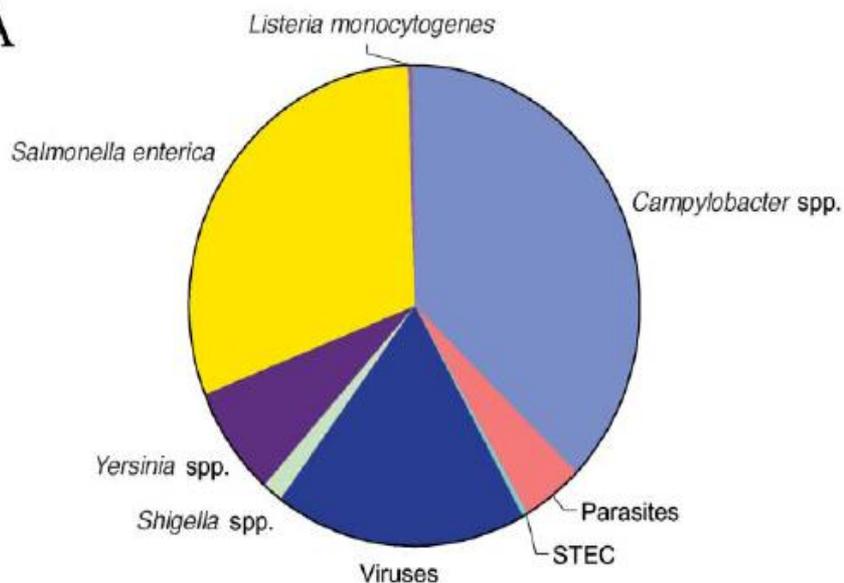


Lähde: THL, Tartuntatautirekisterin tilastotietokanta 1995 - 2008



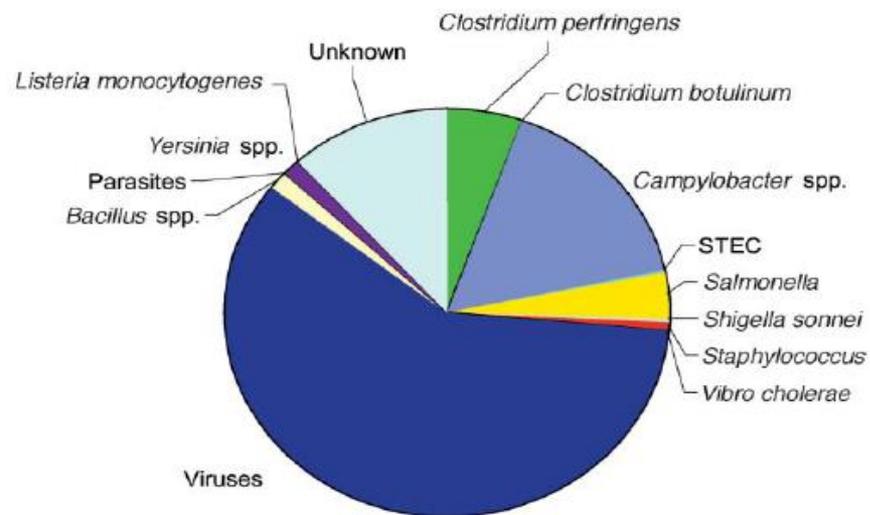
Diarrheal pathogens – Finland

A



Foodborne pathogens 1995-2002, NIDR

B



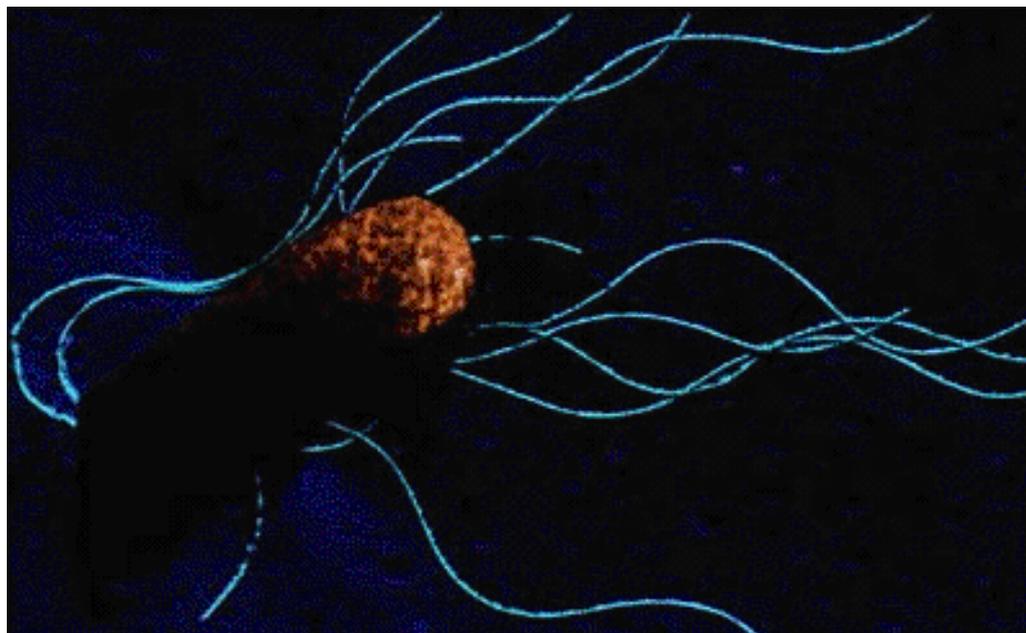
Foodborne epidemics 1995-2002, FFSA

Source: Lukinmaa et al., APMIS 2004



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Salmonella enterica





Salmonella enterica

- If antimicrobial treatment is needed
 - Fluoroquinolones
 - Azithromycin?
 - 3rd generation cephalosporins, carbapenemes
 - (Chloramphenicol)
 - (Ampicillin)
 - (Trimethoprim-sulfa)



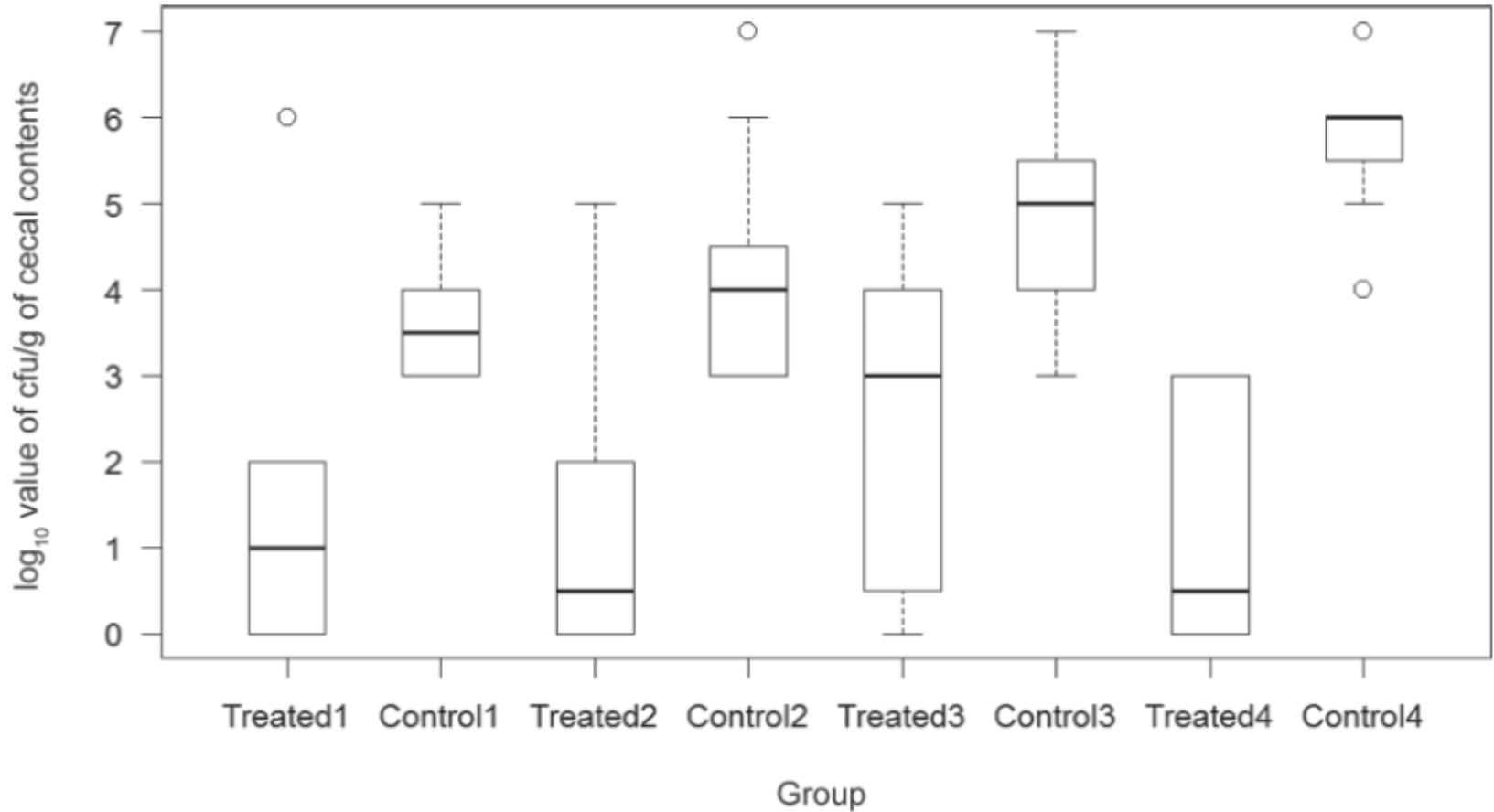
Resistance epidemiology

Antimicrobial resistance among 22 *S. enterica* isolates from food production animals in Finland 2009, Finres-Vet 2007-2009 report

Distribution (%) of MICs (mg l ⁻¹)																						
Antibiotic	%R	95% C.I.	≤0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Ampicillin	22.7	8.7-45.8						50.0	27.3						22.7							
Cefotaxime	0.0	0.0-18.5				22.7	72.7	4.5														
Chloramphenicol	13.6	3.6-35.9								4.5	81.7							13.6				
Ciprofloxacin	13.6	3.6-35.9			22.7	63.6		4.5	9.1													
Gentamicin	0.0	0.0-18.5							4.5	86.4	9.1											
Nalidixic acid	13.6	3.6-35.9										81.8	4.5								13.6	
Streptomycin	18.2	6.0-41.0											9.1	63.6	9.1	4.5	9.1	4.5				
Sulphamethoxazole	22.7	8.7-45.8												72.7	4.5							22.7
Tetracycline	13.6	3.6-35.9								54.5	31.8			4.5	9.1							
Trimethoprim	0.0	0.0-18.5						68.2	31.8													



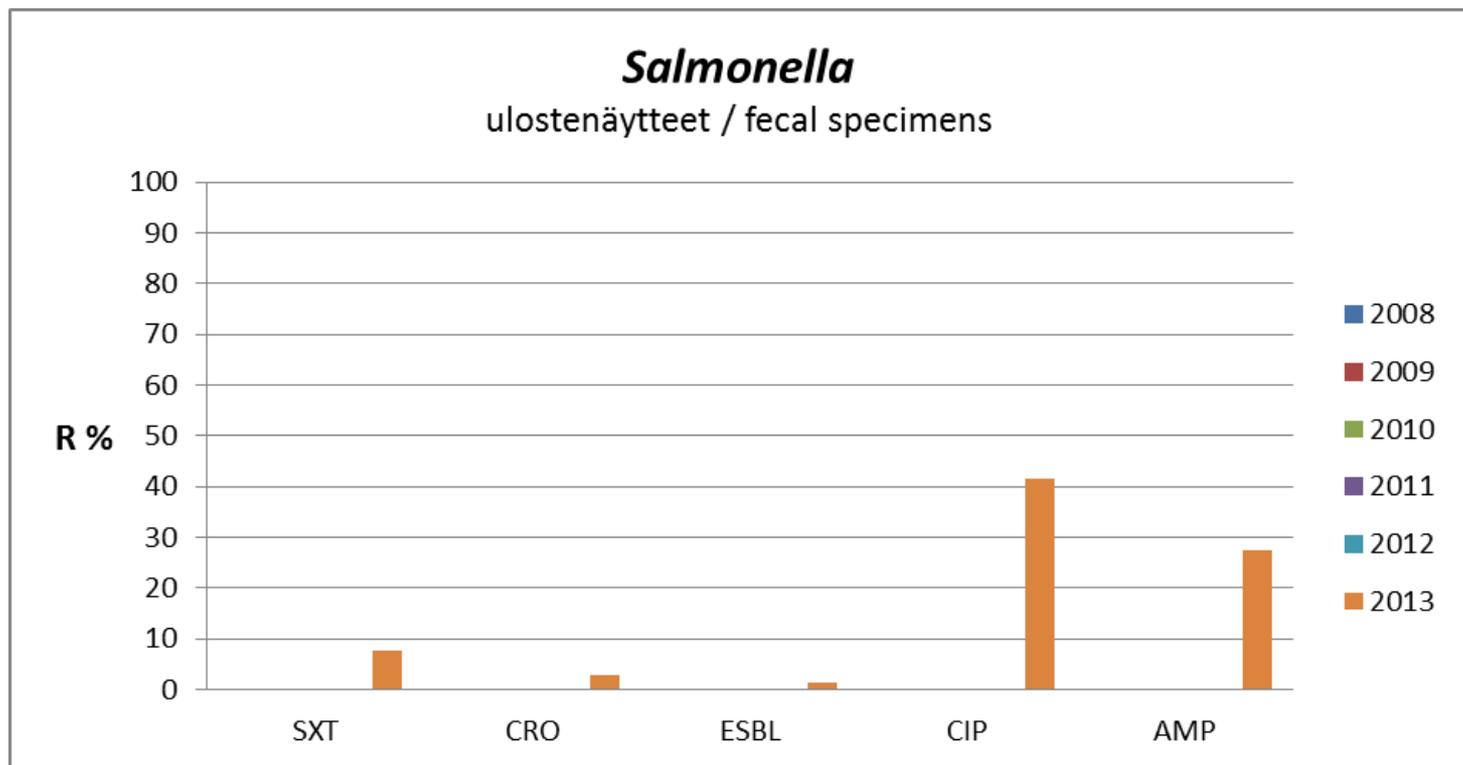
Effect of BROILACT (Orion Corp., Espoo, Finland) on the colonization of ESBL





Resistance epidemiology

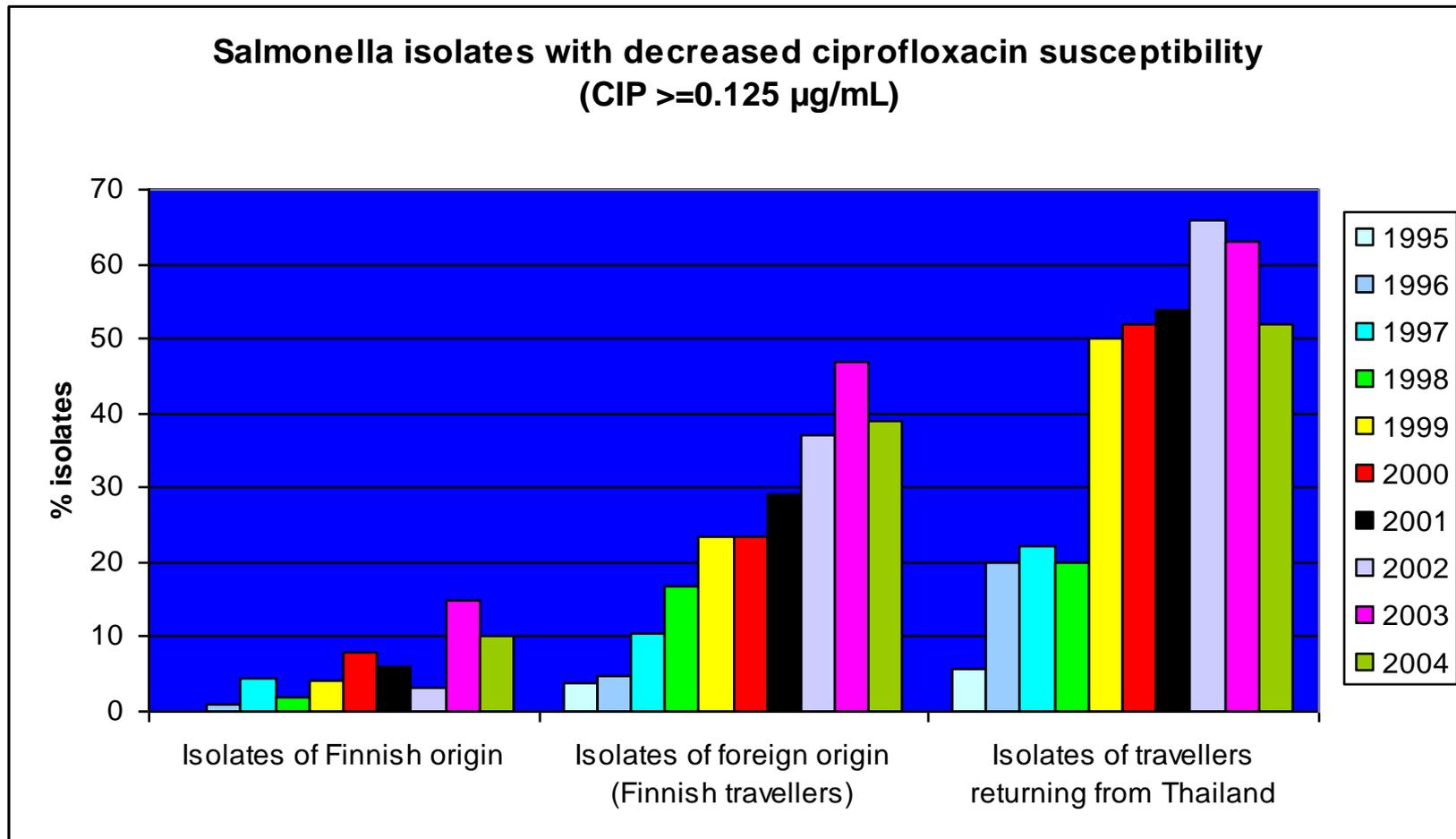
Antimicrobial resistance among *S. enterica*, Finres 2013 report



FINNISH STUDY GROUP FOR ANTIMICROBIAL RESISTANCE



SUOMALAINEN MIKROBILÄÄKERESISTENSSIN TUTKIMUSRYHMÄ

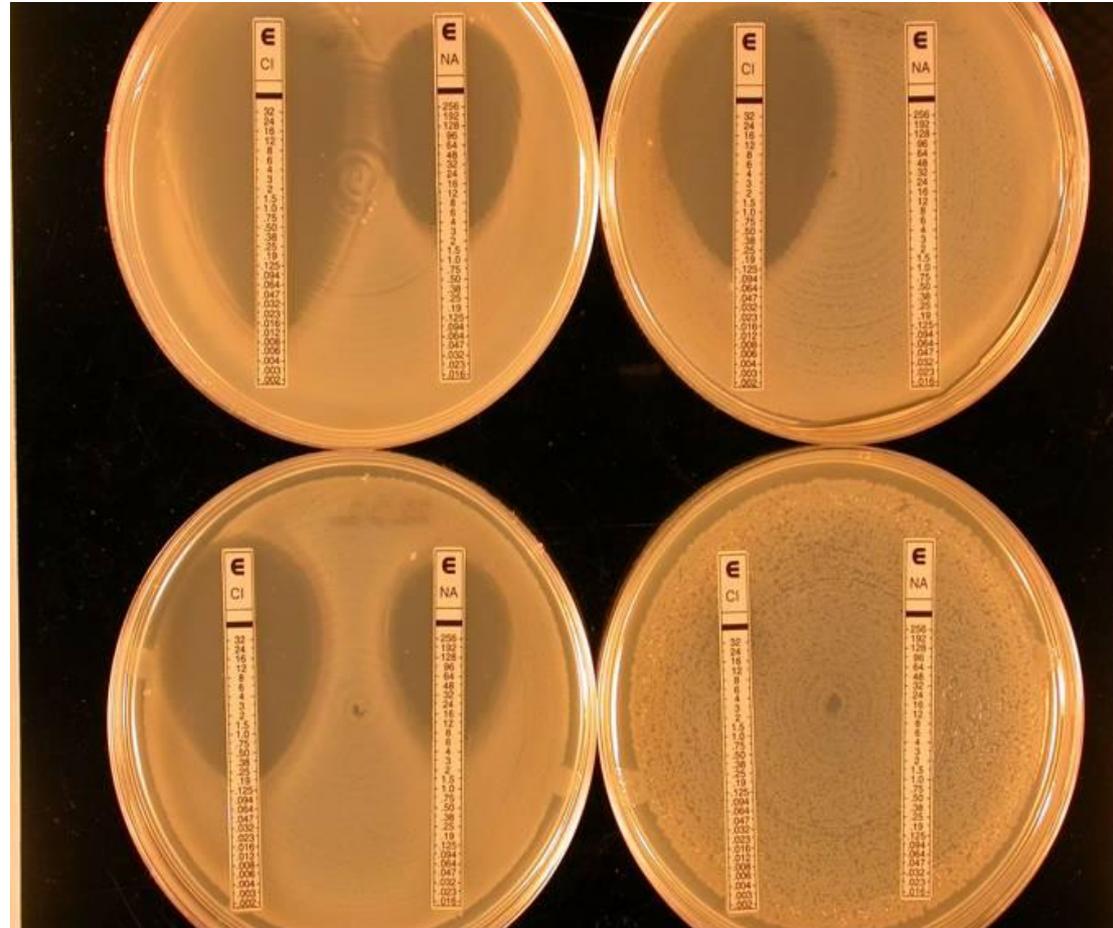


Sources: Hakanen A, EID 2001; Hakanen et al, JAC 2006



Salmonella enterica

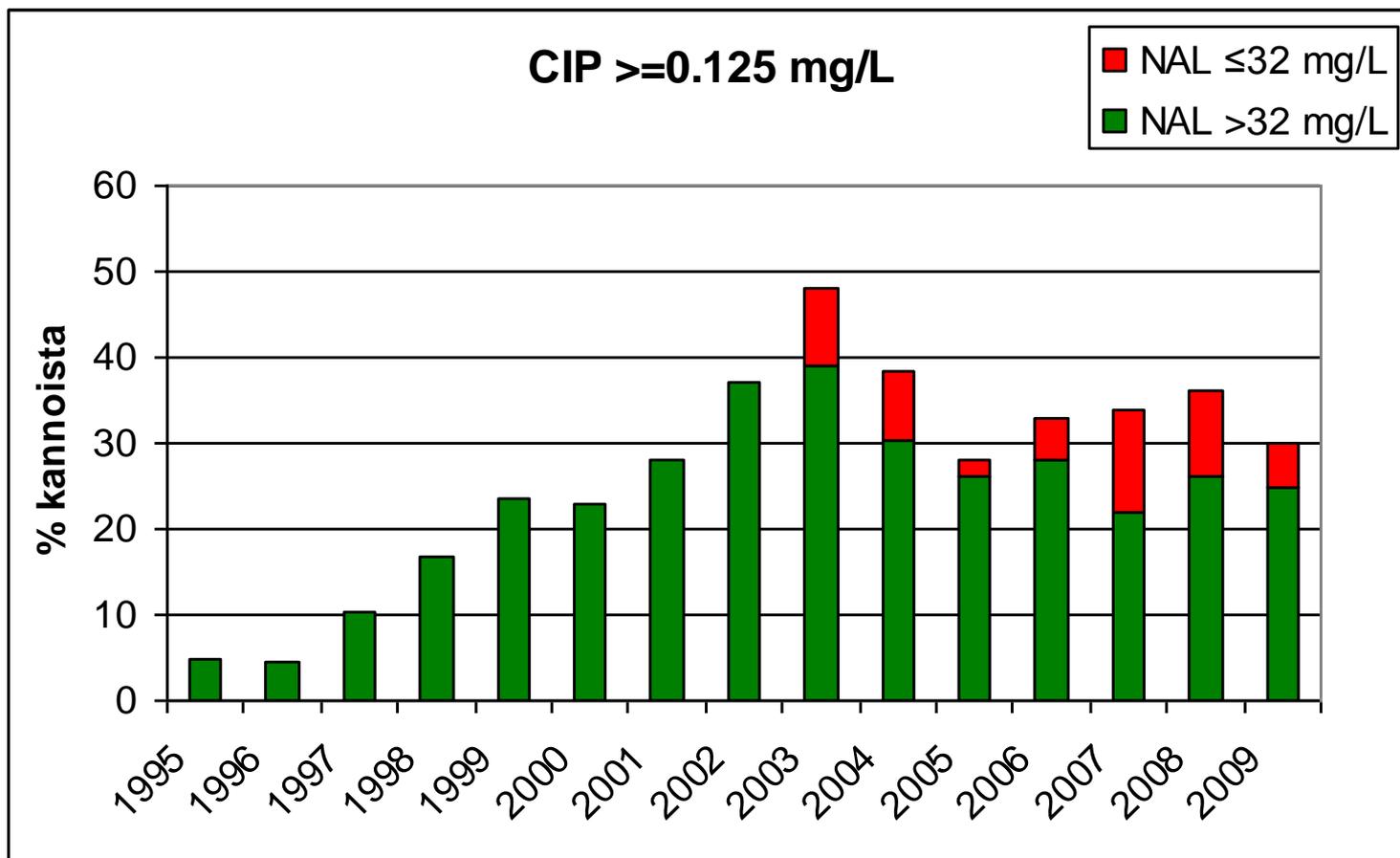
- From 1995 to 2002 all tested *Salmonella* isolates with reduced ciprofloxacin susceptibility (CIP MIC ≥ 0.125 $\mu\text{g/mL}$) were uniformly resistant (MIC ≥ 256 $\mu\text{g/mL}$) to nalidixic acid
- 2003 emergence of a "novel quinolone-resistant phenotype"
(Hakanen, Lindgren, et al, JCM 2005)
- A highly mobile *qnr* genotype confers the novel quinolone-resistant phenotype
(Gunell, et al, AAC 2009)





Salmonella enterica

Two sub-populations among *Salmonella* isolates with decreased ciprofloxacin susceptibility





Enterobacteriaceae

EUCAST kliniska brytpunkter v. 5.0, NordicAST v. 5.1, 2015-04-20

Fluorokinoloner	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)	
	S ≤	R >		S ≥	R <
Ciprofloxacin	0.5	1	5	22	19
Ciprofloxacin, <i>Salmonella</i> spp.	0.06	0.06	5	33	Note
Pefloxacin (screen), <i>Salmonella</i> spp.	NA	NA	5	24	24
Levofloxacin	1	2	5	22	19
Ofloxacin	0.5	1	5	22	19

Kommentarer

Nalidixinsyra har använts för resistensövervakning. Användandet av ECOFF (WT MIC ≤ 16 mg/L, zon 30 µg lapp ≥ 16 mm) har hög känslighet för detektion av kromosomal resistens men inte för plasmidmedierad resistens.

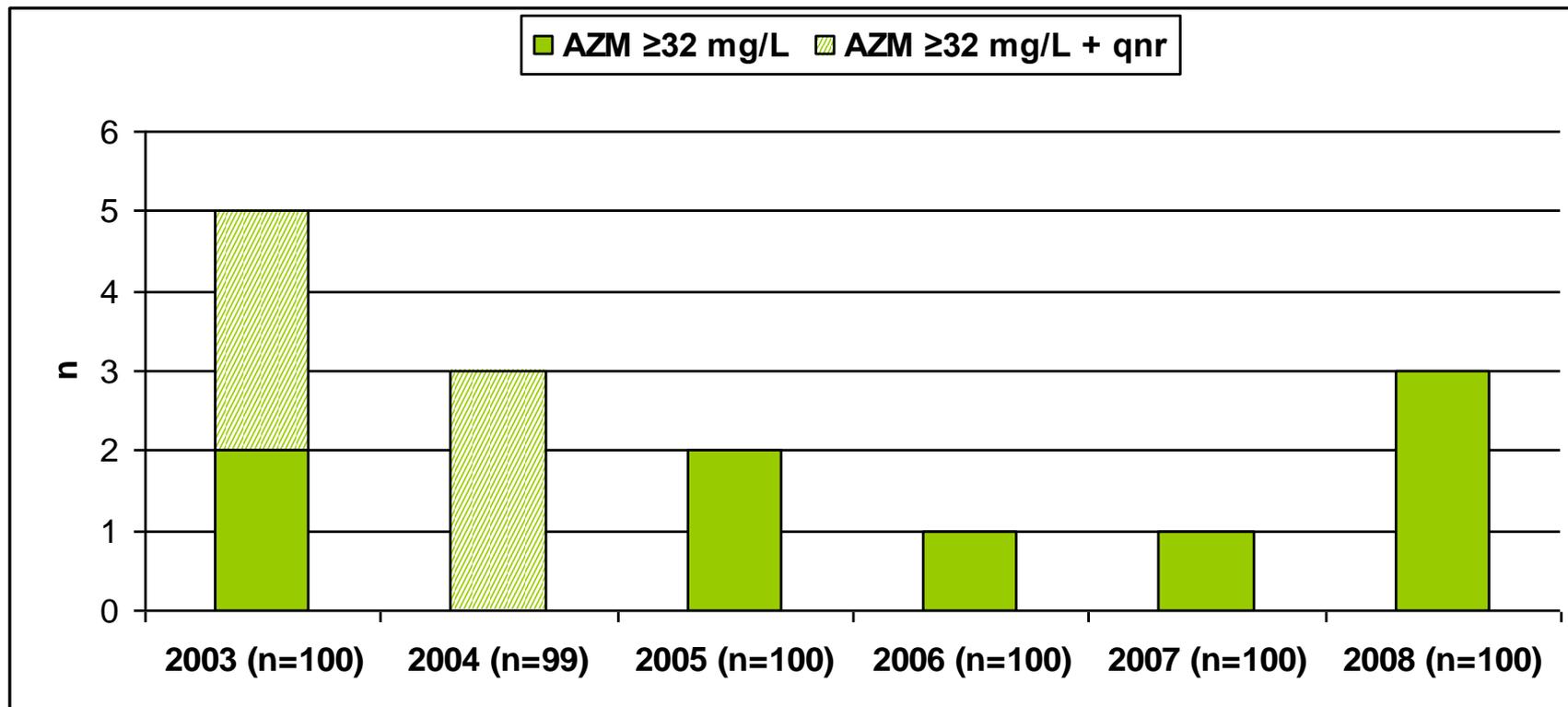
Pefloxacin 5 µg rekommenderas för screening av låggradig kinolonresistens hos *Salmonella* spp. För ciprofloxacin 5 µg gäller följande: Vid zon < 30 mm rapportera R. Vid zon 30-32 mm utför MIC-bestämning eller använd pefloxacin 5 µg.

Pefloxacin kan användas för SIR-kategorisering av *Salmonella* spp. mot ciprofloxacin.



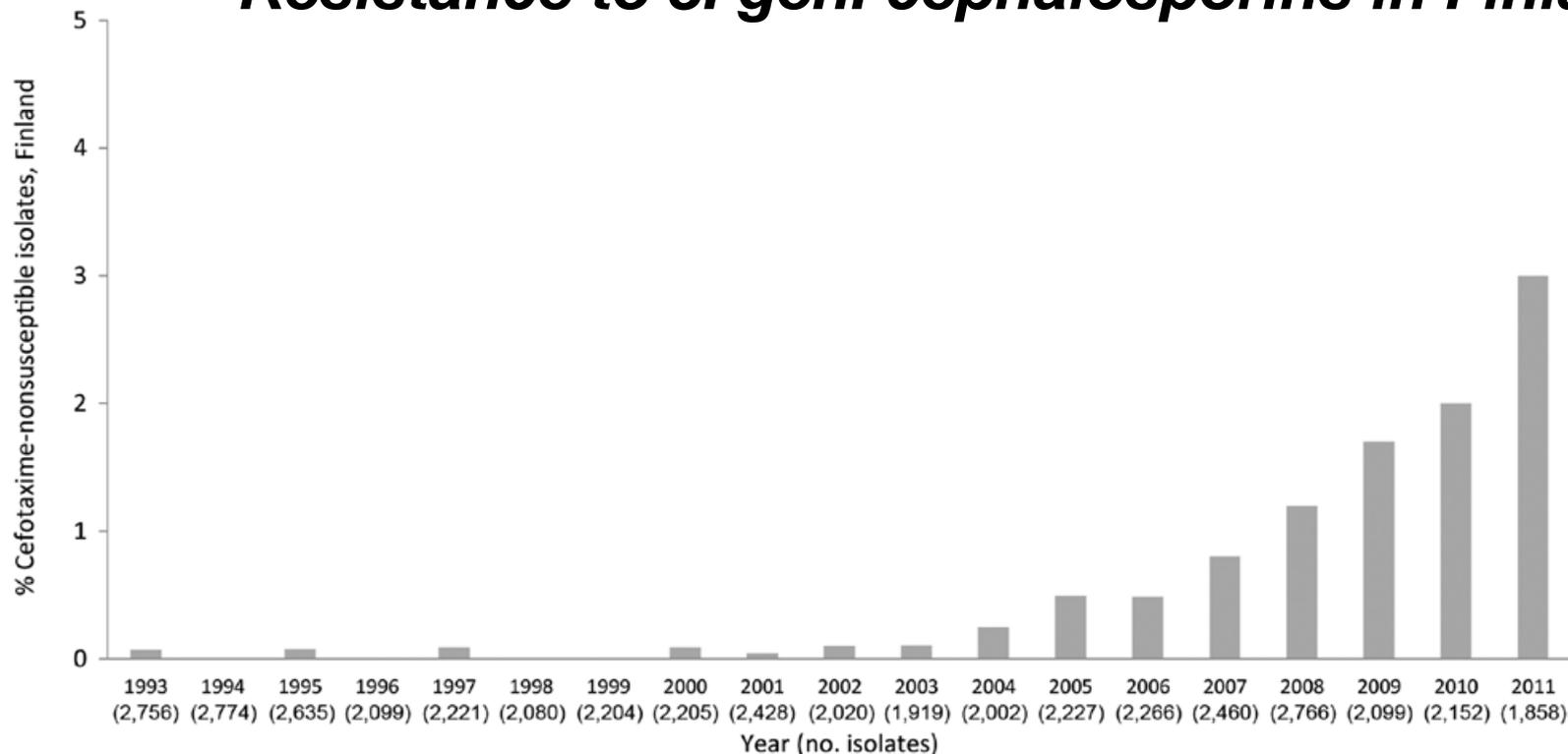
Salmonella enterica

Azithromycin resistance among isolates from Finnish travellers 2003-2008





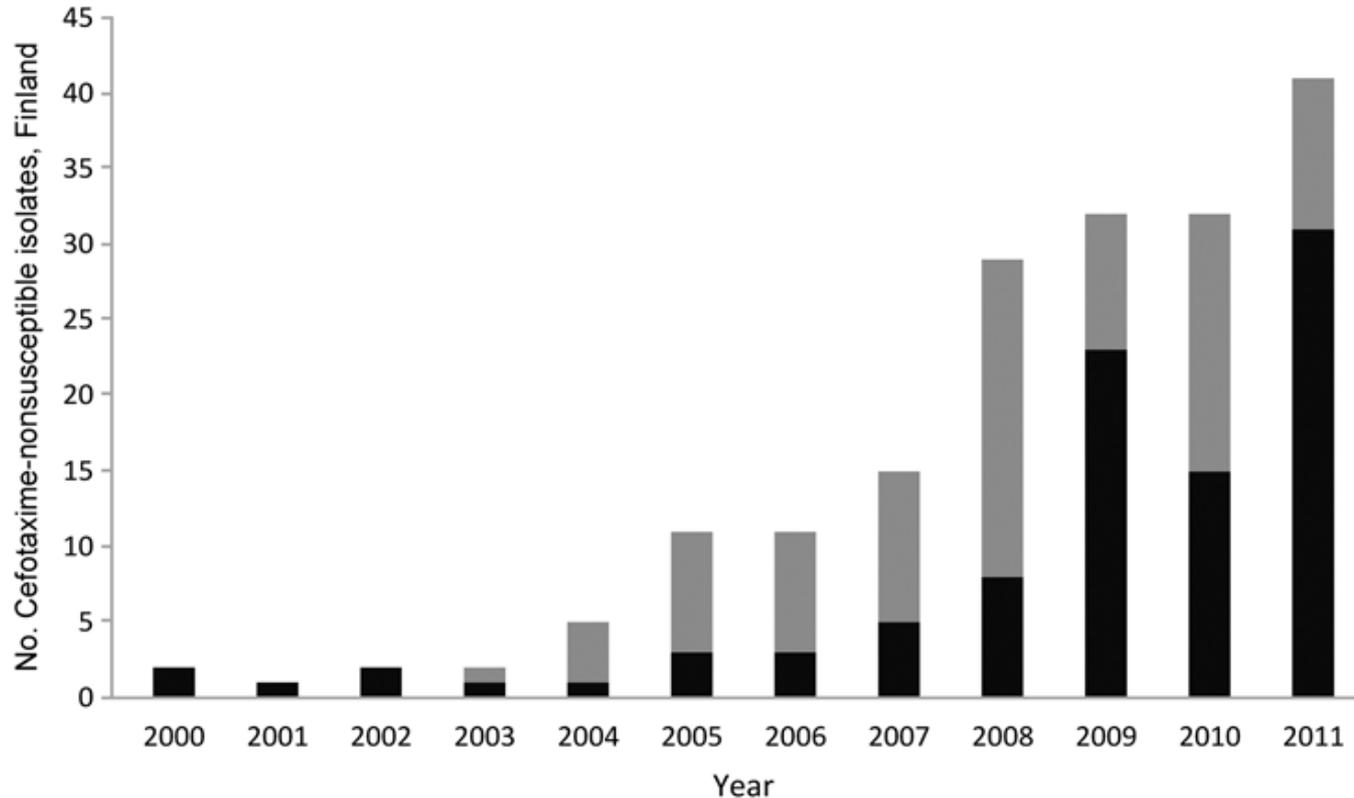
Salmonella enterica – Resistance to 3. gen. cephalosporins in Finland



The increasing trend ($p < 0.001$) in the proportion (%) of cefotaxime-nonsusceptible (30- μ g disk diameter ≤ 22 mm) *Salmonella enterica* isolates in Finland during 1993–2011.



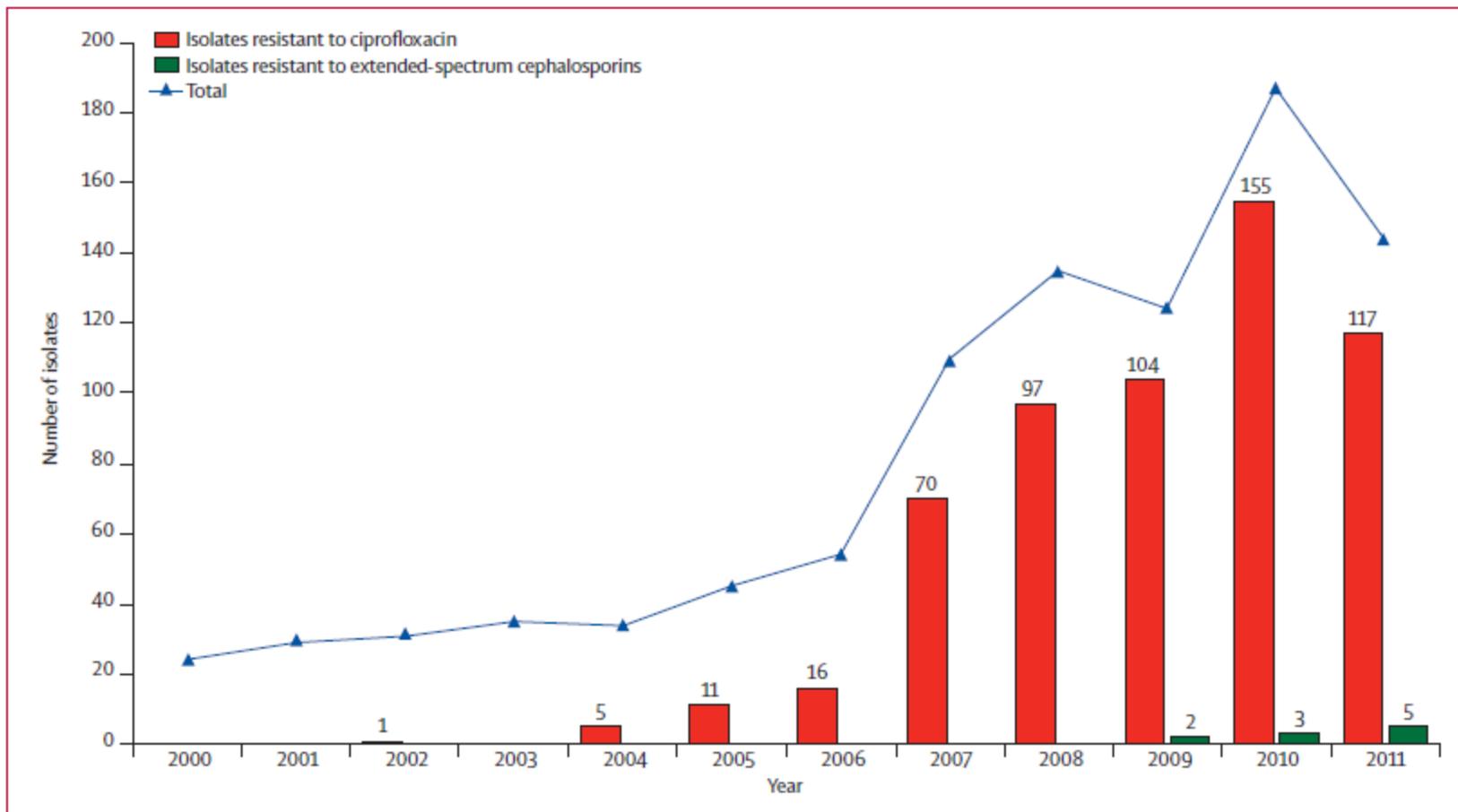
Salmonella enterica – Resistance to 3. gen. cephalosporins in Finland



Number of cefotaxime-nonsusceptible *S. enterica* isolates carrying ESBL (black bars) and AmpC genes (gray bars) in Finland, 1993–2011.



Salmonella enterica serotype Kentucky, France



Two isolates producing carbapenemase OXA-48 from Egypt 2009
Highly drug-resistant isolates producing VIM-2 from Morocco in 2010



Conclusions - Salmonella

- ESBL screening is important not only with *E. coli* and *Klebsiella*, but also with *Salmonella* strains.
- When 3rd generation cephalosporin resistance (combined with fluoroquinolone resistance) among *Salmonella* isolates is increasing, new treatment options are urgently needed.
- (For severely ill patients, MIC-based susceptibility testing should be used.)



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Campylobacter spp.





Campylobacter spp. – History

- 1886, Theodor Escherich observed and described nonculturable spiralshaped bacteria in the colonic mucus of a child who died of “cholera infantum”
- The veterinary era
 - 1906 McFadyean and Stockman – “vibrionic abortion” in sheep
 - 1930s – Enteric infection (also first isolations)
 - 1950s Elizabeth King: Optimum growth temperature of “related vibrio” (*C. jejuni/C. coli*) was 42 °C
- The human medicine era
 - 1972 Jean-Paul Butzler – culturing methods published in JID
 - 1977 Martin Skirrow published in BMJ...and the rest is history



Campylobacter spp. – Taxonomy

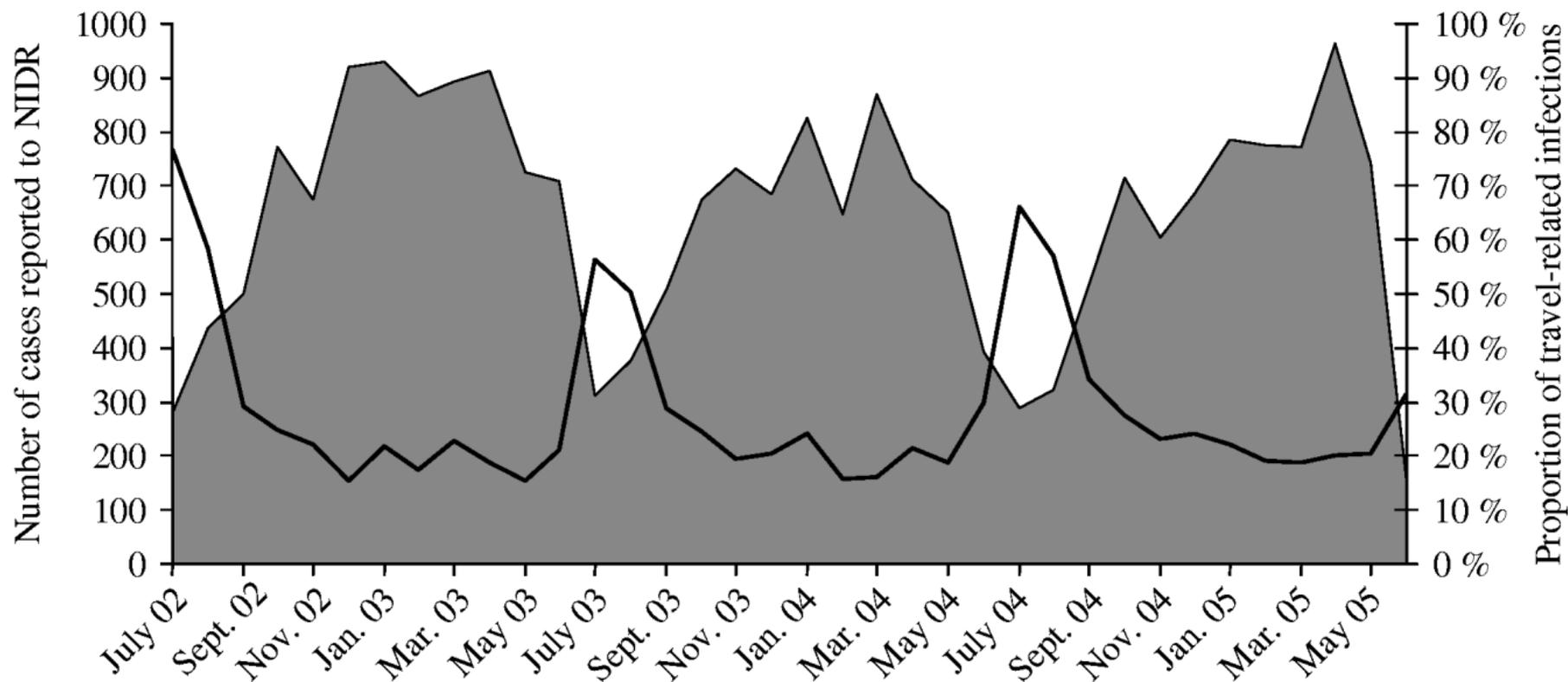
List of valid species and subspecies in the genus *Campylobacter* as of April 30, 2013, and their common hosts and disease associations in humans and animals.

Taxon	Host animal species	Human disease association ¹	Animal disease association ¹
<i>Campylobacter avium</i>	Poultry	None as yet	None as yet
<i>Campylobacter canadensis</i>	Whooping cranes	None as yet	None as yet
<i>Campylobacter coli</i>	Pigs, poultry, ostriches, cattle, sheep	Gastroenteritis	Gastroenteritis, infectious hepatitis
<i>Campylobacter concisus</i>	Humans, domestic pets	Gastroenteritis, periodontitis	None as yet
<i>Campylobacter cuniculorum</i>	Rabbits	None as yet	None as yet
<i>Campylobacter curvus</i>	Humans	Periodontitis, gastroenteritis	None as yet
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	Cattle, sheep, reptiles	Gastroenteritis, septicemia	Spontaneous abortion
<i>Campylobacter fetus</i> subsp. <i>venerealis</i>	Cattle, sheep	Septicemia	Infectious infertility
<i>Campylobacter gracilis</i>	Humans	Periodontitis	None as yet
<i>Campylobacter helveticus</i>	Dogs, cats	Periodontitis	Gastroenteritis
<i>Campylobacter hominis</i>	Humans	None as yet	None as yet
<i>Campylobacter hyointestinalis</i> subsp. <i>hyointestinalis</i>	Cattle, deer, pigs, hamsters	Gastroenteritis	Gastroenteritis
<i>Campylobacter hyointestinalis</i> subsp. <i>lawsonii</i>	Pigs	None as yet	None as yet
<i>Campylobacter insulaenigrae</i>	Seals, porpoises	None as yet	None as yet
<i>Campylobacter jejuni</i> subsp. <i>doylei</i>	Humans	Septicemia, gastroenteritis	None as yet
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	Poultry, cattle, pigs, ostriches, wild birds	Gastroenteritis, Guillain-Barré syndrome	Spontaneous abortion, avian hepatitis
<i>Campylobacter lanienae</i>	Cattle	None as yet	None as yet
<i>Campylobacter lari</i> subsp. <i>concheus</i>	Shellfish	Gastroenteritis	None as yet
<i>Campylobacter lari</i> subsp. <i>lari</i>	Wild birds, dogs, poultry, shellfish, horses	Gastroenteritis, septicemia	Avian gastroenteritis
<i>Campylobacter mucosalis</i>	Pigs	None as yet	None as yet
<i>Campylobacter peloridis</i>	Shellfish	Gastroenteritis	None as yet
<i>Campylobacter rectus</i>	Humans	Periodontitis	None as yet
<i>Campylobacter showae</i>	Humans	Periodontitis	None as yet
<i>Campylobacter sputorum</i>	Humans, cattle, pigs, sheep	Gastroenteritis, abscesses	Spontaneous abortion
<i>Campylobacter subantarcticus</i>	Birds in the subantarctic	None as yet	None as yet
<i>Campylobacter upsaliensis</i>	Dogs, cats	Gastroenteritis	Gastroenteritis
<i>Campylobacter ureolyticus</i>	Humans	Gastroenteritis, Crohn's disease	None as yet
<i>Campylobacter volucris</i>	Black-headed gulls	None as yet	None as yet

¹ Association with a disease is not necessarily proof of causation. For more details on the clinical significance of each taxon, further reading is encouraged.



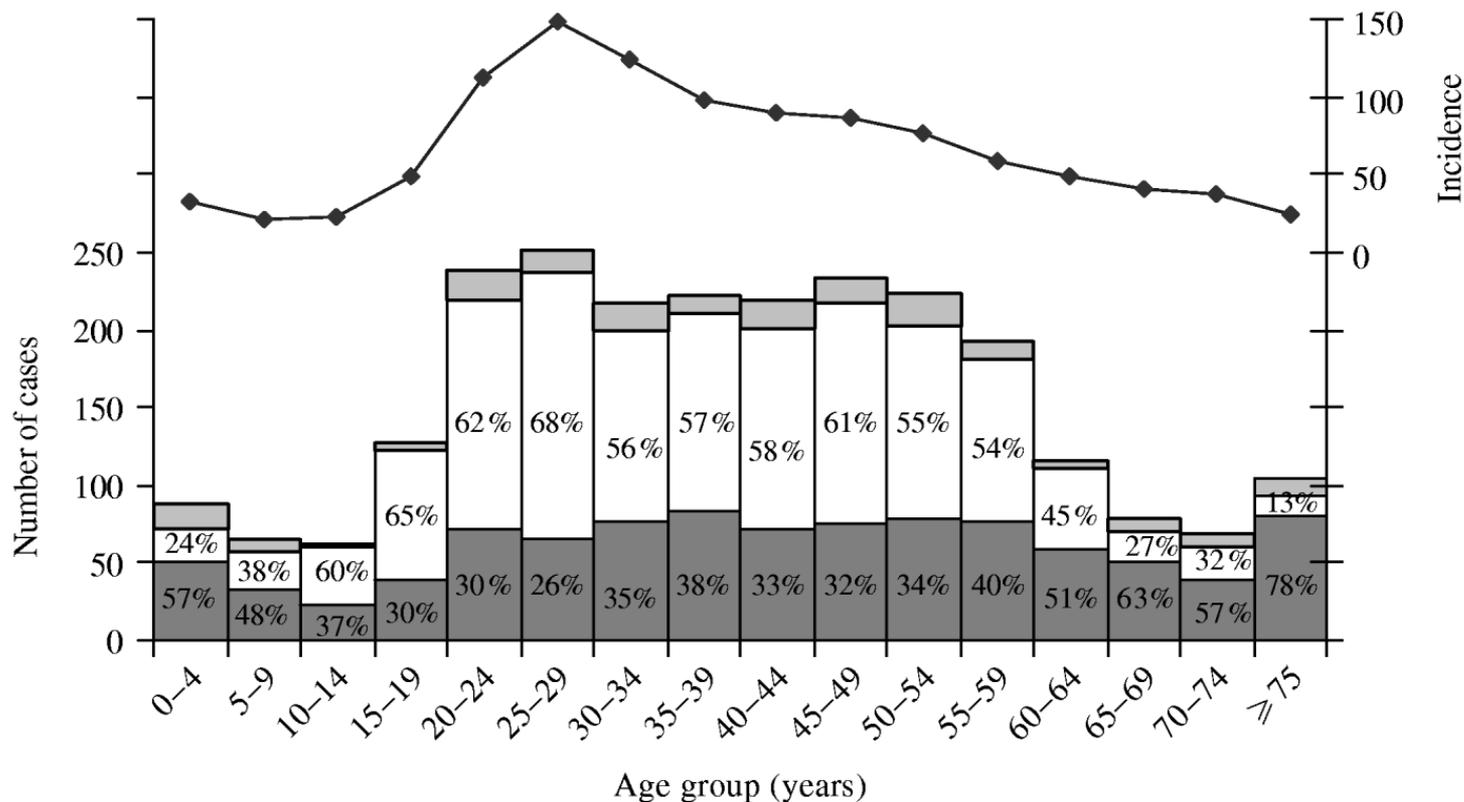
Campylobacter cases reported to the National Infectious Disease Register of Finland between July 2002 and June 2005



Source: Nakari et al, Epidemiol Infect 2010



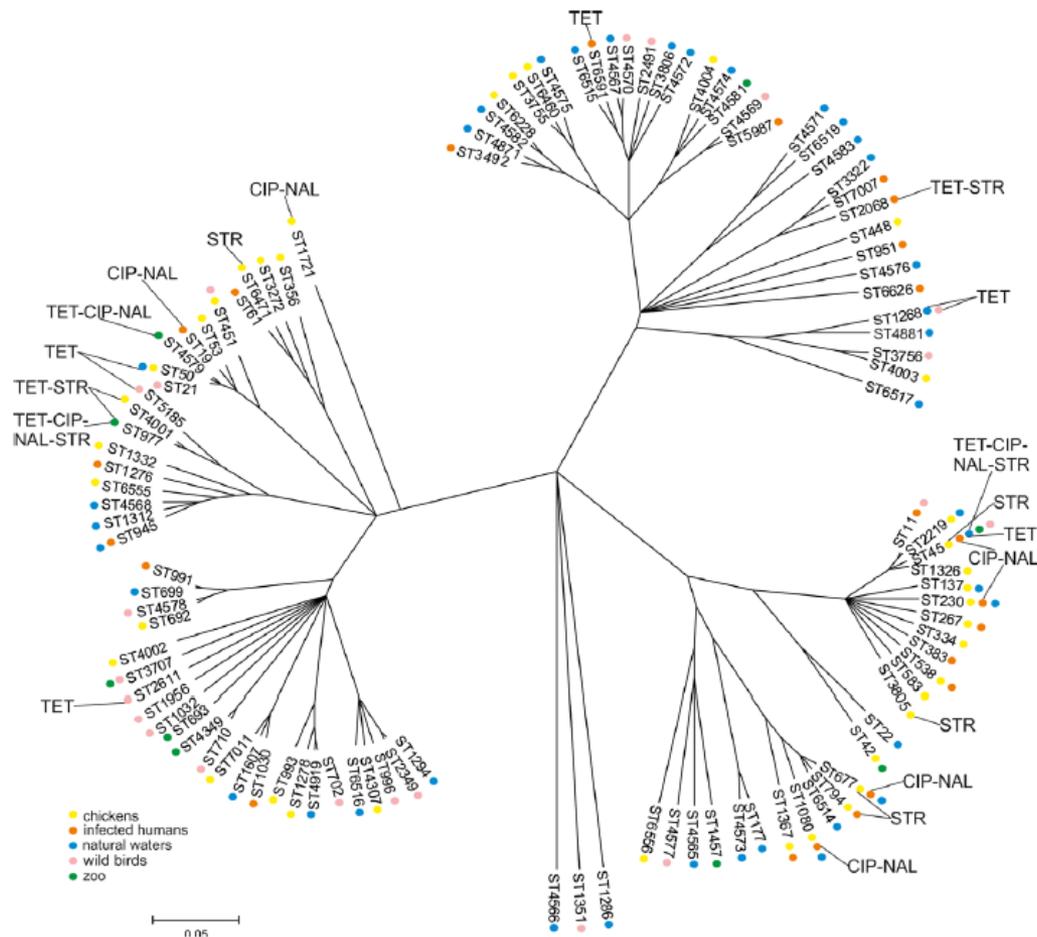
Mean incidence of *Campylobacter* infection in different age groups of patients in Finland between 2002 and 2005



Source: Nakari et al, Epidemiol Infect 2010



ClonalFrame genealogy including all detected *C. jejuni* sequence types (STs) and resistance patterns in different sources





Campylobacter spp.

- If antimicrobial treatment is needed
 - Macrolides
 - Fluoroquinolones
 - Co-amoxiclav?
 - Carbapenems
 - Tigecycline
- Fluoroquinolone resistance is up to 80% but macrolide resistance is still low, 0-4%
 - Poland, Germany, Iran, Ethiopia, Chile, Japan
 - All studies from 1996-2009



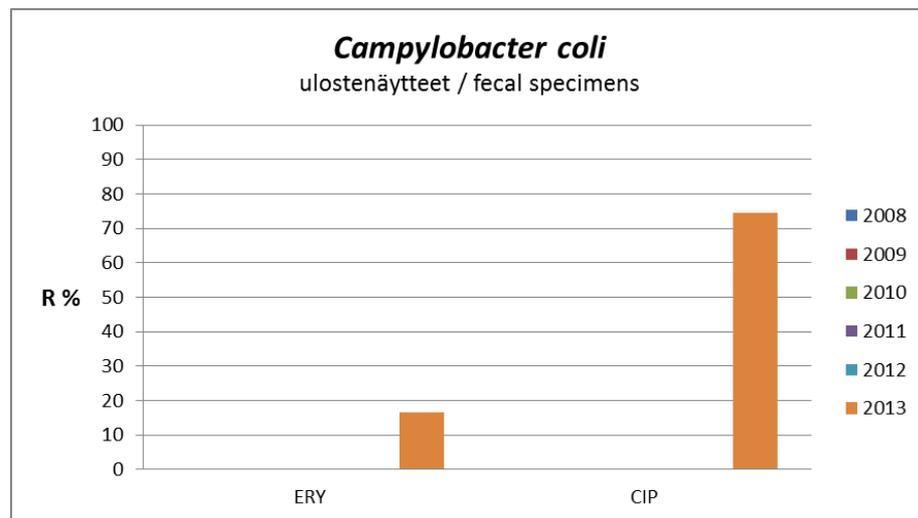
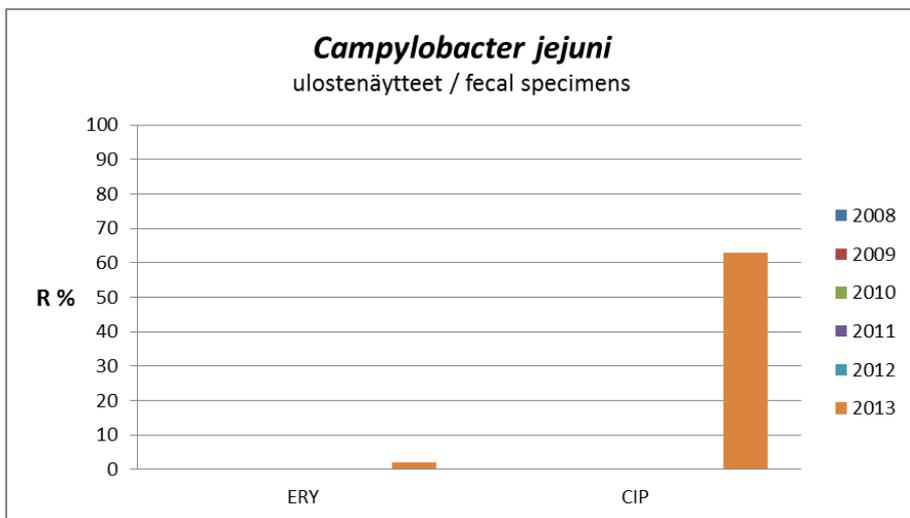
***Campylobacter* spp.**

- The incidence of macrolide resistance among the *Campylobacter* spp. is low, but the macrolide-resistant strains are uniformly multidrug-resistant
- No perorally administered antimicrobial agent reliably covers the macrolide-resistant *Campylobacter* strains



Resistance epidemiology

Antimicrobial resistance among *C.j.* and *C.c.*, Finres 2013 report



FINNISH STUDY GROUP FOR ANTIMICROBIAL RESISTANCE



SUOMALAINEN MIKROBILÄÄKERESISTENSSIN TUTKIMUSRYHMÄ



Resistance epidemiology

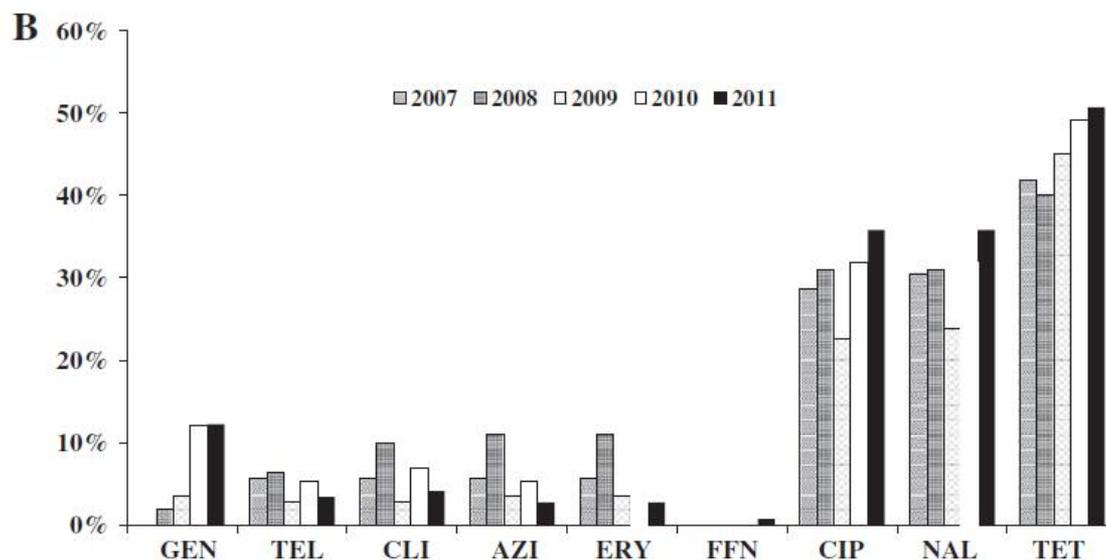
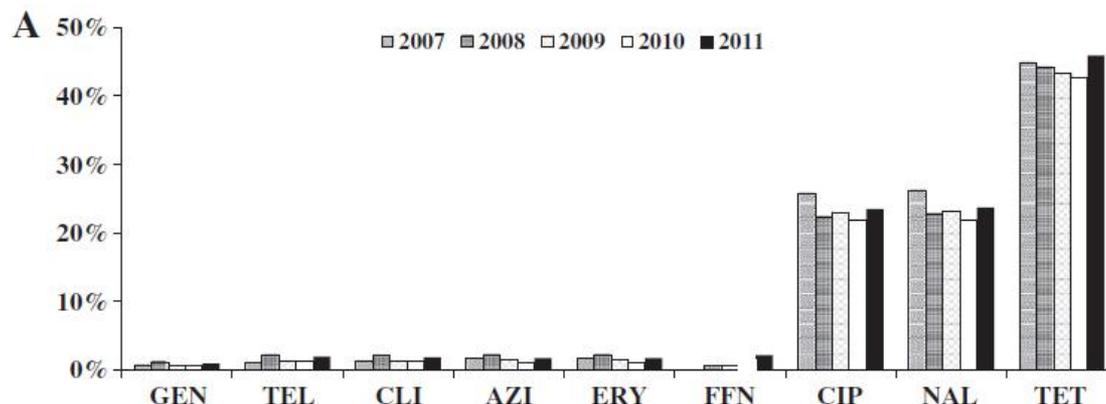
Antimicrobial resistance among 78 *C.jejuni* isolates from chicken in Finland 2009, Finres-Vet 2007-2009 report

Distribution (%) of MICs (mg l ⁻¹)														
Antibiotic	%R	95% C.I.	≤0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Ciprofloxacin	1.3	0.1-7.9	3.8	73.1	19.2	2.6					1.3			
Erythromycin	0.0	0.0-5.8				87.2	9.0	3.8						
Gentamicin	1.3	0.1-7.9			1.3	52.6	44.9	1.3						
Nalidixic acid	1.3	0.1-7.9							56.4	39.7	2.6			1.3
Tetracycline	2.6	0.5-9.9		85.9	10.3			1.3			1.3	1.3		



Resistance epidemiology

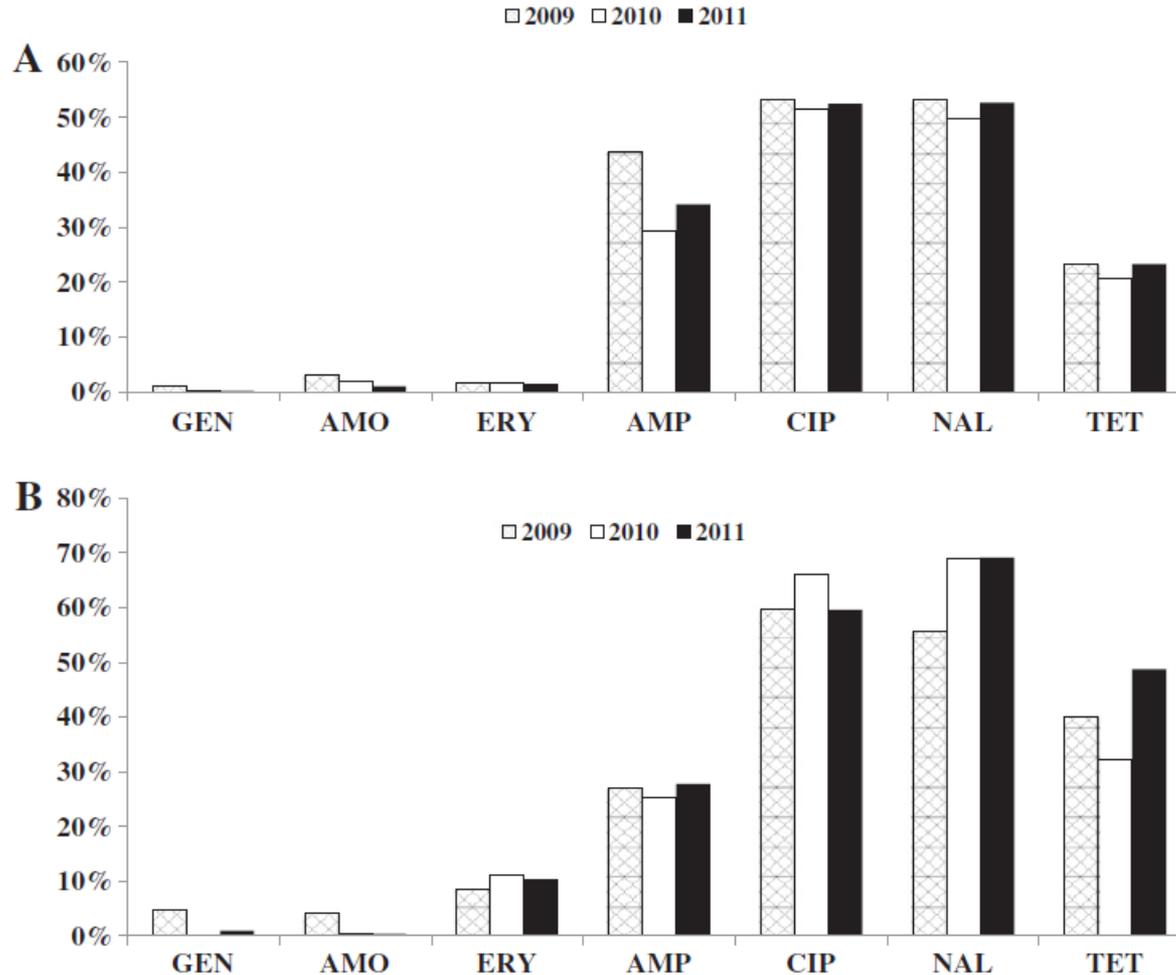
Antimicrobial resistance among *C.j.* (A) and *C.c.* (B), U.S. NARMS





Resistance epidemiology

Antimicrobial resistance among *C.j.* (A) and *C.c.* (B), EFSA-ECDC report





***Campylobacter* spp. – Susceptibility testing**

- CLSI disk diffusion method may not be a reliable tool for *Campylobacter* spp.
- For 17 (10%) of 174 *Campylobacter* strains, the variation was substantial for both erythromycin and ciprofloxacin.
- For seven antimicrobial agents, only macrolide-resistant strains (to macrolides) did not have a substantial variation between 2-4 times repeated disk diffusion tests.



Susceptibility testing

Table 1

Interpretive criteria for *Campylobacter* susceptibility testing recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008, 2010), the European Committee on Antimicrobial Susceptibility (EUCAST) (EUCAST, 2012a, 2013a), the British Society for Antimicrobial Chemotherapy (BSAC) (BSAC, 2012), and the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (CA-SFM, 2010).

Antimicrobial class ^a	Antimicrobial agent	Organization ^b	Disk diffusion method			Dilution method ^c			
			Disk content	Zone diameter (mm) interpretive criteria ^d		MIC (µg/mL) interpretive criteria			
				S	I	R	S	I	R
Macrolides	Erythromycin	CLSI	15 µg	>6	>6	6	≤8	16	≥32
		EUCAST, <i>C. jejuni</i>	15 µg	≥20	-	<20	≤4	-	>4
		EUCAST, <i>C. coli</i>	15 µg	≥24	-	<24	≤8	-	>8
		BSAC	5 µg	≥22	-	≤21	≤4	-	>4
		CA-SFM	15 UI	≥22	-	<17	≤1	-	>4
Quinolones	Ciprofloxacin	CLSI	5 µg	>6	>6	6	≤1	2	≥4
		EUCAST	5 µg	≥26	-	<26	≤0.5	-	>0.5
		BSAC	1 µg	≥26	-	≤25	≤0.5	1	>1
		CA-SFM	5 µg	≥25	-	<22	≤0.5	-	>1
		Nalidixic acid	BSAC	30 µg	≥20	-	≤19	-	-
CA-SFM	30 µg		≥20	-	<15	≤8	-	>16	
Tetracyclines	Tetracycline	CLSI	-	-	-	-	≤4	8	≥16
		EUCAST	30 µg	≥30	-	<30	≤2	-	>2
		CA-SFM	30 UI	≥19	-	<17	≤4	-	>8
	Doxycycline	CLSI	-	-	-	-	≤2	4	≥8

^a Erythromycin can be used to determine susceptibility to azithromycin (CLSI and EUCAST) and clarithromycin (CLSI, EUCAST, BSAC, and CA-SFM); quinolone resistance is most reliably detected in tests with nalidixic acid disks (BSAC); and tetracycline can be used to determine susceptibility to doxycycline (EUCAST).

^b All published breakpoints from CLSI, EUCAST, and BSAC are shown in the table. CA-SFM publishes interpretive criteria for additional 9 antimicrobials (data not shown).

^c These MIC interpretive criteria apply to both agar dilution and broth microdilution methods.

^d According to CLSI, no zone of inhibition (growth up to the edge of a 6-mm disk) indicates resistance to macrolides or ciprofloxacin, respectively. Appearance of any zone of inhibition would require MIC determination for accurate categorization of susceptibility.



Susceptibility testing

Table 2

Standardized in vitro antimicrobial susceptibility testing methods for *Campylobacter* approved by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008, 2010) and the European Committee on Antimicrobial Susceptibility (EUCAST) (EUCAST, 2012a,b; ISO, 2006).

Testing parameters	CLSI			EUCAST ^a	
	Disk diffusion	Agar dilution	Broth microdilution	Disk diffusion	Broth microdilution
Medium	Mueller–Hinton agar with 5% defibrinated sheep blood	Mueller–Hinton agar with 5% defibrinated sheep blood	Cation-adjusted Mueller–Hinton broth with lysed horse blood (2.5–5% v/v)	Mueller–Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) ^b	Cation-adjusted Mueller–Hinton broth with 5% lysed horse blood and 20 mg/L β-NAD
Inoculum	McFarland 0.5			McFarland 0.5	
Incubation	Microaerobic atmosphere (10% CO ₂ , 5% O ₂ , and 85% N ₂), 36–37 °C for 48 h or 42 °C for 24 h			Microaerobic environment (10% CO ₂ , 5% O ₂ , and 85% N ₂), 41 ± 1 °C for 24 h ± 30 min ^c	
Quality control organism	<i>C. jejuni</i> ATCC 33560			<i>C. jejuni</i> ATCC 33560	

^a Agar dilution test parameters are not available by EUCAST.

^b The MH-F plates should be dried (at 20–25 °C overnight, or at 35 °C, with the lid removed, for 15 min) prior to inoculation to reduce swarming.

^c Some *C. coli* isolates may not have sufficient growth after 24 h incubation. These are re-incubated immediately and inhibition zones read after a total of 40–48 h incubation.



EUCAST methodology

Media	MH-F (pre-dried plates to prevent swarming)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41°C 24 h ± 30 min*
Reading	EUCAST standard reading

* *Campylobacter coli* isolates with insufficient growth are reincubated immediately and read after a total of 40-48 h incubation



C. jejuni and *C. coli*

EUCAST kliniska brytpunkter v. 5.0, NordicAST v. 5.1, 2015-04-20

Fluorokinoloner	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)	
	S ≤	R >		S ≥	R <
Ciprofloxacin	0.5	0.5	5	26	26

Makrolider	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)	
	S ≤	R >		S ≥	R <
Azitromycin	Note	Note		Note	Note
Klaritromycin	Note	Note		Note	Note
Erytromycin <i>C. jejuni</i>	4	4	15	20	20
Erytromycin <i>C. coli</i>	8	8	15	24	24

Tetracycliner	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)	
	S ≤	R >		S ≥	R <
Doxycyclin	Note	Note		Note	Note
Tetracyclin	2	2	30	30	30



Conclusions - Campylobacter

- Use EUCAST disk diffusion method for *Campylobacter* susceptibility testing!
- For severely ill patients, MIC-based susceptibility testing should be used
 - But how?
- There is a need for international harmonization of *Campylobacter* susceptibility testing.



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Thank you!

Tack!

Kiitos!