Antimicrobial resistance monitoring: building up capacities and key observations

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Agenda

- ✓ Laboratory capacities – NVRI experiences
- ✓ *Salmonella* resistance
  - ✓ Serovar differences
  - ✓ Quinolone resistance
  - ✓ Multiresistance, incl. ACSSuT profile
  - ✓ Aminoglicoside resistance
  - ✓ Phenicols resistance
  - ✓ Rationale for cut-off value aproach
- ✓ *E. coli* resistance
  - ✓ Variability by source
  - ✓ MDR
  - ✓ Seasonal trends
  - ✓ ???

- ✓ presumably & hopefully
- ✓ no answers
- ✓ no conclusions
- ✓ puzzles and questions for further studies

minimum efforts to recognise AMR epi situation

Antimicrobial susceptibility testing at NVRI, NRL-*Salmonella*

- ✓ Resistance - important *Salmonella* feature
  - ✓ research projects
  - ✓ diffusion method, commercial tests
  - ✓ publications: 1997, 1998, 1999, 2001
- ✓ Important epidemiological issue
  - ✓ ARBAO-II
  - ✓ Five-year research project (1 Nov. 2003 – 30 Sept. 2008)
  - ✓ MIC testing: DKMVN1, PLVM, EUVMS
  - ✓ Directive 2003/99/EC
  - ✓ National Antimicrobial Protection Program (MH, 2004)
  - ✓ NRL-AR (2006……)
  - ✓ Commission Decision 2007/407/EC

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Laboratory capacities

- Personel
  - skills, training, networking
- Facilities
- Method
  - EN ISO 20776-1:2006 (MIC, reference)
  - antimicrobials, dilution range, interpretation
- Recommendations (i.e. EFSA's)
- Equipment
  - TREK D.S. (Sensititre: EUVMS, AutoInoculator, Sensitouch, SWIN,
  - VetMIC
- Software (WHONet)
- Legislation: 2003/99/EC, 2007/407/EC

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Antimicrobial resistance monitoring: building up capacities

”Do not ask what country can do for you, ask yourself what you can do for country”

(J.F. Kennedy, 1964)

”Do not ask what **resistance monitoring** can do for you, ask yourself what you can do for **resistance monitoring** ”

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Resistance in *Salmonella*

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Salmonella strains

2001-2007: N ≅ 16.000

Source of isolation	Number of isolates	% of isolates
poultry	8393	55,6%
turkey	1707	11,3%
geese	834	5,5%
swine	314	2,1%
ducks	283	1,9%
pigeons	87	0,6%
other animals	193	1,3%
feed	676	4,5%
food	2542	16,8%
environment & sewage	72	0,5%

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Salmonella AST

	antibiotic	No of strains			
		DKMVN1	PLVM	EUVMS	total
1	Ampicillin	430	906	x	1336
2	Chloramphenicol	430	906	x	1336
3	Colistin	430	906	x	1336
4	Florfenicol	430	906	x*	1336
5	Gentamicin	430	906	x	1336
6	Nalidixic acid	430	906	x	1336
7	Streptomycin	430	906	x	1336
8	Sulfamethoxazole	430	906	x	1336
9	Tetracycline	430	906	x	1336
10	Trimethoprim	430	906	x	1336
11	Apramycin	430	906		1336

	antibiotic	No of strains			
		DKMVN1	PLVM	EUVMS	total
12	Ceftiofur	430	906		1336
13	Cephalothin	430	906		1336
14	Neomycin	430	906		1336
15	Spectinomycin	430	906		1336
16	Amoxicillin/ Clavulanic acid	430			430
17	Cefuroxime		906		906
18	Ciprofloxacin	430		x	430
19	Enrofloxacin		906		906
20	Cefotaxime			x*	0
21	Ceftazidime			x*	0
22	Kanamycin			x*	0

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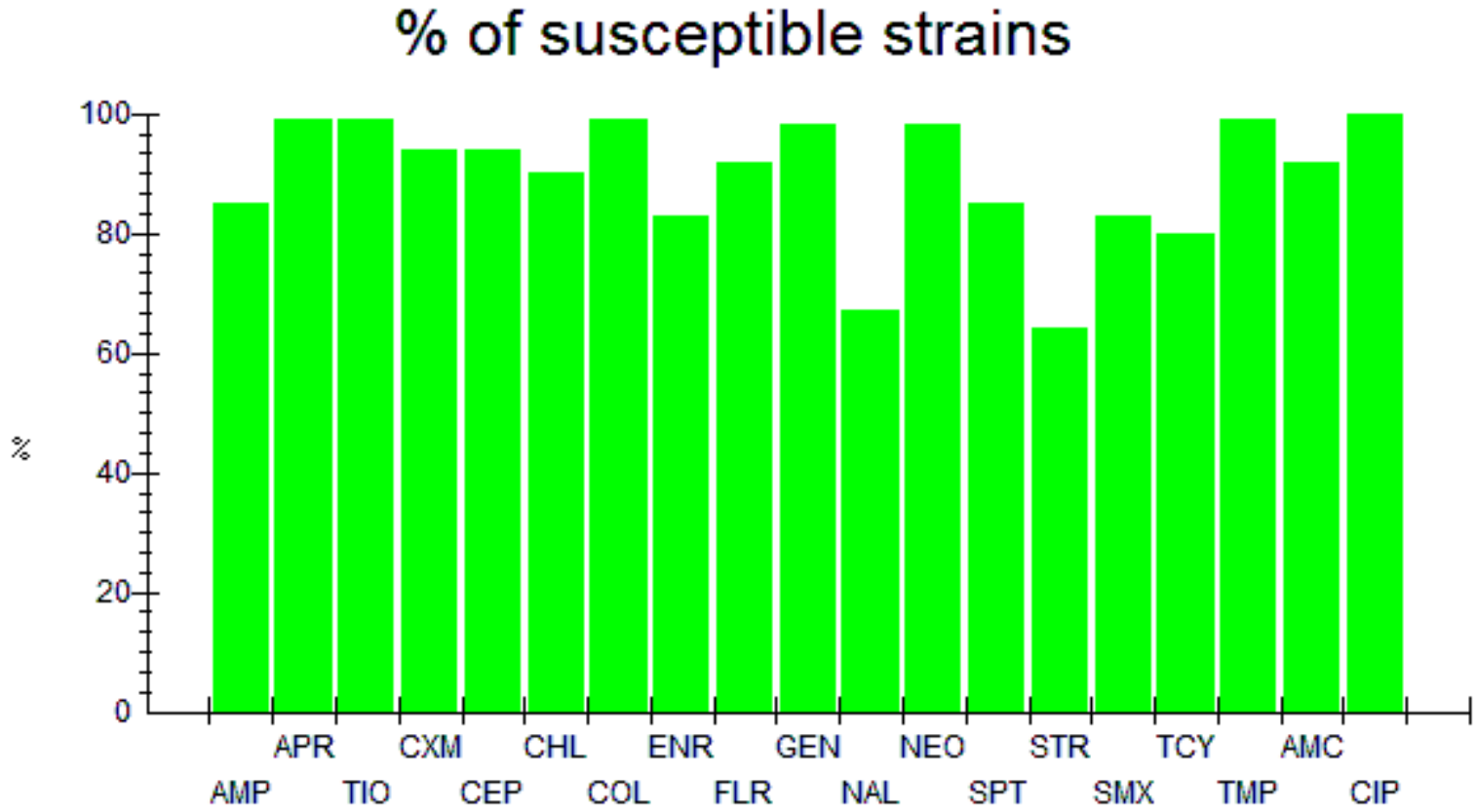
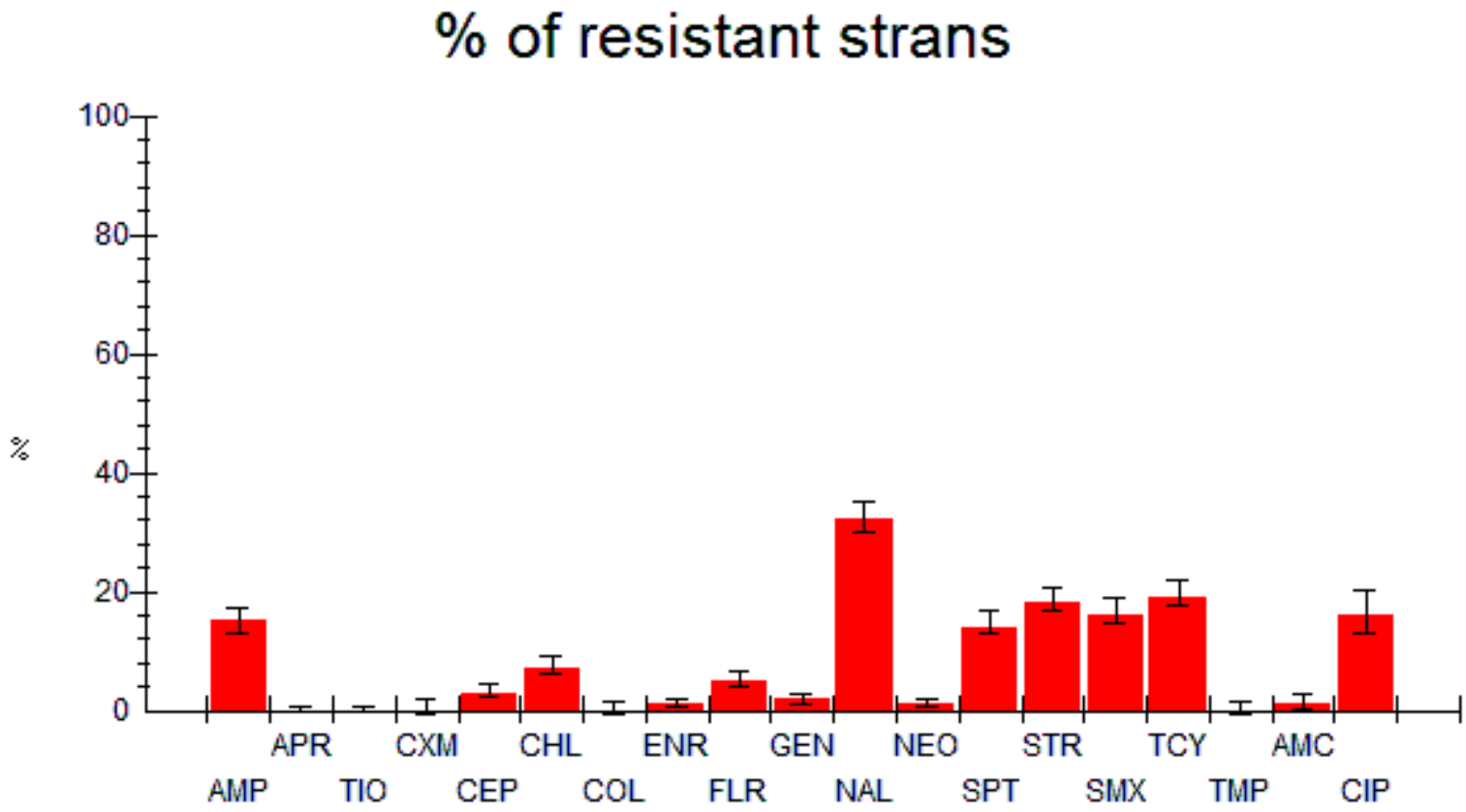
„How can I cure *Salmonella* infection?”



interpretation acc. to CLSI clinical breakpoins

What is the level of *Salmonella* resistance?



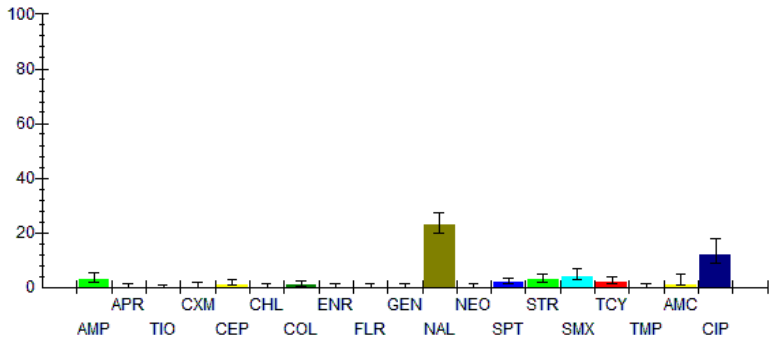
interpretation acc. to EUCAST epidemiological cut off value (if available) or CLSI clinical breakpoins

Factors influencing *Salmonella* resistance

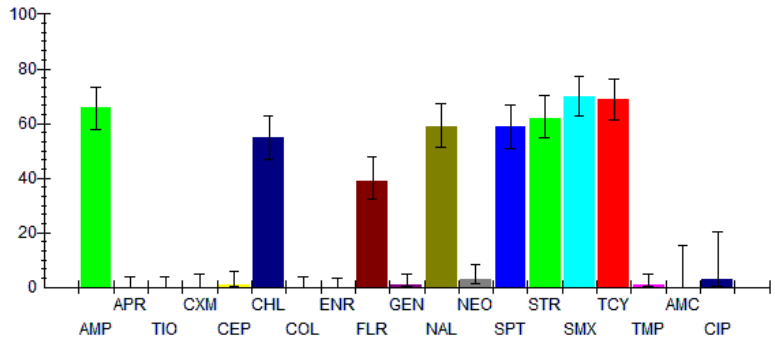
- ✓ serovar
- ✓ year-to year variations (within serovar)
- ✓ source of isolation (AMU)
- ✓ ….

AMR variability in *Salmonella* serovars

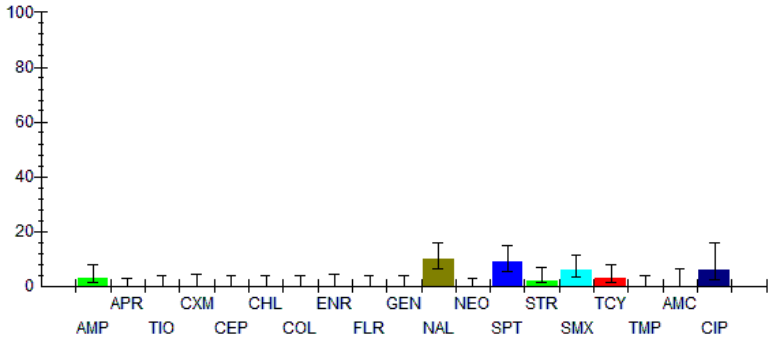
S. Enteritidis (N=564)



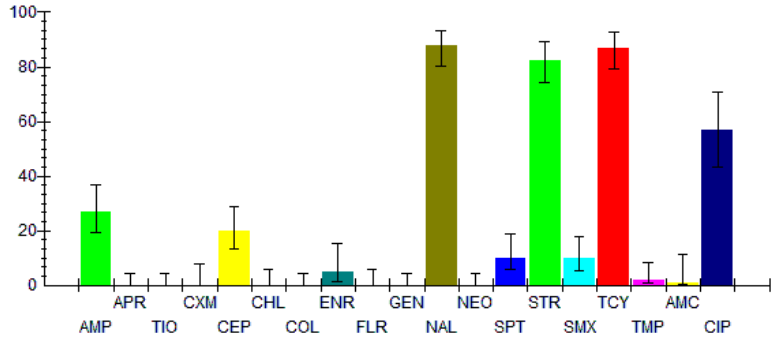
S. Typhimurium (N=156)



S. Infantis (N=174)

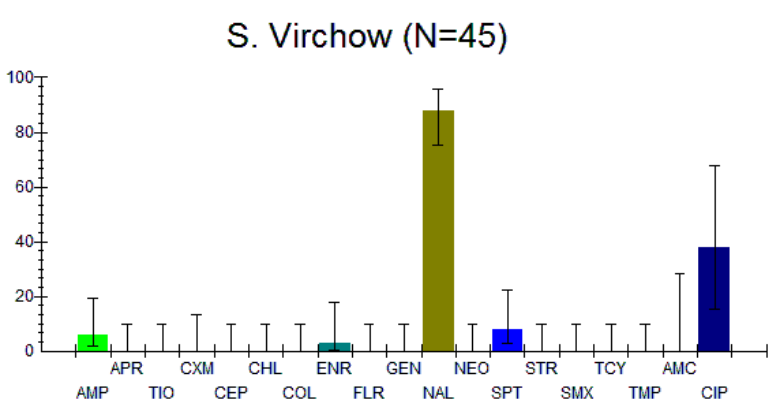


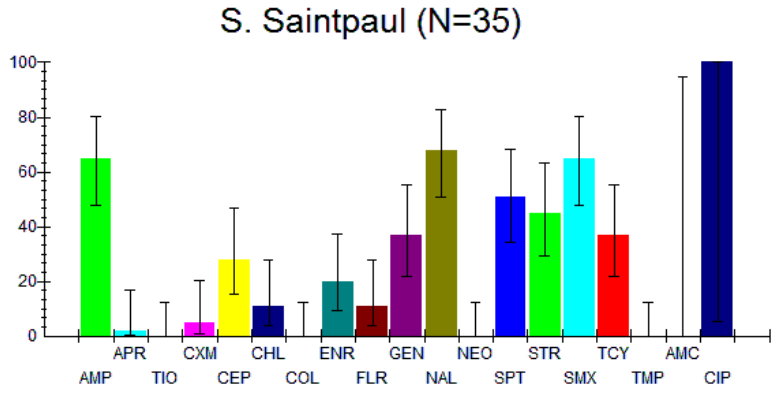
S. Hadar (N=110)

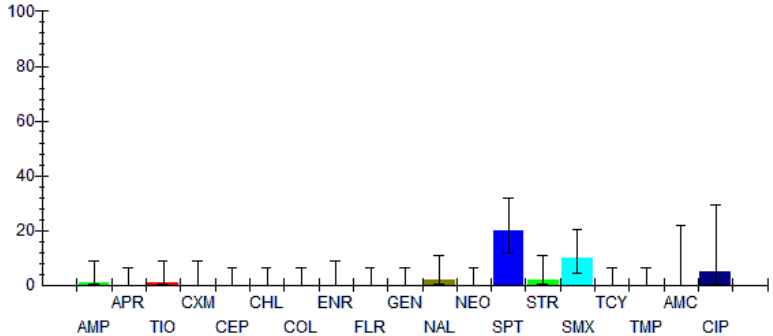


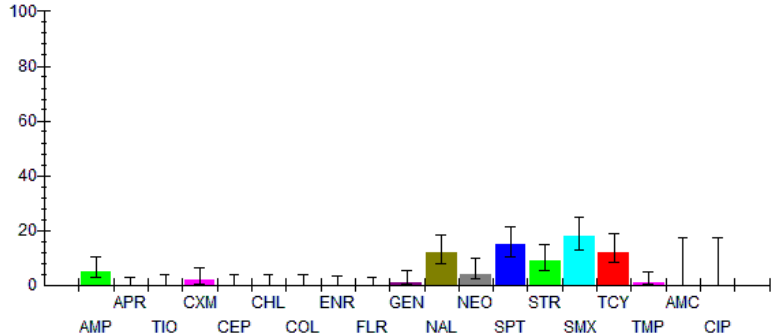
AMC, CIP, TIO, AMC – few isolates tested

AMR variability in *Salmonella* serovars (2)









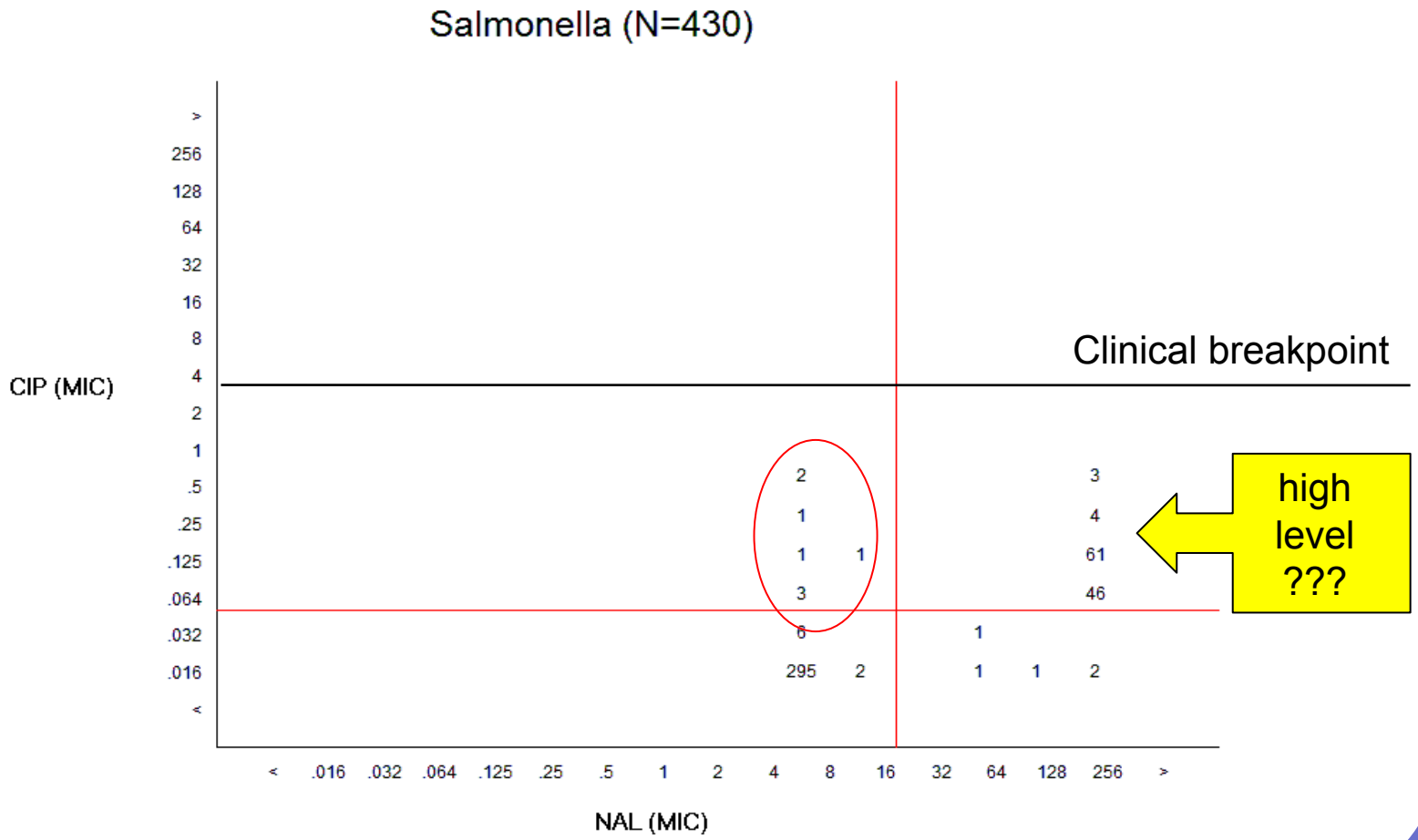
AMC, CIP, TIO, AMC – few isolates tested

Quinolones: resistance mechanisms

1. not transferable (chromosomal) - gerase
   - mutations: *gyrA/B/S, parC/E*
   - gradual:
     - single mutation = resistance to NAL+ reduced FQ susceptibility
     - two-step mutations = resistance to all quinolones

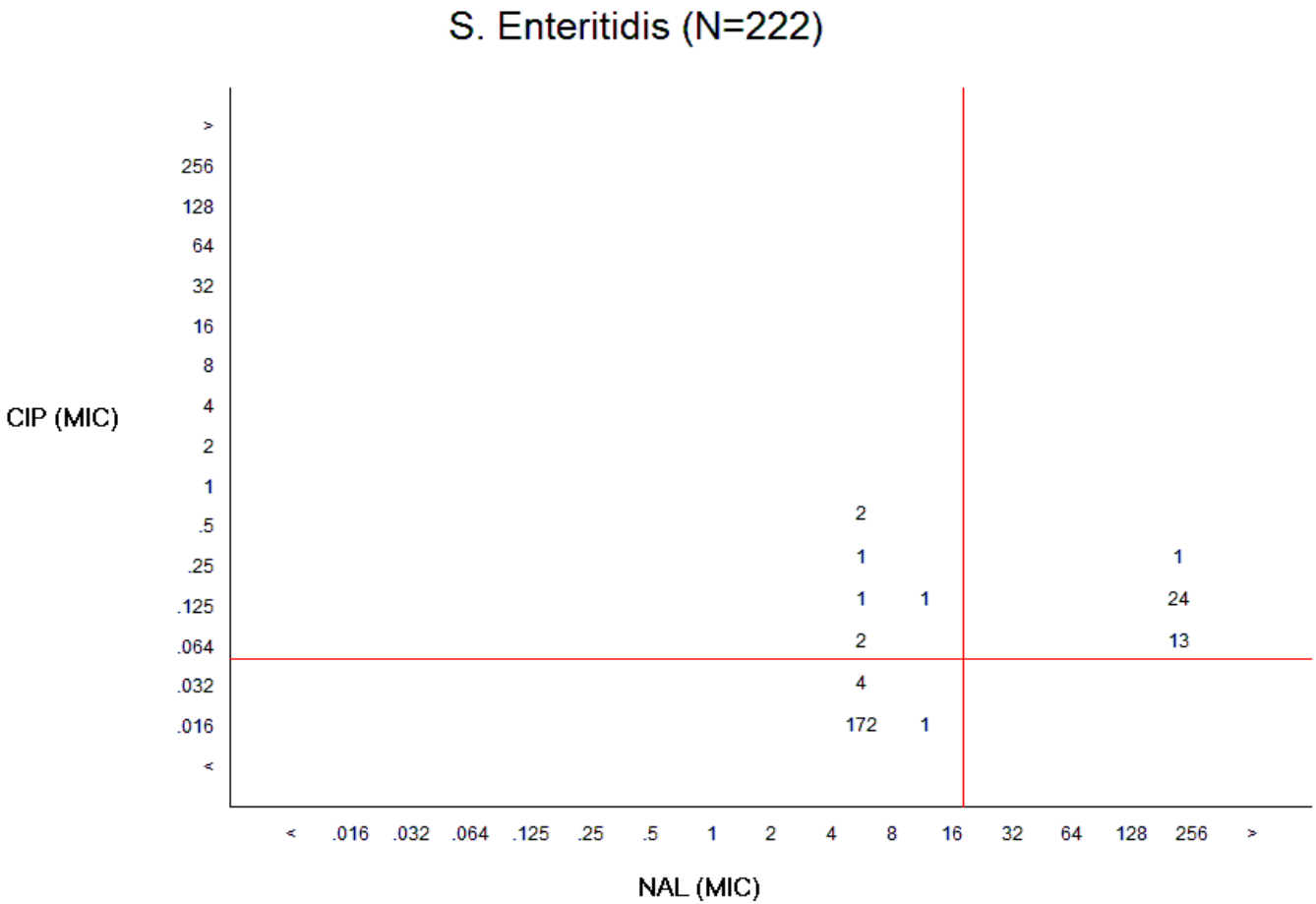
   ⇒ NAL for screening for FQ resistance
2. transferable (plasmid-mediated)
   - *qnrA* gene (1994) – protein that blocks FQ action
     - gyrase protection = reduced suscetiblility for FQ + increased mutation frequency
     - plasmid carring other resistance genes (co-resistance)
     - not necessarily full NAL resistance = screeining unrealiable
   - *aac(6')Ib* gene (2006) – modified aminoglycoside acetyltransferase = resistance to exclusively for CIP
     - resistance plasmid (including ceftazidime)

Quinolone resistance in *Salmonella*



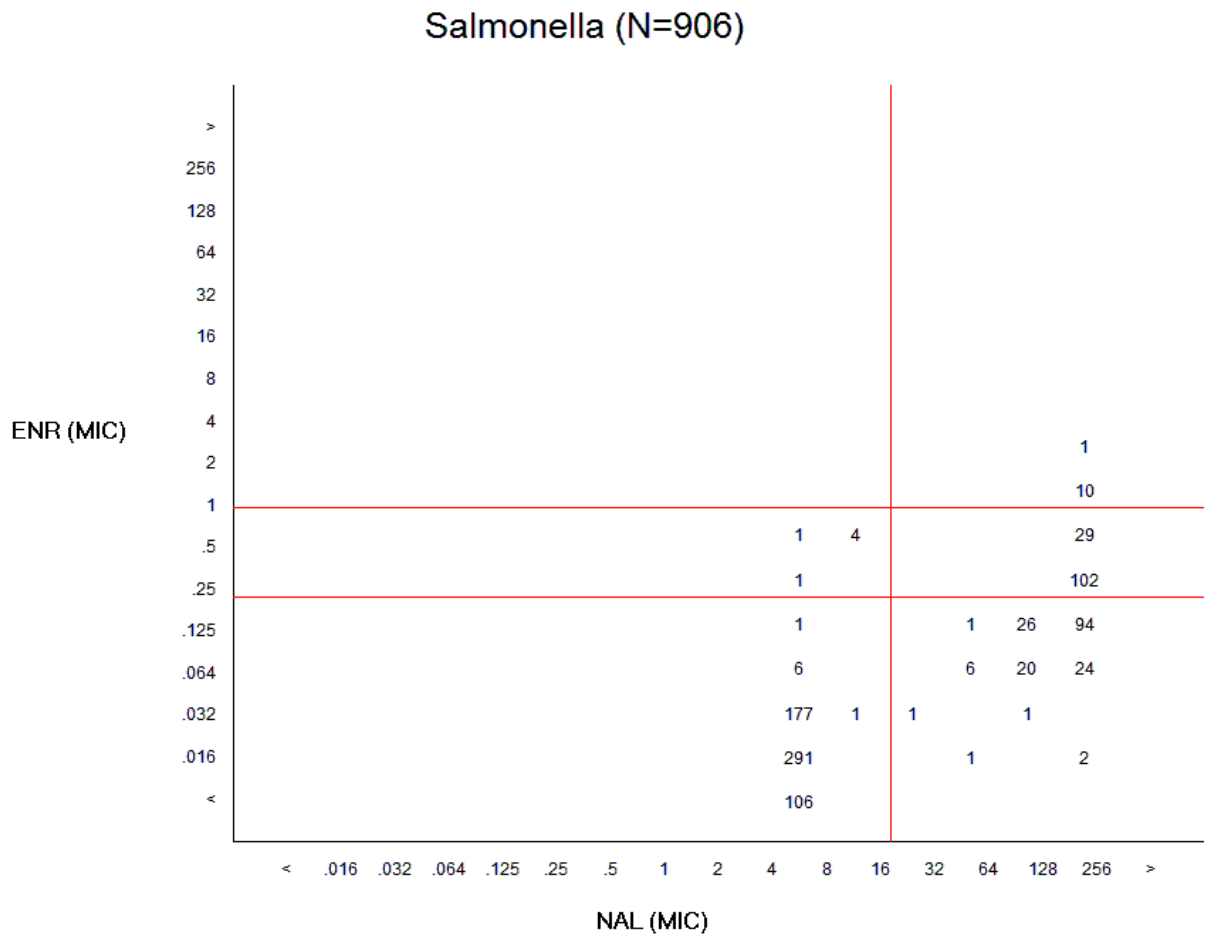
EUCAST criteria

Quinolone resistance in *S.* Enteritidis



EUCAST criteria

Quinolone resistance in *Salmonella* (2)



high level?

Nal - EUCAST criterion, Enr – CLSI criterion

Resistance profiles in ciprofloxacin-resistant *Salmonella*

Strain ID	Source	Year	Serovar	R-profile
398/04	pet food	2004	Hadar	CipST
N191A	layers	2005	Enteritidis	Cip
N189A	layers	2005	Enteritidis	Cip
N316D	layers	2005	Enteritidis	Cip
N131A	layers	2005	Enteritidis	Cip
N58E	layers	2005	Enteritidis	CipCepA
N192A	layers	2005	Enteritidis	Cip
N214F	layers	2005	Enteritidis	Cip

Resistance profiles in enrofloxacin-resistant *Salmonella*

Strain ID	Source	Year	Serovar	R-profile
R-255	broilers	2005	Infantis	EnrA
R-155	broilers	2005	Mbandaka	EnrAS
N362A	layers	2005	Enteritidis	Enr
R-319	broilers	2005	Enteritidis	Enr
975/07	food	2007	Sandiego	EnrAST
BST-SZ-090-C	turkey	2007	Saintpaul	EnrAST
BST-SZ-111-A	turkey	2007	Saintpaul	EnrAST
BSSP-KAT-372L	swine	2007	Newport	EnrA
BST-KAT-069-B	turkey	2007	Saintpaul	EnrASTSu
1640/07	poultry	2007	Enteritidis	Enr
86./07	poultry	2007	Enteritidis	EnrA
637/07	poultry	2007	Enteritidis	Enr
236/07	compost	2007	Hadar	EnrST

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Multiresistance in *Salmonella*

	Typhimurium	Hadar	Saintpaul
No of strains tested	156	110	35
No of strains resistant	124 (79%)	107 (97%)	34 (97%)
No of R-profiles	30	23	20
Diversity index (D)	0.792	0.756	0.951
No of antimicrobials/classes*	≤7	≤6	≤6
Most frequent R-profile	ACSSuTNa (66)	STNa (51)	ASSuNaG (6)
Most complexed R-profile	ACSSuTNaCip (1)	ACSSuTNa (1)	ACSSuTNa (1)

* only antimicrobials mentioned in 2007/407/CE were taken into accout

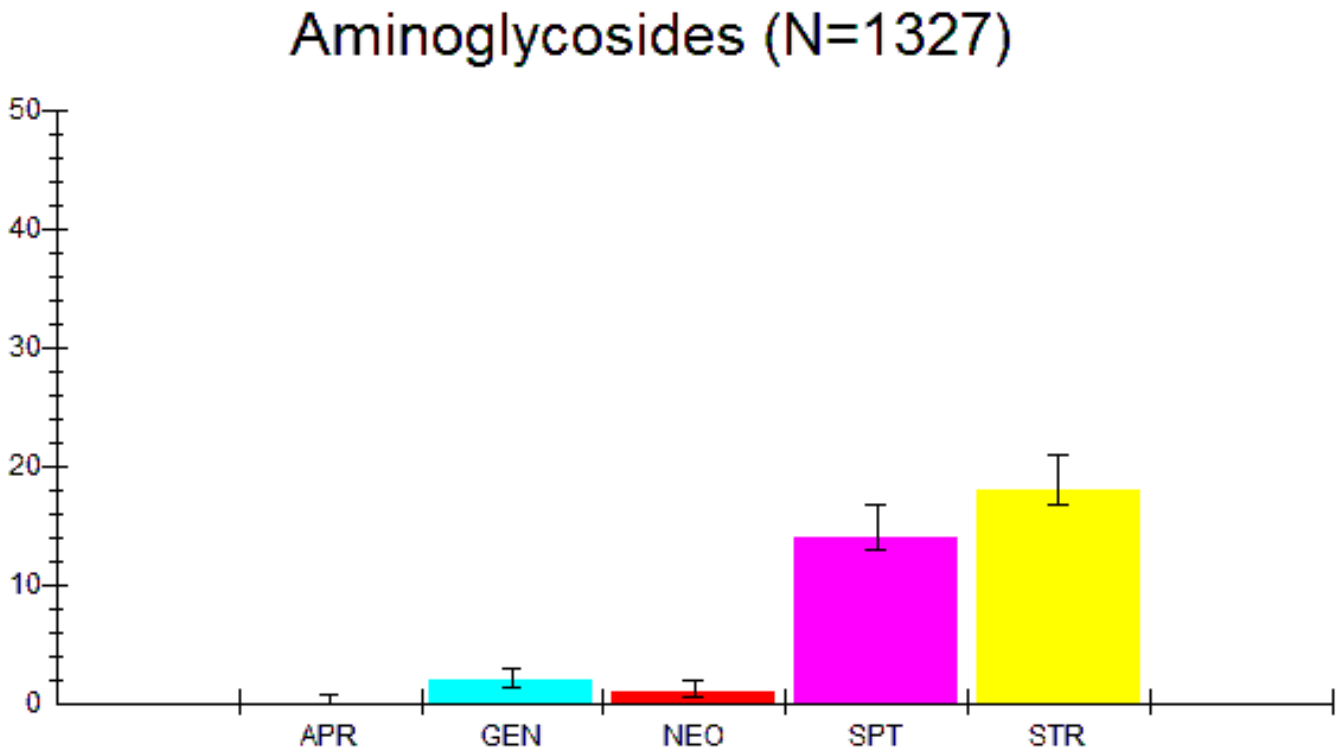
86 resistance profiles in 584 *Salmonella* strains (19 compounds)

ACSSuT phenotype

- genome integrated, class-1 integron mediated resistance
- *S.* Typhimurium DT104

Salmonella	No of strains
Typhimurium	78
Enteritidis	1
Hadar	1
Infantis	1
Saintpaul	1

Aminoglycoside resistance



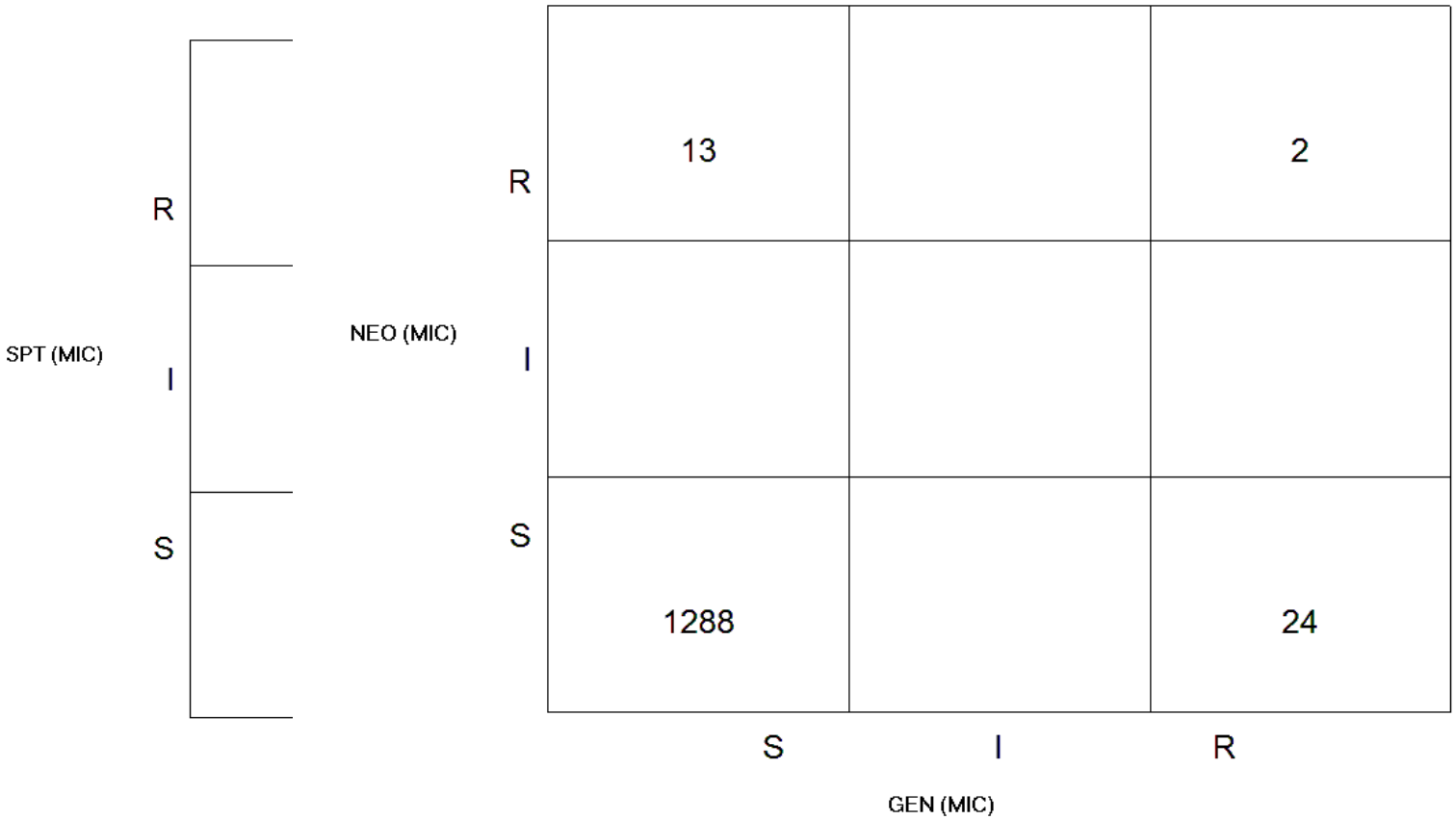
Aminoglycosides: resistance mechanisms

1. drug modification:
   - acetylotransferases
   - adenylotransferases
   - phosphotransferases
2. ribosomal target modification
   - methyltransferases
   - point-mutations (16S rRNA)
3. efflux
4. altered membrane permeability

$\Rightarrow$ transferable or genome integrated

Consequense: cross-resistance occurs, but incomplete and depends on the gene(s) present

Aminoglycosides cross- and co-resistance



High Level Aminoglycoside Resistance

1. HLAR phenotype: Gentamycin MIC ≥ 512 mg/l
   - ✓ *amrA* - encoding a protein similar to 16S rRNA methylases found in aminoglycoside-producing Actinomycetes
   - ✓ amrA - Enterobacteriaceae – plasmid mediated,
     - ✓ Europe, Japan
     - ✓ human & animals

2. *amrA* in *Salmonella* - rare (new/emerging)
   - ✓ Spain,
   - ✓ Poland: humans: Oranienburg (n=4), Enteritidis (n=3) and Typhimurium (n=1) [plasmid: *armA* + *blaCTX-M*]

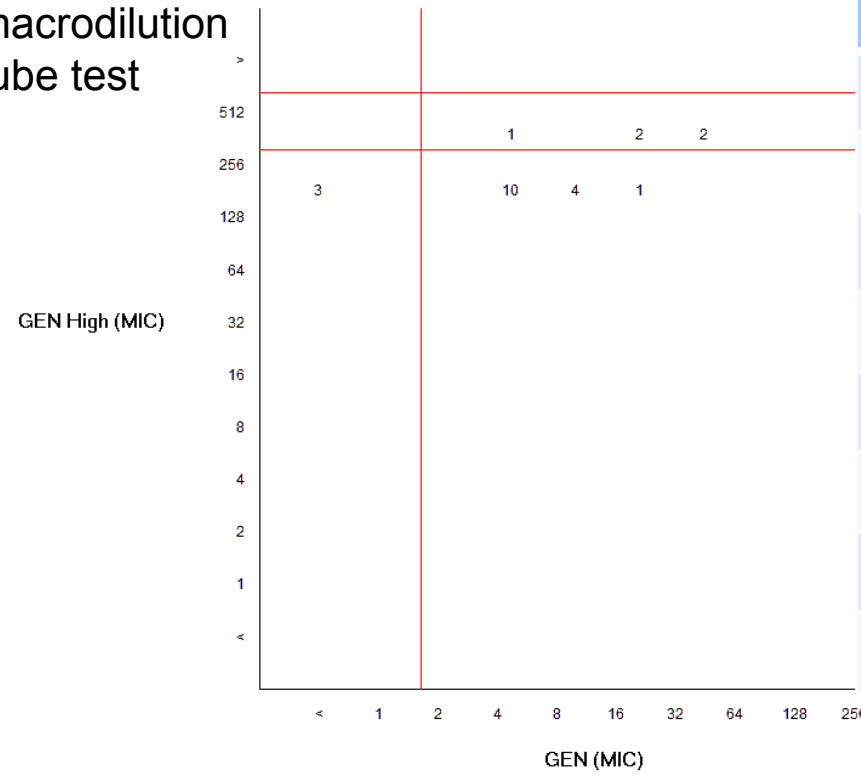
Gentamycin-resistant *Salmonella*

Serovar	# strains
Albany	1
Derby	1
Enteritidis	3
Infantis	1
Saintpaul	13
Senftenberg	1
Typhimurium	1
total	21

isolation	year				
source	2002	2004	2005	2006	2007
layers		1	4		
broilers				2	
swine	1				1
turkey					12

HLAR

macrodilution tube test

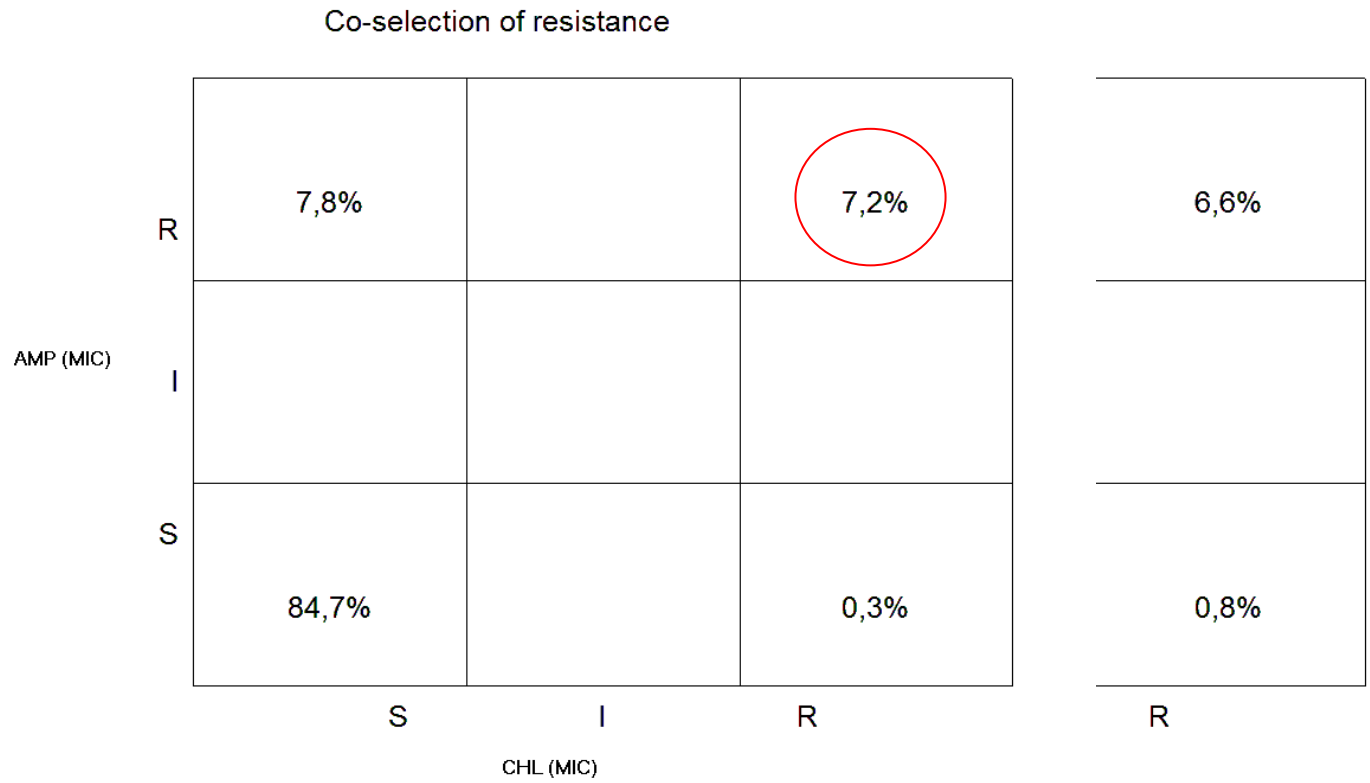


Serovar	GEN	GEN High
Albany	1	
Derby	1	1 (T/07)
Enteritidis	3	2 (B/05, L/04)
Infantis	1	
Saintpaul	**13**	**1 (T/07)**
Senftenberg	1	
Typhimurium	1	1 (S/02)
total	21	

not methylases - just enzymatic modification and …….

Amphenicols resistance

- ✓chloramphenicol use in food animals banned for 20 years
- ✓ 7,5% of resistant *Salmonella* strains
- ✓co-selection of resistance – ACSSuT



Amphenicols: resistance mechanisms

1. *floR - part of integron gene casette*
   - ✓ *chloramphenicol & florfenicol cross-resistance*
2. *CAT - chloramphenicol acetyltransferase*
   - ✓ *chloramphenicol resistance*

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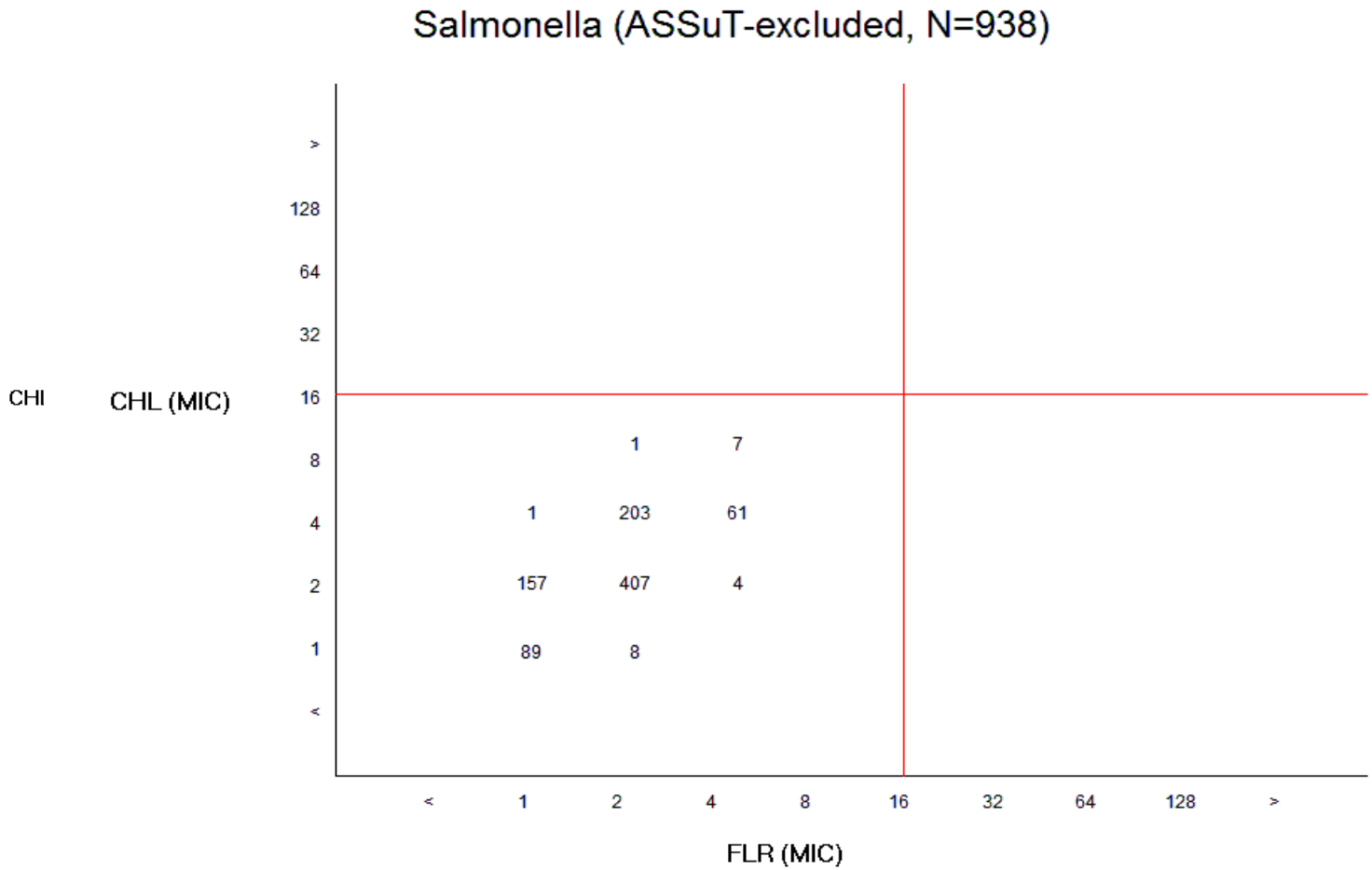
Chloramphenicol & florfenicol MIC values distribution



Conclusion: amphenicols resistance due to *floR gene* and co-selection with other resistance mechanisms

EUCAST epidemiological cut-off values: new European approach

- Commission Decision of 12 June 2007 on a harmonised monitoring of antimicrobial resistance in *Salmonella* in poultry and pigs (2007/407/EC). Official Journal of the European Union 2007; L 153: 26-29.
- European Food Safety Authority. Report including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers. The EFSA Journal 2007; 96: 1-41.
- European Food Safety Authority-Working Group on Developing Harmonised Schemes for Monitoring Antimicrobial Resistance in Zoonotic Agents. Harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from food animals in the European Union. Clin Microbiol Infect 2008; 14: 522-33.

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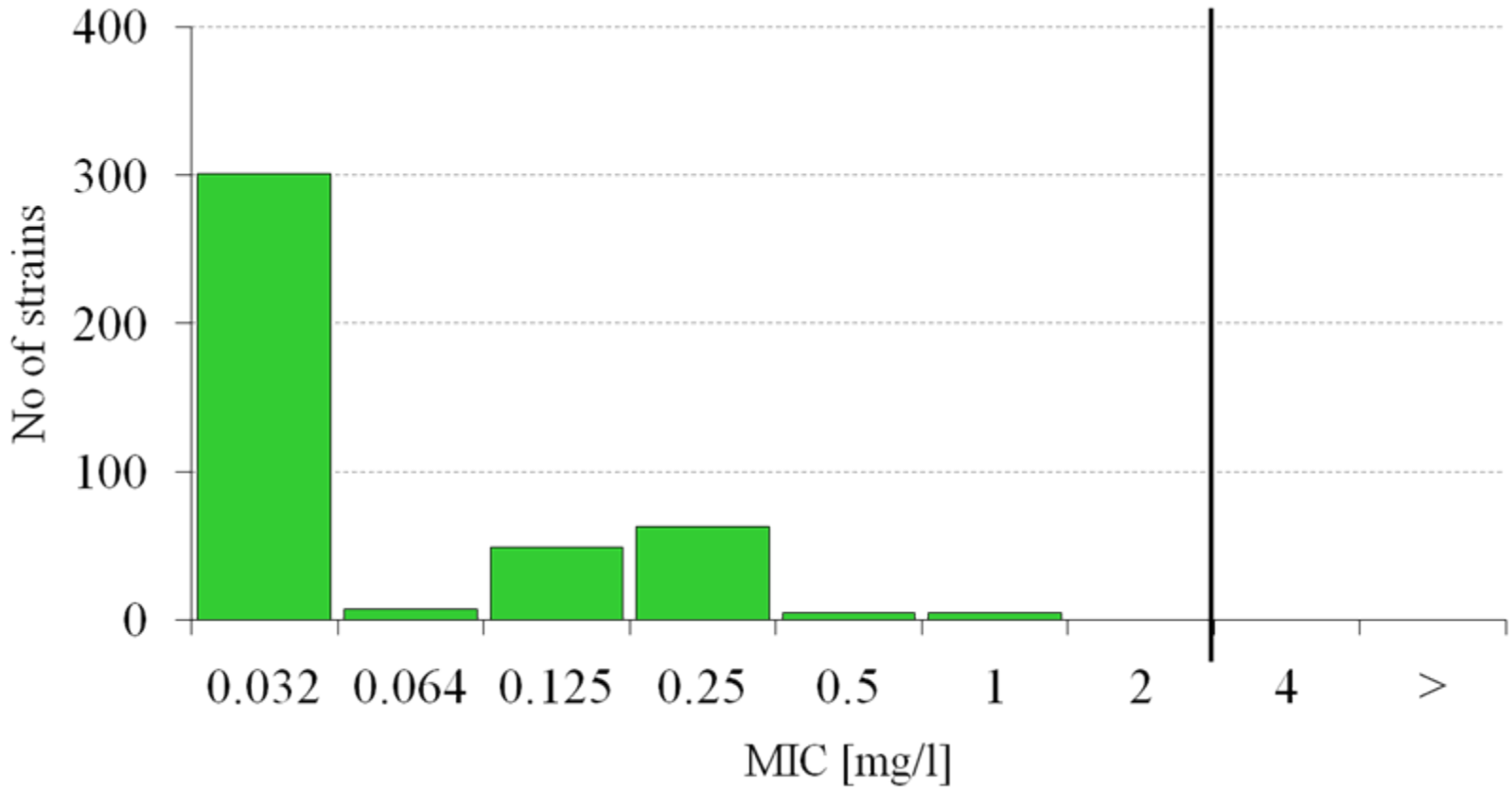
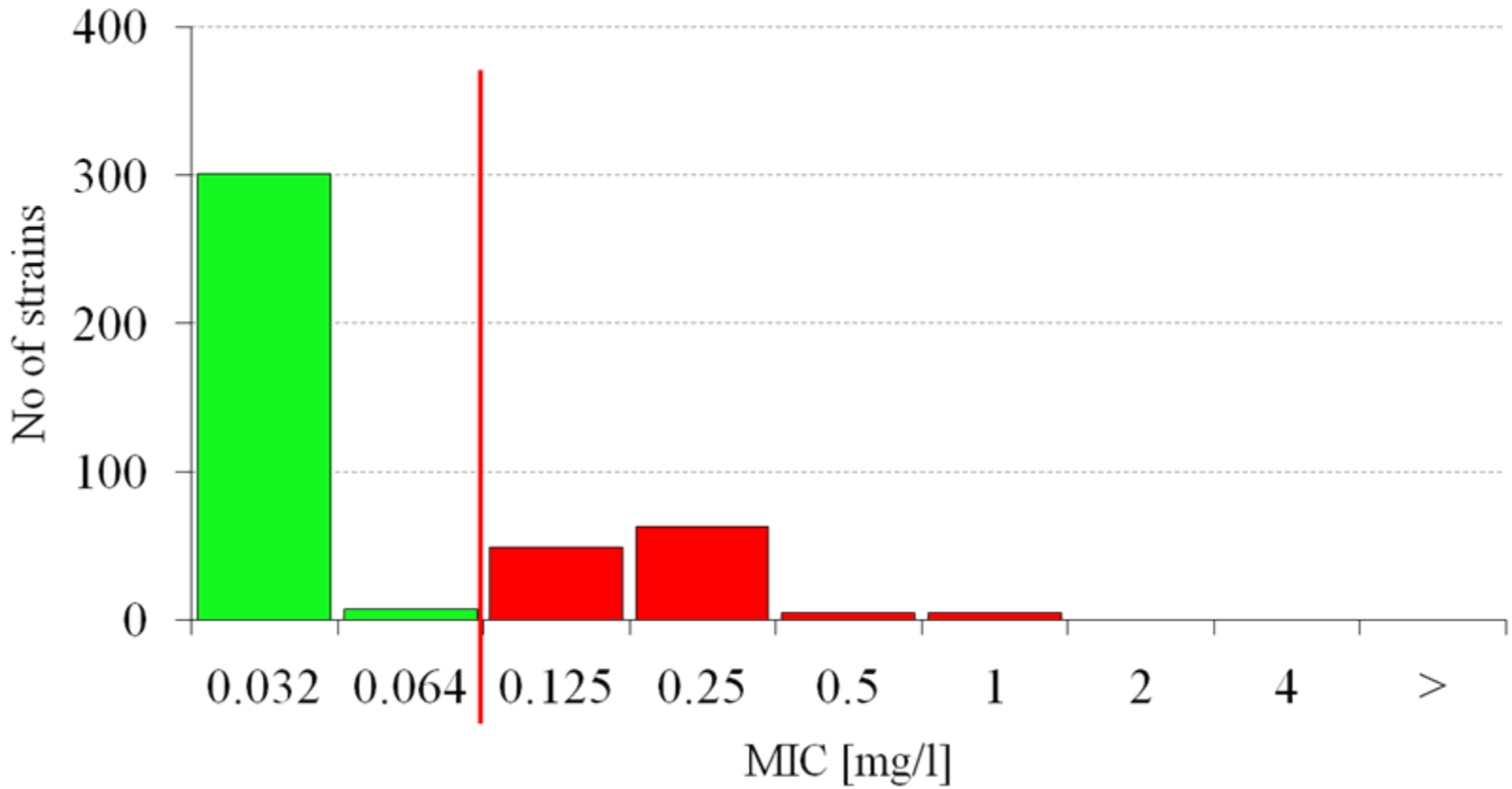
Re-evaluation of *Salmonella* antimicrobial resistance

- No change:
  - chloramphenicol (7,4%),
  - nalidixic acid (32,6%),
  - sulfamethoxazole (17,1%),
  - trimethoprim (19,5%)
- Lower resistance:
  - streptomycin (18,7% *vs*. 22,8% acc. CLSI)
- Higher resistance:
  - ampicillin (15,0% *vs*. 14,3% acc. CLSI),
  - gentamicin (2,0% *vs.* 1,0% acc. CLSI),
  - ciprofloxacin (28,4% *vs*. 0,0% acc. CLSI)

Ciprofloxacin – CLSI (R ≥ 4 mg/l)



Ciprofloxacin – EUCAST (R > 0.6 mg/l)



AMR in indicator *E. coli*

Five-year Research Project (1 Nov. 2003 – 30 Sept. 2008)

Aim:

- ✓ observe AMR patterns/resistance level
- ✓ as a possible selective preasure of antimicrobial use
- ✓ year-to-year variations

PIWet

E. coli – study setup

- ✓ Healthy animals sampled at slaughter
- ✓ Slaugherhouses
  - ✓ cattle (10), pigs (9), broilers (5), geese (4), turkey (5)
- ✓ Samples
  - ✓ rectal swabs – cattle and pigs; caecum contents – poultry
  - ✓ 200 /species/year
  - ✓ streaked directly onto MacConkey agar
- ✓ single lactose-fermenting colony for biochemical confirmation
- ✓ agar diffusion method – CLSI criteria:
  - ✓ Ampicillin
  - ✓ Chloramphenicol
  - ✓ Nalidixic acid
  - ✓ Streptomycin
  - ✓ Sulphonamides
  - Cefuroxime
  - Gentamicin
  - Enrofloxacin
  - Tetracycline
  - Trimethoprim

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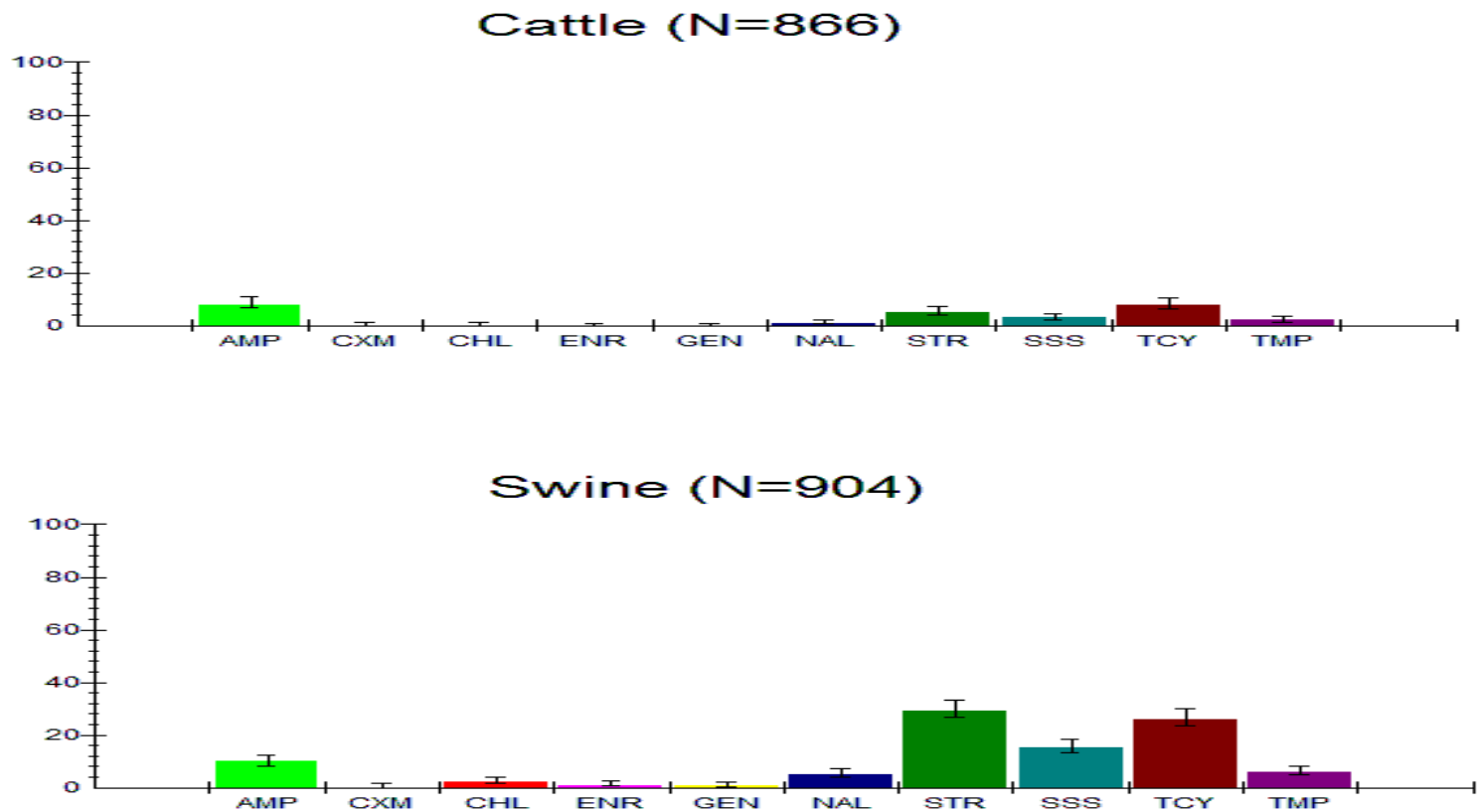
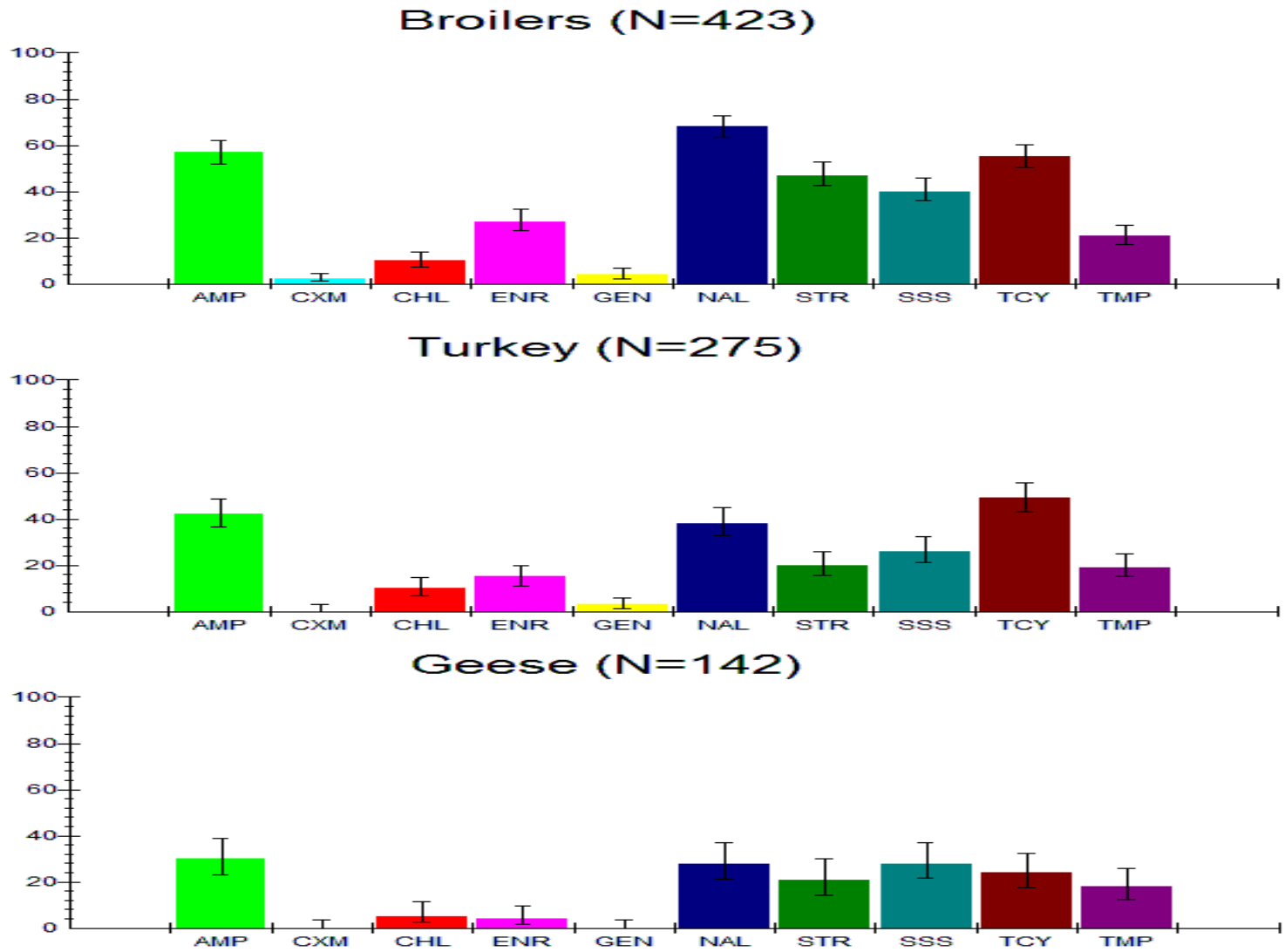
E. coli strains

Animal species	No of isolates				
	2004	2005	2006	2007	Total
Broilers	105	71	22	225	423
Cattle	228	182	195	261	866
Geese	49	37	17	39	142
Swine	313	337	92	162	904
Turkey	48	26	122	79	275
total	743	653	448	766	2610

AMR in *E. coli* by source of isolation



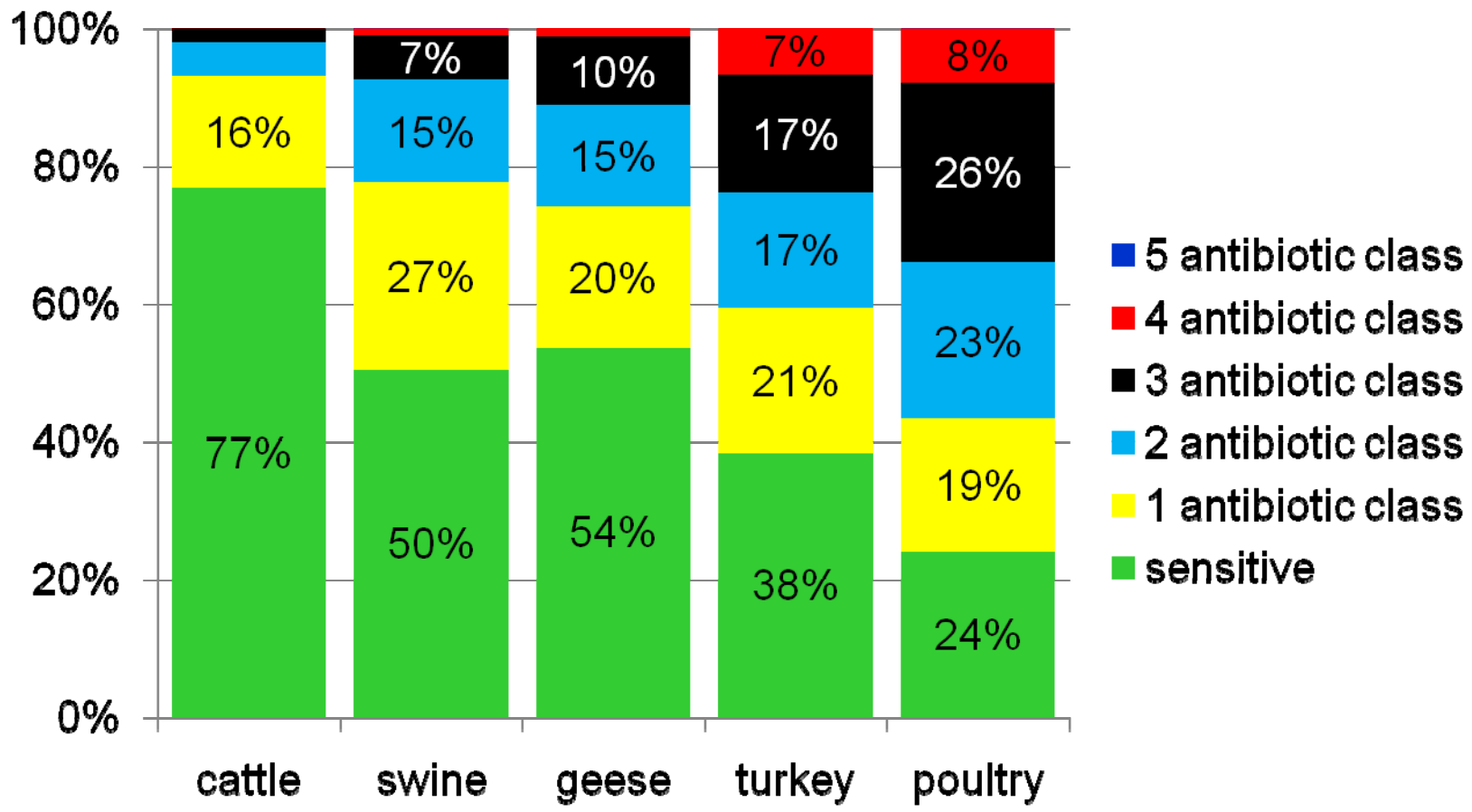
AMR in *E. coli* by source of isolation (2)



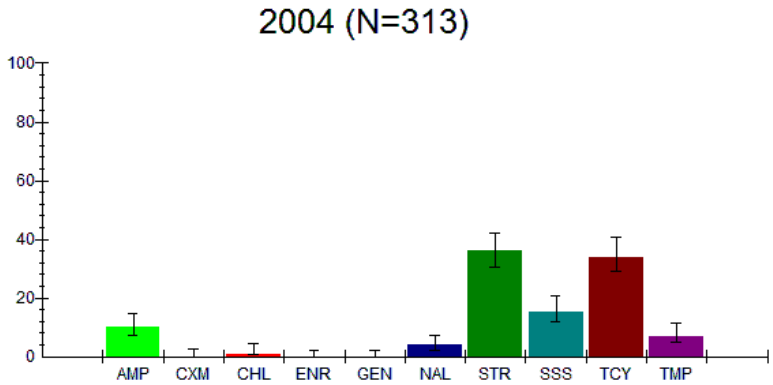
Multiresistance in *E. coli*

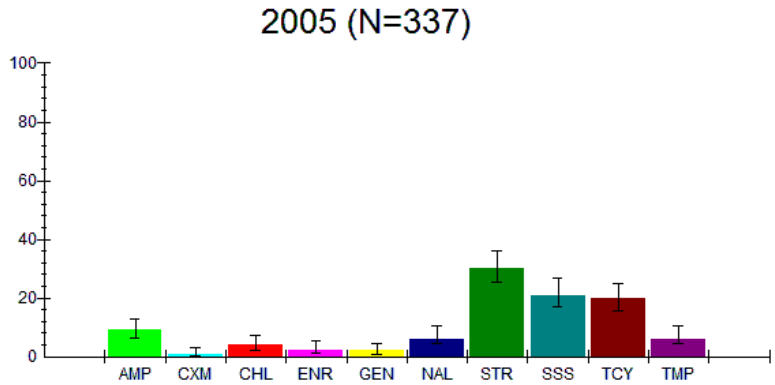
	cattle	swine	broilers	turkey	geese
No of resistant strains	202	450	323	170	66
% of resistant strains	23%	50%	76%	62%	46%
No of R-profiles	27	46	38	26	16
Diversity index (D)	0.879	0.806	0.933	0.903	0.897
No of antimicrobial classes	4	5	5	4	4
Most frequent R-profile	A (48)	S (235)	AST (53)	AT (31)	A (17)
Most complexed R-profile	CSTCxm	ACxmCGST	ACxmCGST	ACxmCGST	ACST

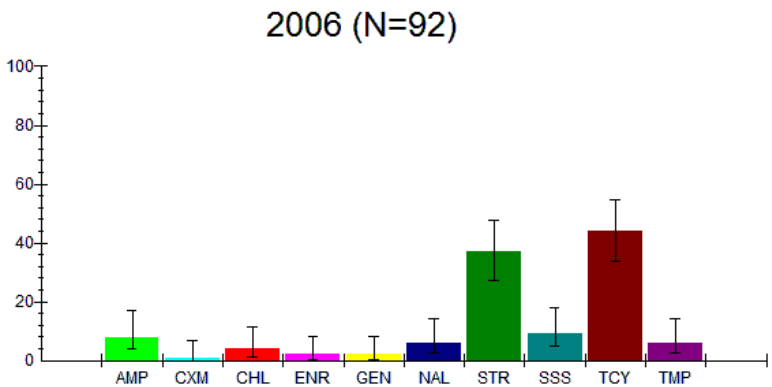
Multiresistance in *E. coli*

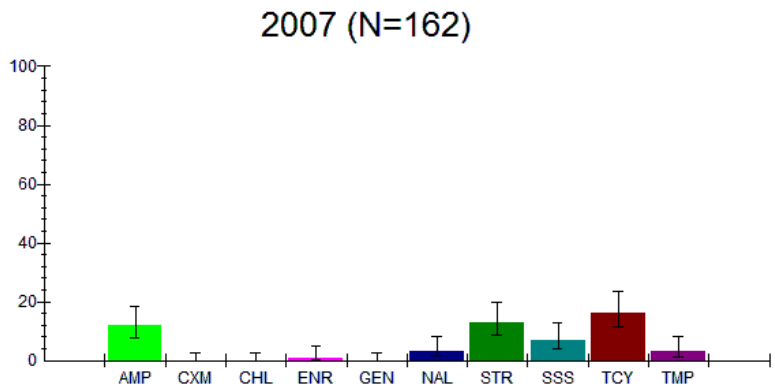


Trends in *E. coli* resistance: swine isolates









Does it prove decrease of resistance?

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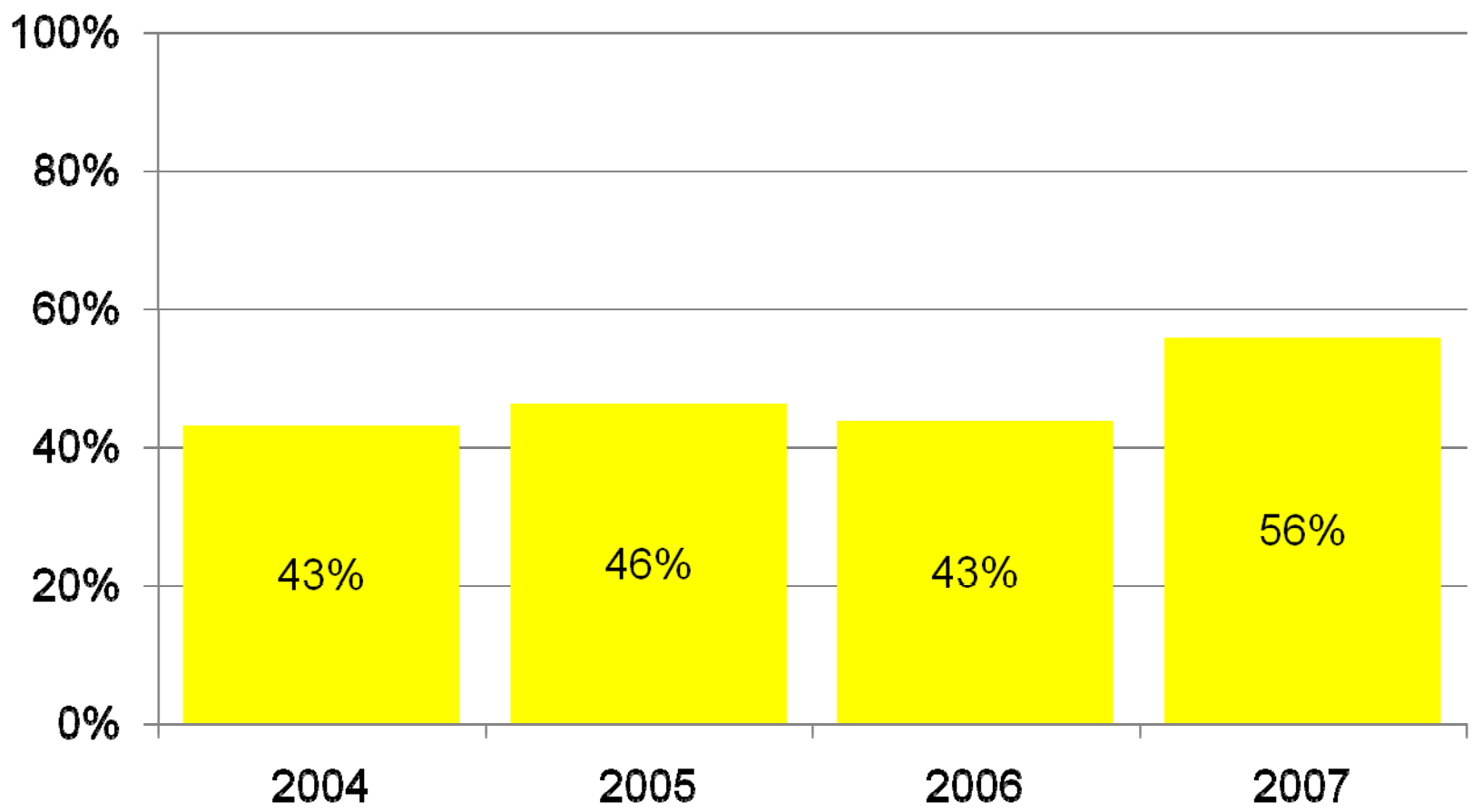
Resistant *E. coli* swine isolates over years: inconclusive



HLAR due to 16S rRNA methylases (Bruno Gonzalez-Zorn, ASM Conference, S10:11)

- ✓ 23 *Enterobacteriaceae* from humans in Poland
- ✓ no animal source …..?

NVRI data:

- ✓ 35/2610 (1,3%) Gentamycin resistant strains (R ≤ 12 mm)
- ✓ 17/2610 (0,7%) Gentamycin = 6mm
- ✓ 12 strains Gen (6mm)+Amp (R)

	2004	2005	2006	2007	Total
turkey			2		
broilers	1	1		2	4
pigs		1	1		2
total	1	2	3	6	12

Cxm R

Summary: limited methodology although enough to recognise the background of resistance

” Never was so much owed by so many to so few strains tested

W. Churchill, 1940