National Veterinary Research Institute

Puławy, Poland

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Dariusz Wasyl, DVM, PhD, ScD

National Reference Laboratory for *Salmonella* & Antimicrobial Resistance

Department of Microbiology

National Veterinary Research Institute

Puławy, Poland

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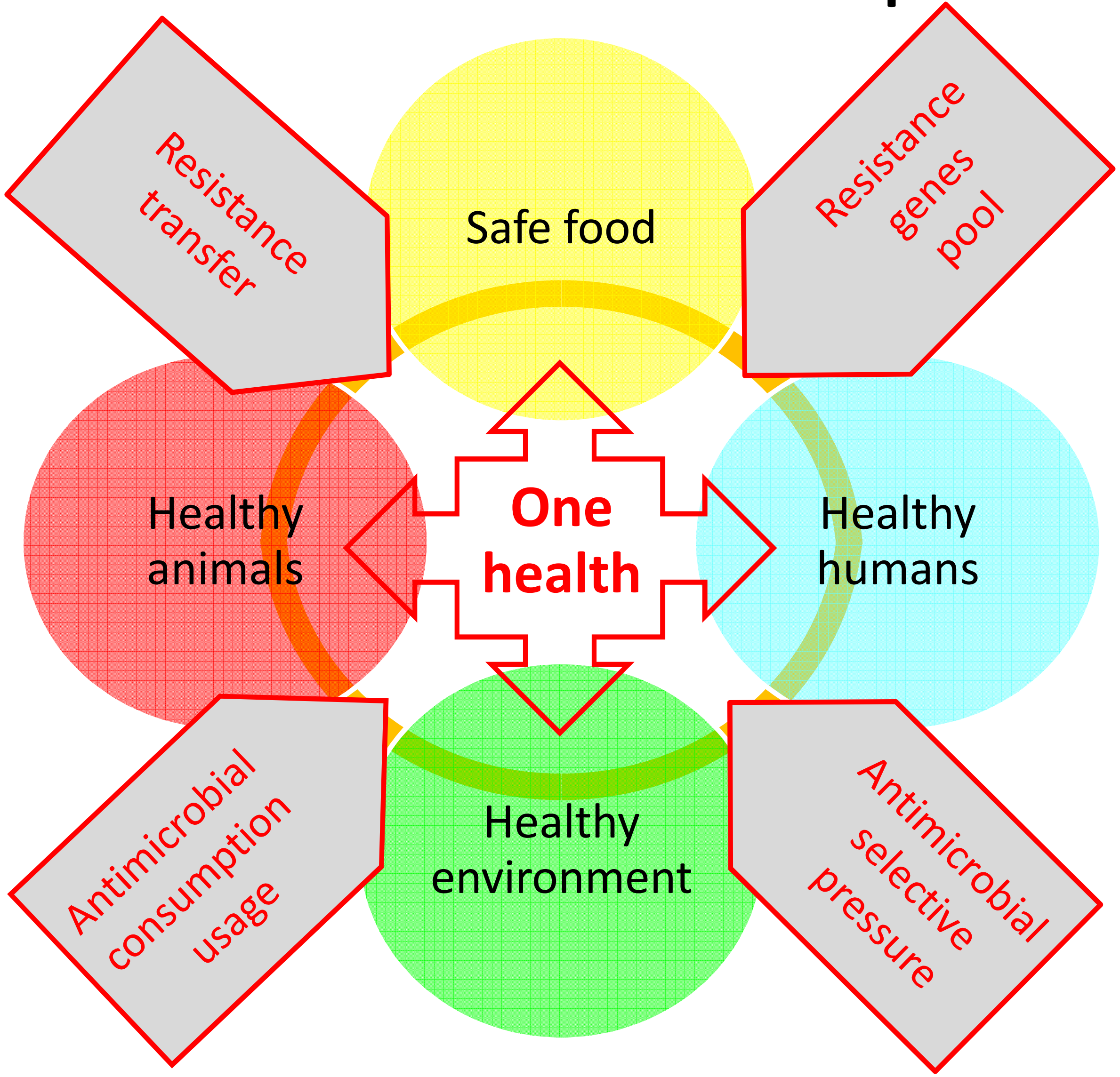
Antimicrobial resistance under One Health concept



Resistance in Poland: humans

J Antimicrob Chemother 2015; **70**: 1981–1988
doi:10.1093/jac/dkv055 Advance Access publication 10 March 2015

Journal of Antimicrobial Chemotherapy

Survey of metallo-β-lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008–11

C. C. Papagiannitsis[1,2]†, R. Izdebski[1]†, A.
M. J. M. Bonten[3], Y. Car
and M. Gniadkowski[1]* or

[1]National Medicines Institute, Warsaw, Poland; [2]Faculty of … University Medical Center Utrecht, Utrecht, The Netherlands; [4]Tel-Aviv Sourasky … University of Antwerp, Antwerp, Belgium; [6]INSERM, U957 & Université Paris-Est, Créteil, France

carbapenemases

CTX-M-3: 80,6% (-15)

SHV: 17,5% (-2, -5, -12)

TEM: 0,7% (-19, -48)

CMY: *P. mirabilis* 20,5% (-12, -15)

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AAC
Journals.ASM.org

The First NDM Metallo-β-Lactamase-Producing *Enterobacteriaceae* Isolate in Poland: Evolution of IncFII-Type Plasmids Carrying the bla_{NDM-1} Gene

J. Fiett,[a] A. Baraniak,[a] R. Izdebski,[a] I. Si… …wski[a]
National Medicines Institute, Warsaw, Poland

NDM

Antimicrobial Agents and Chemotherapy, Nov. 2007, p. 3789–3795 Vol. 51, No. 11
0066-4804/07/$08.00+0 doi:10.1128/AAC.00457-07

Complete Nucleotide Sequence of the pCTX-M3 Plasmid and Its Involvement in Spread of the Extended-Spectrum β-Lactamase Gene $bla_{CTX-M-3}$

M. Gołębiewski,[1]† I. Kern-Zdanowicz,[1]* M. Zienkiewicz,[1]‡ M. Adamczyk,[1] J. Żylińska,[1] A. Baraniak,[2] M. Gniadkowski,[2] J. Bardowski,[1] and P. Cegłowski[1]

Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences,[1] and National Medicines Institute,[2] Warsaw, Poland

ESBLs

Antimicrobial Agents and Chemotherapy, July 2008, p. 2449–2454 Vol. 52, No. 7
0066-4804/08/$08.00+0 doi:10.1128/AAC.00043-08

Molecular Survey of β-Lactamases Conferring Resistance to Newer β-Lactams in *Enterobacteriaceae* Isolates from Polish Hospitals

Joanna Empel, Anna Baraniak,† Elżbieta Literacka,† Agnieszka Mrówka,† Janusz Fiett, Ewa Sadowy, Waleria Hryniewicz, Marek Gniadkowski,* and the Beta-PL Study Group‡

National Medicines Institute, 00-725 Warsaw, Poland

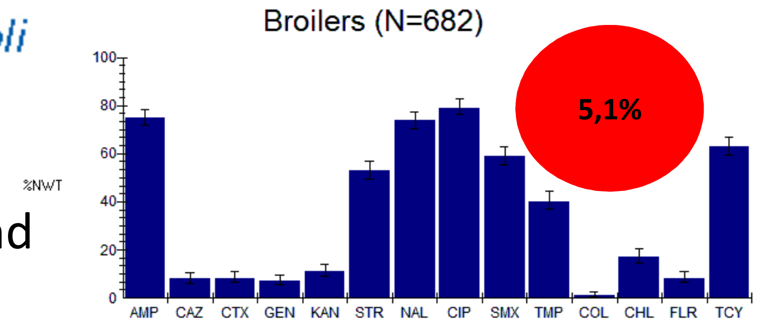
Received 11 January 2008/Returned for modification 23 March 2008/Accepted 25 April 2008

Resistance in Poland: food animals

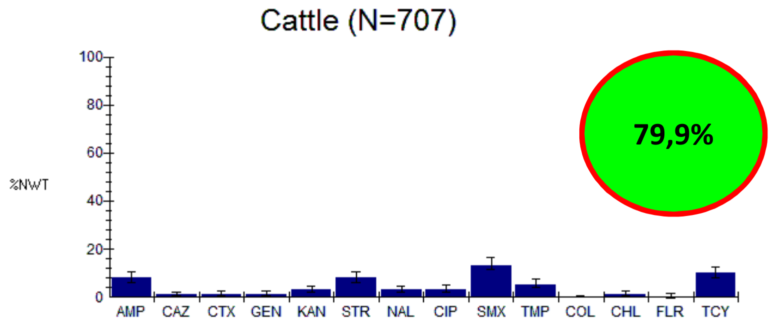
frontiers in
MICROBIOLOGY
ORIGINAL RESEARCH ARTICLE
published: 05 August 2013
doi: 10.3389/fmicb.2013.00221

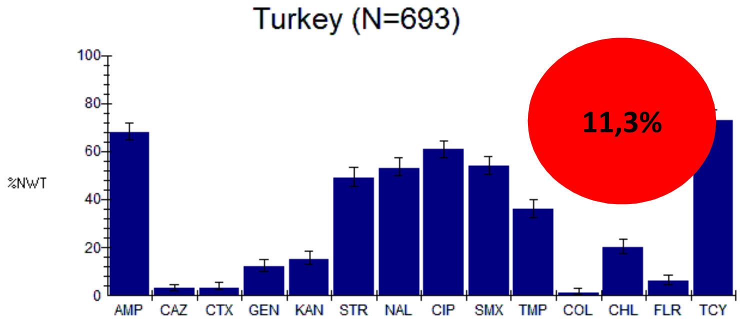
Antimicrobial resistance in commensal *Escherichia coli* isolated from animals at slaughter

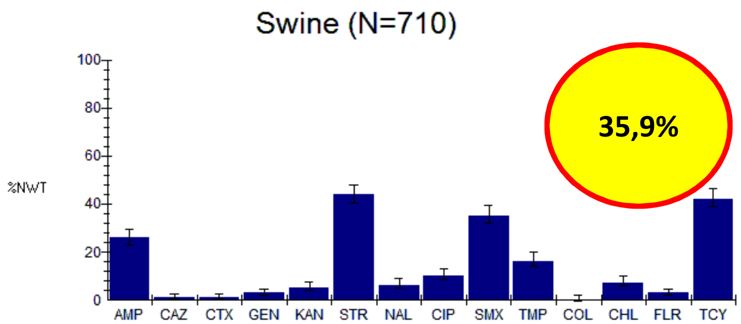
Dariusz Wasyl*, Andrzej Hoszowski, Magdalena Zając and Krzysztof Szulowski

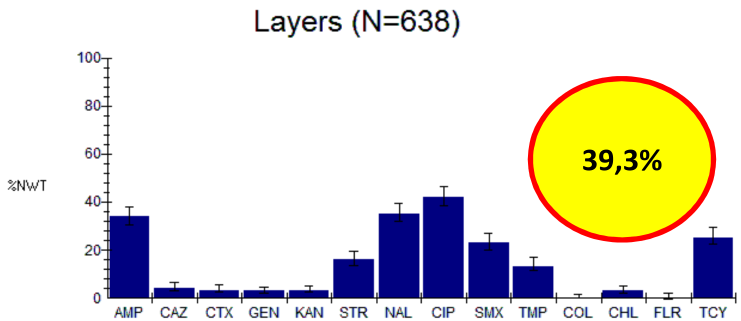


Resistance reflects antimicrobial usage policies and management practices different animal species









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Resistance in Poland: food animals

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MICROBIOLOGY

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published: 05 August 2013
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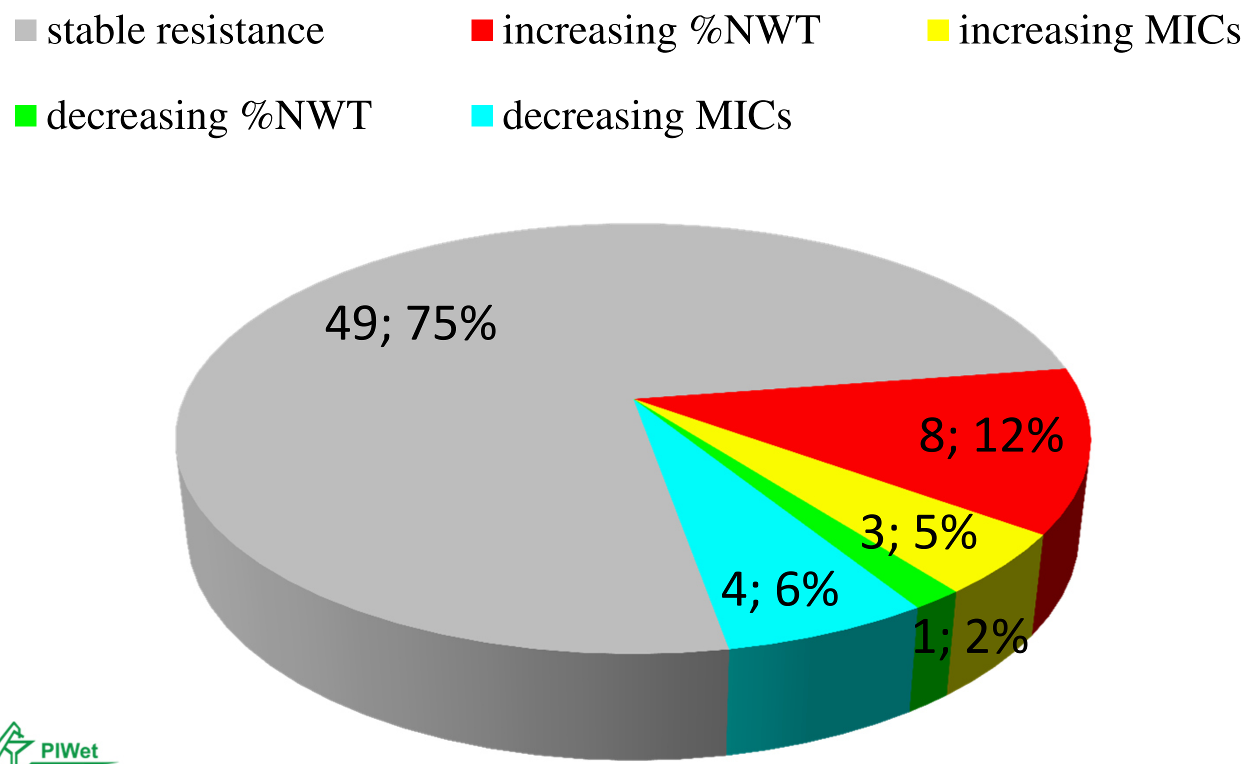
Antimicrobial resistance in commensal *Escherichia coli* isolated from animals at slaughter

Dariusz Wasyl*, Andrzej Hoszowski, Magdalena Zając and Krzysztof Szulowski

Trends over 4-years period



broilers: Amp, Ctx
layers: Ctx, Ctz
turkeys: Amp, Ctz, Gen, Su

cattle: Ctx, Ctz, Gen

layers: Str

layers: Chl, Flr, Tet
cattle: Chl

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Resistance in Poland: food animals

Bull Vet Inst Pulawy 54, 147-151, 2010

SIMPLE AND EFFICIENT SCREENING METHOD FOR THE DETECTION OF CEPHALOSPORIN RESISTANT *ESCHERICHIA COLI*

DARIUSZ WASYL, ANDRZEJ HOSZOWSKI, MAGDALENA ZAJĄC, AND MAGDALENA SKARŻYŃSKA

Department of Microbiology, National Veterinary Research Institute, 24-100 Pulawy, Poland
wasyl@piwet.pulawy.pl

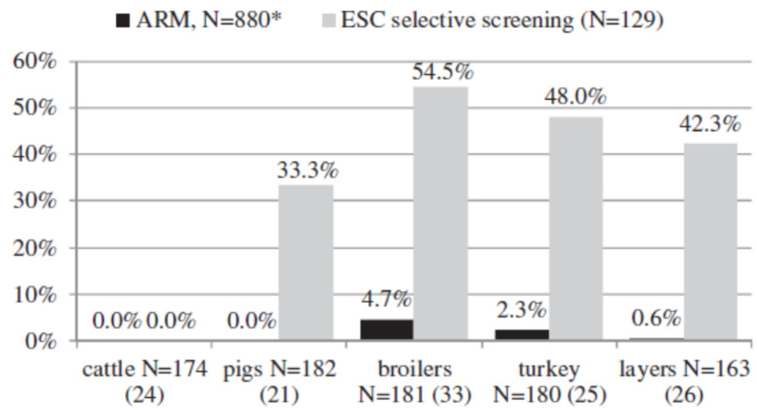


FIG. 1. ESC prevalence in *Escherichia coli* isolated from food animals (digits in brackets stands for number of samples

huge burden of cephalosporin resistance in food animals

MICROBIAL DRUG RESISTANCE
Volume 00, Number 00, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/mdr.2011.0033

VETERINARY MICROBIOLOGY

Prevalence and Characterization of Cephalosporin Resistance in Nonpathogenic *Escherichia coli* from Food-Producing Animals Slaughtered in Poland

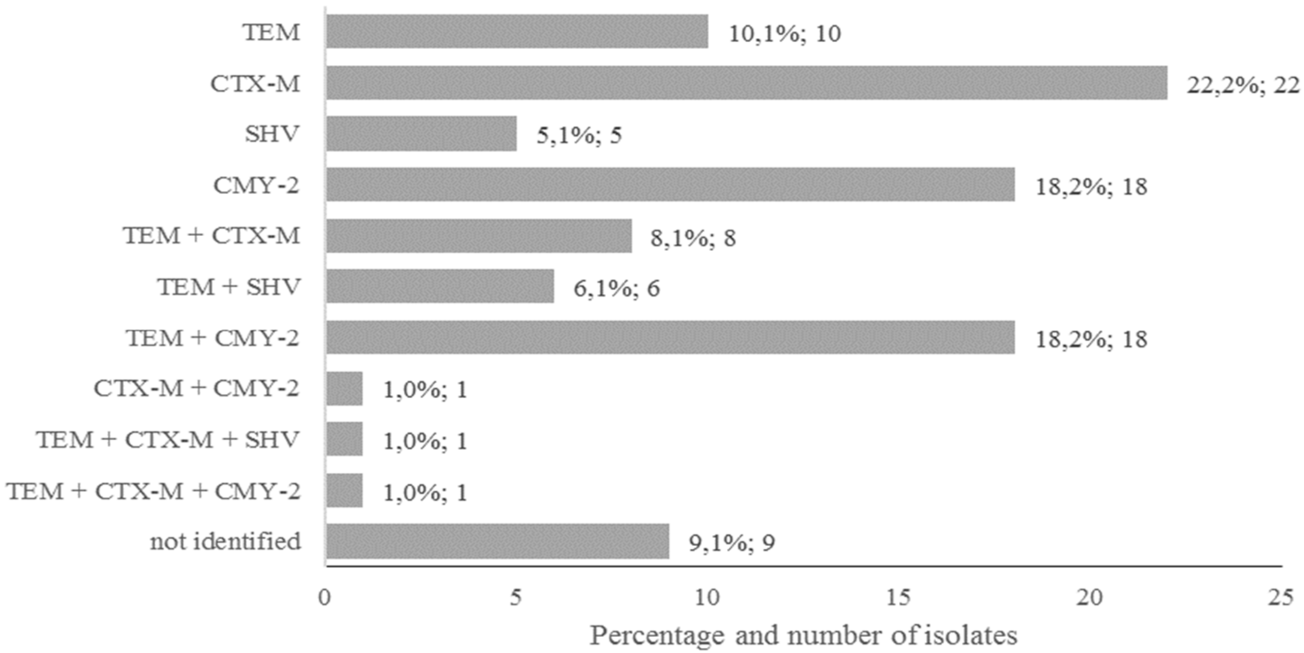
Dariusz Wasyl,[1] Henrik Hasman,[2] Lina M. Cavaco,[2] and Frank M. Aarestrup[2]

genetic background different from humans

TABLE 1. RESISTANCE MECHANISMS BY SOURCE OF *ESCHERICHIA COLI* ISOLATION

Phenotype	*Resistance mechanism*	No. of strains				
		Pigs	*Layers*	*Broilers*	*Turkeys*	*Total*
ESBL (*n* = 15)	CTX-M-1	2	3[a]		3	8
	CTX-M-1+TEM-1b	1	2	1	1	5
	SHV-12				1	1
	Not identified	1				1
ampC (*n* = 33)	CMY-2		4[b]	10[b]		14
	CMY-2+*ampC* overexpression:					
	C-88T+A-82G+G-18A+C-1T+C58T	1		2	1	4
	C-73T+G-28A+C58T			2		2
	Not tested	2	2	3	6	13

Resistance in Poland: food animals



Lalak *et al.*, submitted

Isolation source	Cephalosporin resistance genes (bla)					
	TEM-1	TEM-135	TEM-52/-92	SHV-12	CTX-M-1/61	CMY-2
cattle	1					1
pigs	4			2	4	2
broilers	4	1		5	5	6
turkey	5			4	4	6
layers	2	2	1		4	4
Total	16	3	1	11	17	19

Resistance in Poland: food animals

Contents lists available at ScienceDirect
Veterinary Microbiology
journal homepage: www.elsevier.com/locate/vetmic

High-level fluoroquinolone resistant *Salmonella enterica* serovar Kentucky ST198 epidemic clone with IncA/C conjugative plasmid carrying $bla_{CTX-M-25}$ gene

Dariusz Wasyl[a,1,*], Izabela Kern-Zdanowicz[b,1], Katarzyna Domańska-Blicharz[c], Magdalena Zając[a], Andrzej Hoszowski[a]

Contents lists available at ScienceDirect
Food Research International
journal homepage: www.elsevier.com/locate/foodres

First isolation of ESBL-producing *Salmonella* and emergence of multiresistant *Salmonella* Kentucky in turkey in Poland

D. Wasyl*, A. Hoszowski

Contents lists available at ScienceDirect
Veterinary Microbiology
journal homepage: www.elsevier.com/locate/vetmic

Short communication

Genetic lineages of *Salmonella enterica* serovar Kentucky spreading in pet reptiles

Magdalena Zając[a,*], Dariusz Wasyl[a], Andrzej Hoszowski[a], Simon Le Hello[b], Krzysztof Szulowski[a]

frontiers in MICROBIOLOGY
ORIGINAL RESEARCH ARTICLE
published: 18 December 2013
doi: 10.3389/fmicb.2013.00395

The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain

Simon Le Hello[1*], Amany Bekhit[1,2], Sophie A. Granier[3], Himel Barua[4], Janine Beutlich[5], Magdalena Zając[6], Sebastian Münch[7], Vitali Sintchenko[8], Brahim Bouchrif[9], Kayode Fashae[10], Jean-Louis Pinsard[11], Lucile Sontag[1], Laetitia Fabre[1], Martine Garnier[1], Véronique Guibert[1], Peter Howard[9], Rene S. Hendriksen[12], Jens P. Christensen[13], Paritosh K. Biswas[4], Axel Cloeckaert[14,15], Wolfgang Rabsch[7], Dariusz Wasyl[6], Benoit Doublet[14,15] and François-Xavier Weill[1]

FOODBORNE PATHOGENS AND DISEASE
Volume 9, Number ■, 2012
© Mary Ann Liebert, Inc.
DOI: 10.1089/fpd.2012.1154

Original Article

Occurrence and Characterization of Monophasic *Salmonella enterica* Serovar Typhimurium (1,4,[5],12:i:-) of Non-Human Origin in Poland

Dariusz Wasyl and Andrzej Hoszowski

Resistance in *Salmonella* – clonal spread

PIWet

Resistance in Poland: food animals

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp.

Dariusz Wasyl *, Andrzej Hoszowski, Magdalena Zając

Table 2
Gyrase (*gyrA*, *gyrB*) and topoisomerase VI (*parC*) genes substitutions within quinolone resistance determining region by ciprofloxacin MIC values and *Salmonella* serovar: SA – Adelaide, SAg – Agona, SE – Enteritidis, SH – Hadar, SI – Infantis, SL – Lexington, SM – Mbandaka, SN – Newport, SS – Saintpaul, SSt – Stanley, STe – Tennessee, ST – Typhimurium, SV – Virchow. Plasmid-mediated quinolone resistance (*qnrS1*/3, *qnrS2*) presence was indicated. No relevant substitutions were found in *parE*.

gyrA		*gyrB*	*parC*		Number of isolates by $MIC_{Cip(mg/L)}$ (*Salmonella* serovar codes)						Total
Ser83	Asp87	Leu470	Thr57	Ala141	0.125	0.25	0.5	1	2	4	
–	Asn[a]	–	–	–		4 (ST 3, SS 1)					4
–	Tyr[b]	–	–	–	2 (SE, SV)	5 (SE 2, SV 2, SS 1)			1 (SE, *qnrS2*)		8
Phe[c]	–	–	–	–		1 (SV)	1 (SV)	2 (SE)			4
Tyr[d]	–	–	–	–		1 (SE)	3 (SE)				4
–	Asn[a]	–	Ser[e]	–	1 (SH)	1 (SH)					2
–	Gly[f]	–	Ser[e]	–	1 (SN)						1
–	Tyr[b]	–	Ser[e]	–	1 (SSt)	5 (SH, SA 2, STe 2)					6
Leu[g]	Asn[a]	–	–	–	1 (SV)						1
Phe[c]	–	–	Ser[e]	–		3 (SN 2, SM)					3
Tyr[d]	–	–	Ser[e]	–		1 (SN)	3 (SAg 1, SI 2)	3 (SI 2, SN 1)	1 (SN)	1 (SN, *qnrS1/S3*)	9
–	–	–	Ser[e]	Ser[h]		1 (SL)					1
Tyr[d]	–	(Met)[i]	Ser[e]	–			1 (SI)				1
No. of tested isolates					6	22	8	5	2	1	44

Table 3
Plasmid mediated quinolone resistance genes.

Salmonella	PMQR (*N* = 92)				QRDR		Total (%)		Percentage of PMQR-positive isolates within serovar (95% CI)
	qnrB10/B19	*qnrS1/S3*	Negative	Not tested	*qnrS1/S3*	*qnrS2*			
Agona		1	1[a]				2	2.1%	3.7% (1.0–12.5%)
Enteritidis	3	5	5[a]	1		1	15	16.0%	1.5% (0.7–2.2%)
Indiana	3						3	3.2%	10.7% (3.7–27.2%)
Infantis		1	2[a]				3	3.2%	1.1% (0.0–2.4%)
Mbandaka		3					3	3.2%	2.0% (0.0–4.2%)
Montevideo				1			1	1.1%	n.a.[b]
Newport	8	37	2	4	1		52	55.3%	41.3% (32.7–49.9%)
Oranienburg			1[a]				1	1.1%	5.6% (1.0–25.8%)
Saintpaul	1	3					4	4.3%	10.3% (4.1–23.6%)
Typhimurium	9						9	9.6%	3.6% (1.3–6.0%)
Virchow			1[a]				1	1.1%	0.9% (0.0–2.6%)
Total	24	50[c]	12	6	1	1	94	100.0%	

MICROBIAL DRUG RESISTANCE
Volume 00, Number 00, 2014

DOI: 10.1089/mdr.2014.0061

MECHANISMS

Prevalence and Characterization of Quinolone Resistance Mechanisms in Commensal *Escherichia coli* Isolated from Slaughter Animals in Poland, 2009–2012

Table 2. Prevalence of Plasmid-Mediated Quinolone Resistance Phenotype by Year and Source of *Escherichia coli* Isolation

	No. of PMQR-suspected E. coli *(No. of tested)*						
	By year				*Total*		
Isolation	*2009*	*2010*	*2011*	*2012*	n	*%*	*95% CI*
Source							
Cattle	6 (173)	0 (173)	1 (173)	1 (189)	8 (708)	1.1	0.3–2.0%
Swine	7 (185)	4 (170)	7 (172)	11 (199)	29 (726)	4.0	2.6–5.4%
Broilers	10 (189)	15 (192)	11 (170)	19 (193)	55 (744)	7.4	5.5–9.3%
Layers	12 (168)	13 (170)	14 (155)	18 (161)	57 (654)	8.7	6.5–10.9%
Turkeys	16 (185)	18 (170)	10 (171)	26 (193)	70 (719)	9.7	7.6–11.9%
Total							
n	51 (900)	50 (875)	43 (841)	75 (935)	219 (3,551)		
%	5.7	5.7	5.1	8.0		6.2	
95% CI	4.2–7.2%	4.2–7.3%	3.6–6.6%	6.3–9.8%			5.4–7.0%

Quinolone resistance
diverse mechanisms

ANTIMICROBIAL RESISTANCE IN WILDLIFE

Study aims:

1. to evaluate antimicrobial resistance in indicator *E. coli* isolated from hunted wild boars and deer,
2. to assess the prevalence of cephalosporin resistance in wildlife,
3. to characterize cephalosporin and quinolone resistance mechanisms as well as their carrier plasmids.

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Resistance in wildlife

Hunting seasons

- Oct 2012 ÷ Jan 2013
- Oct 2013 ÷ Jan 2014

Faecal samples

- red deer
- roe deer
- fallow deer
- European bison
- wild boars

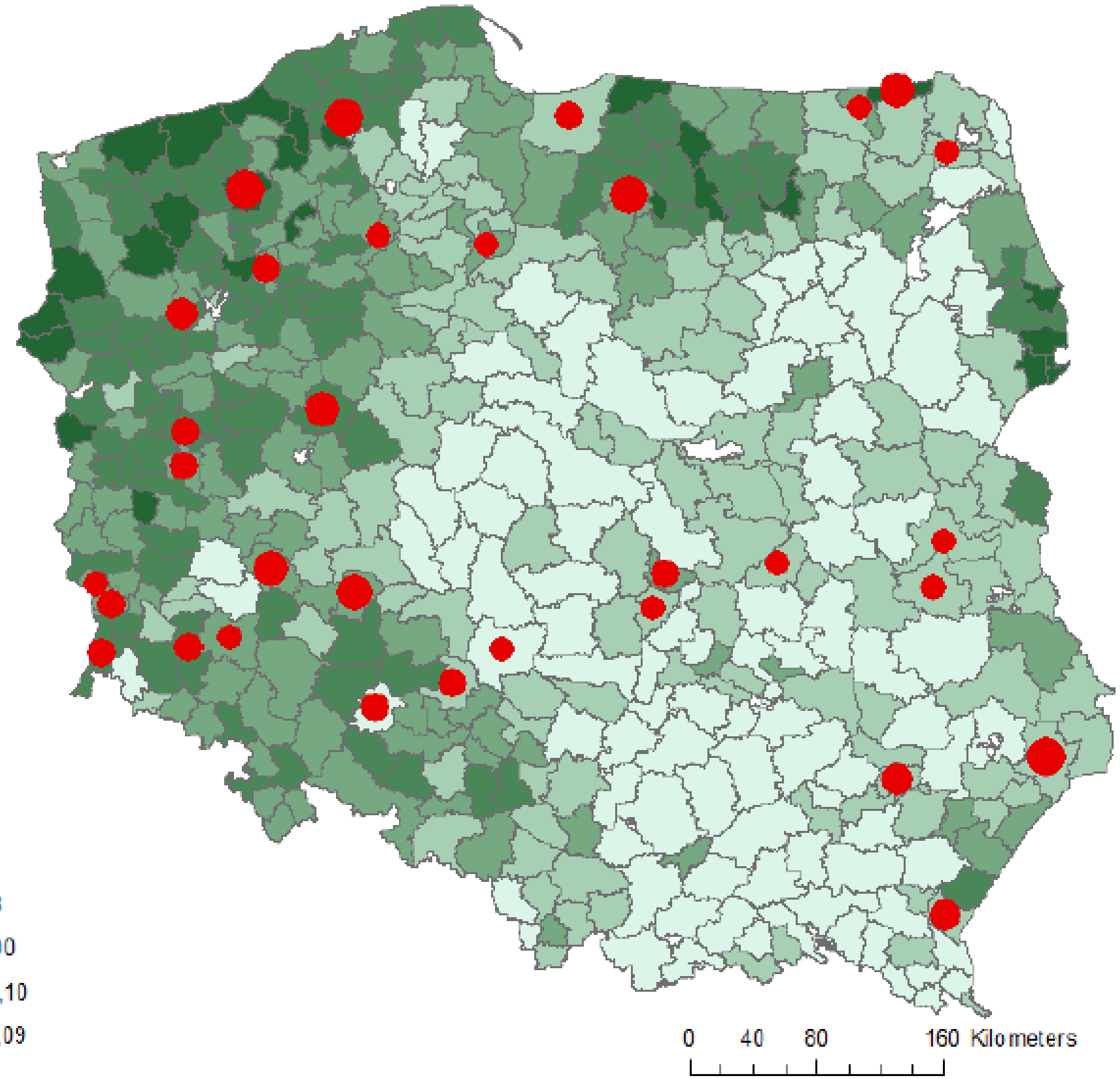
Wild boar sampling

Number of samples (animals) tested
N = 332
42 hunts

Number of samples
1 - 3
6 - 10
12- 14
16 - 19
21 - 30

Population density (per 1000 ha)

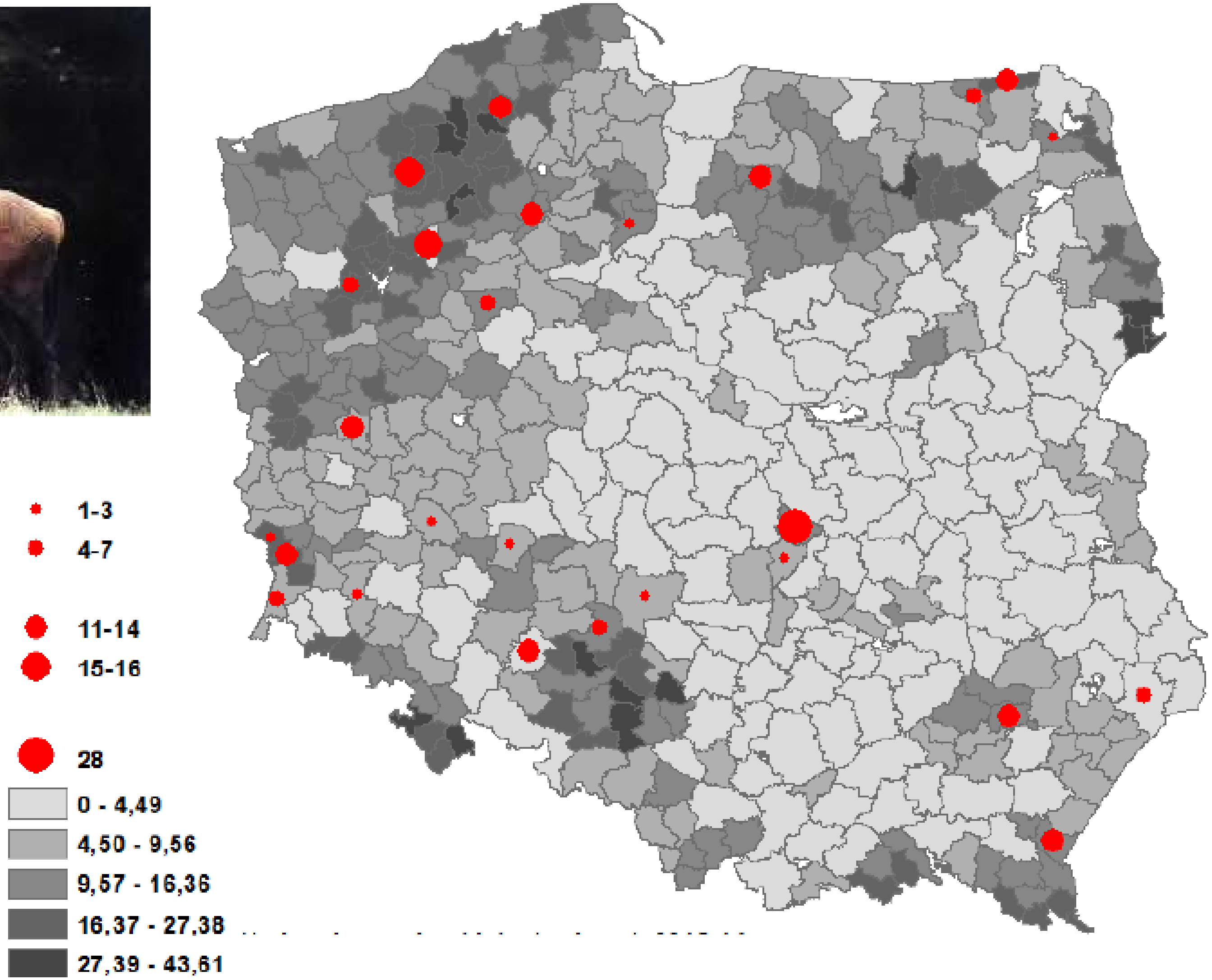
Population density
0 - 5,42
5,43 - 9,33
9,34 - 14,00
14,01 - 20,10
20,11 - 34,09



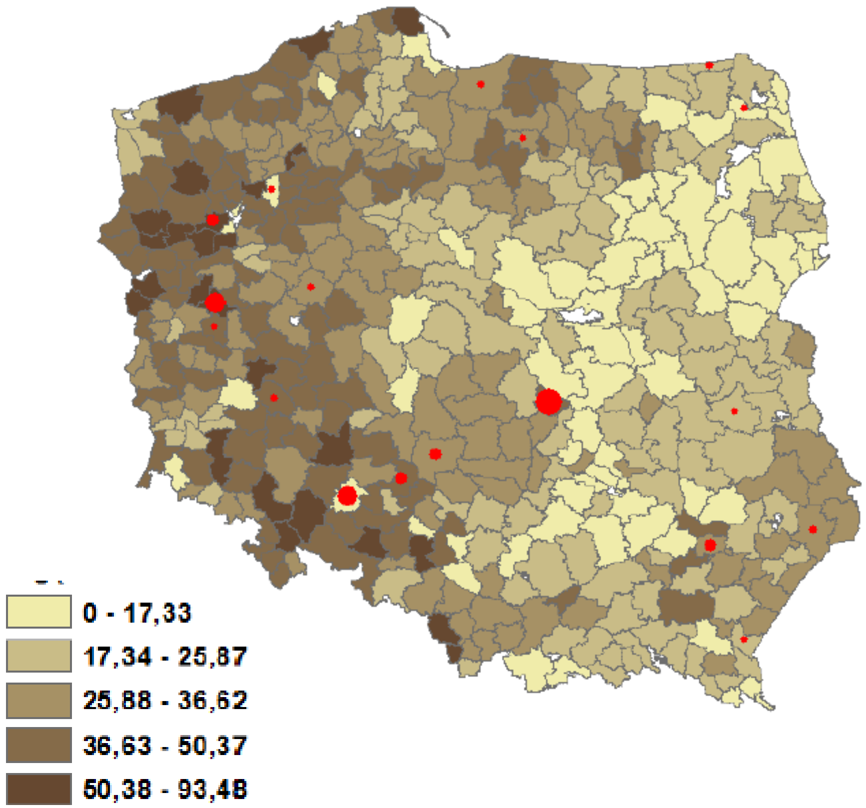
Red deer sampling

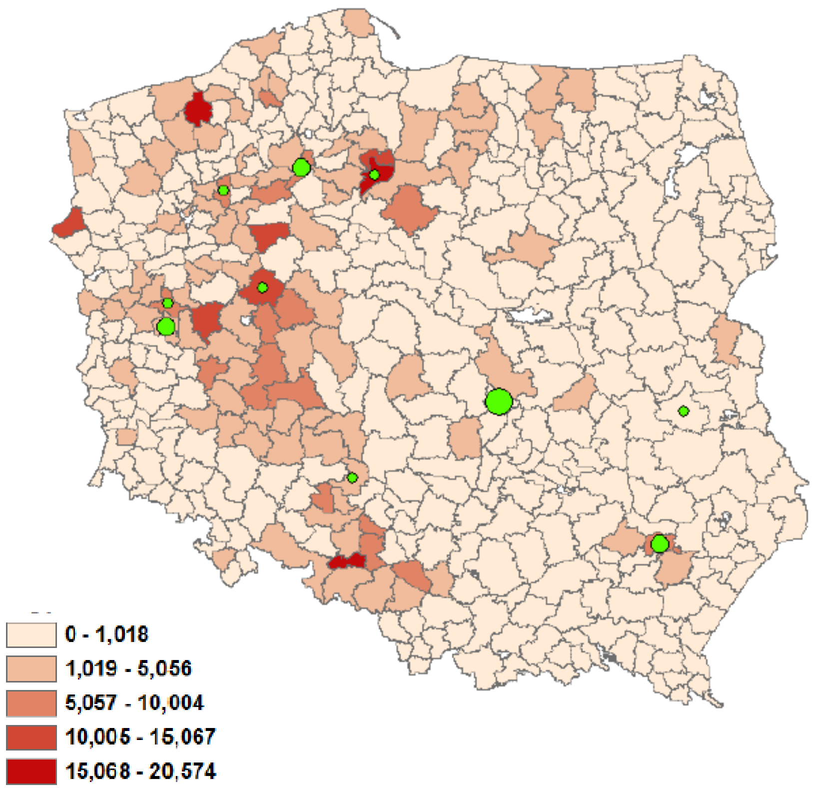
Number of samples (animals) tested
N = 225
42 hunts

Population density (per 1000 ha)



Roe deer (n=76), fallow deer (n=24), European bison (n=3) sampling





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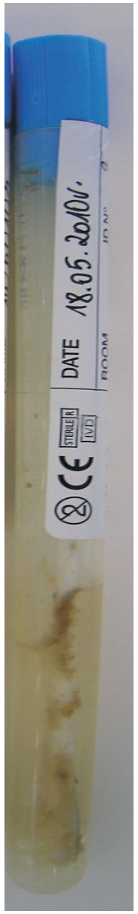
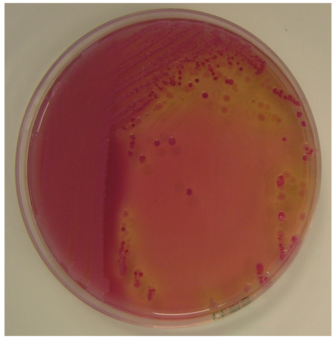
Isolation of *Escherichia coli*



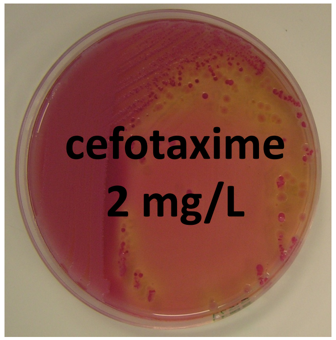
direct streak

Indicator *E. coli*



MacConkey Agar

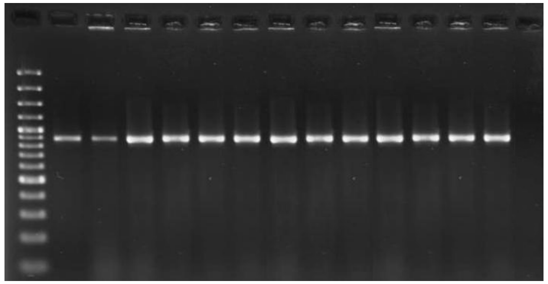
Cephalosporin-resistant *E. coli*



MacConkey Agar

identification

PCR: *uspA*



Chen, J. et al. Lett. Appl. Microbiol. 1998, 27(6): 369-371

Antimicrobial resistance testing

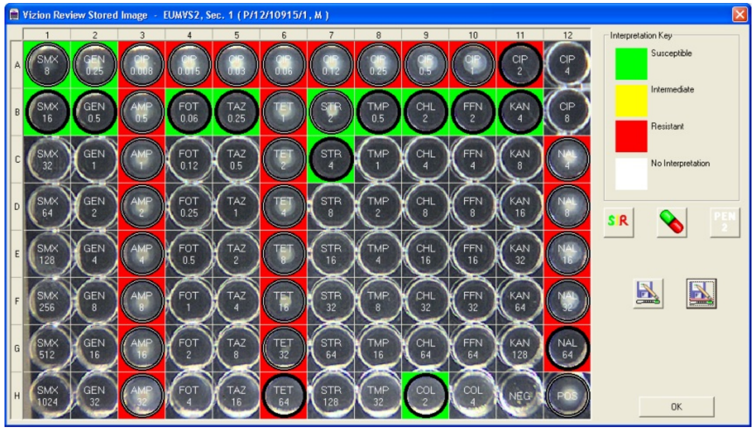
- Minimal Inhibitory Concentrations:
  14 antimicrobials/9 classes

TREK DIAGNOSTIC SYSTEMS
MAGELLAN BIOSCIENCES

- Interpretation: EUCAST epidemiological cut-off

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

microbiological resistance (NWT)



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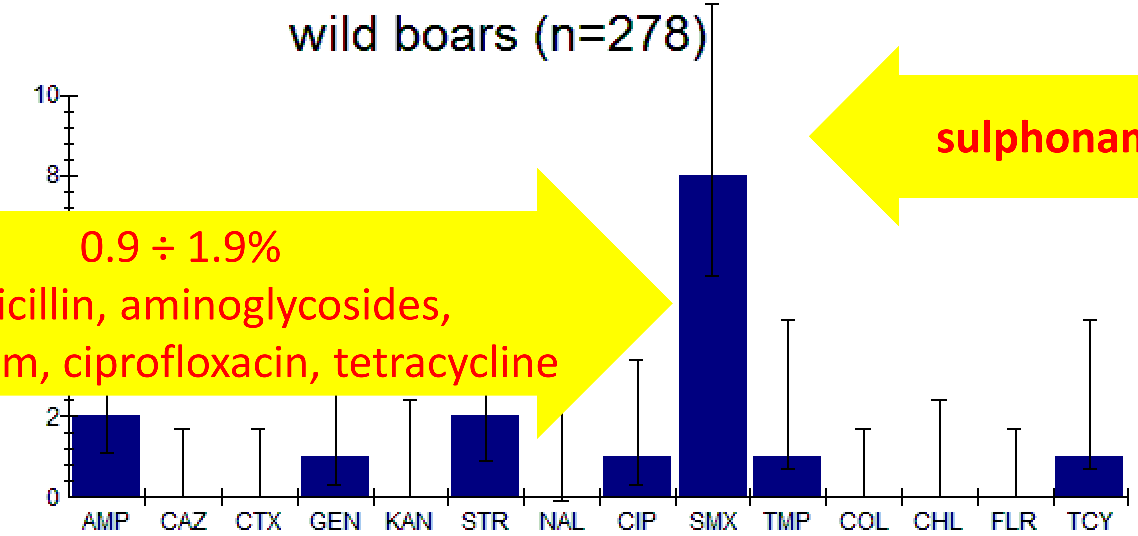
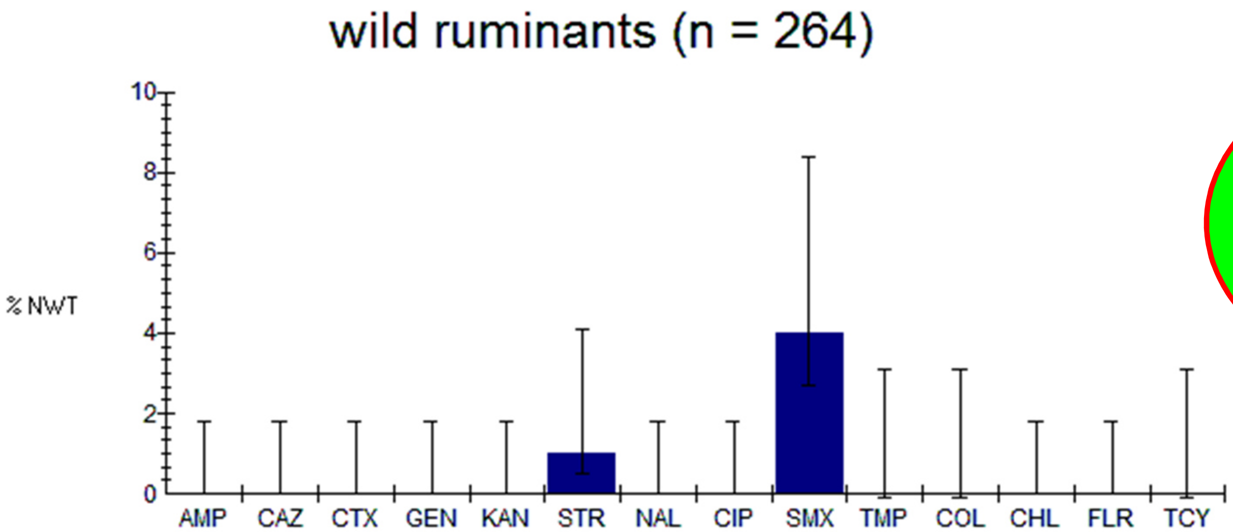
Antimicrobial resistance in *E. coli* from wild boars



sulphonamides (5.5 ÷ 12.3%)

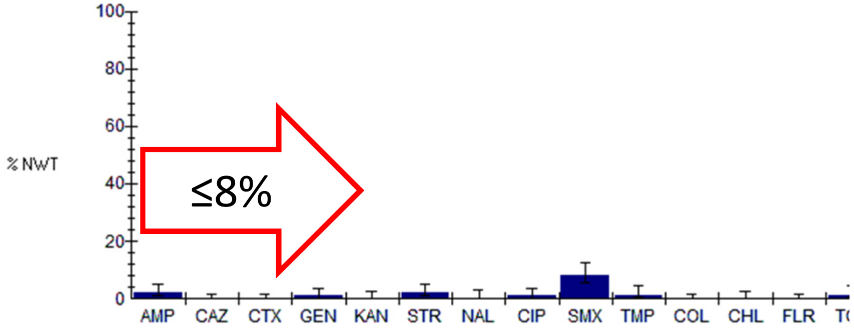
0.9 ÷ 1.9%
ampicillin, aminoglycosides,
trimetoprim, ciprofloxacin, tetracycline



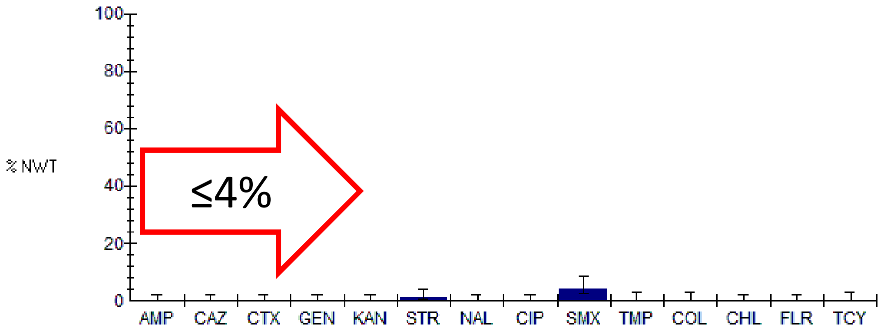
Not significant differences

Antimicrobial resistance in *E. coli* from wild boars

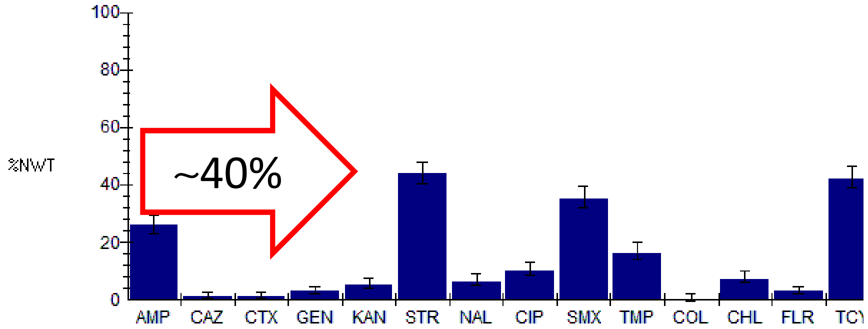
wild boars (n = 278)



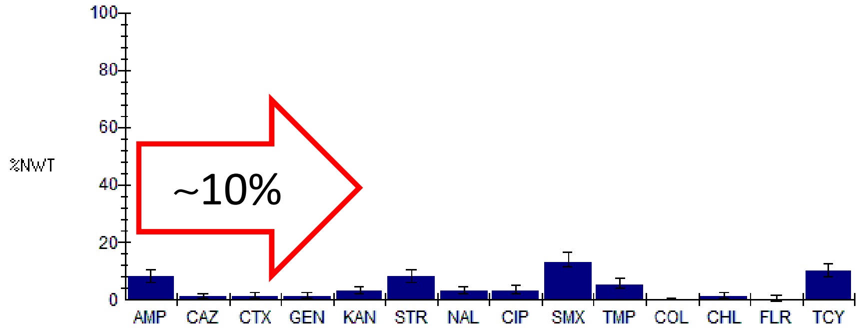
wild rumminants (n = 264)



Swine (N=710)



Cattle (N=707)



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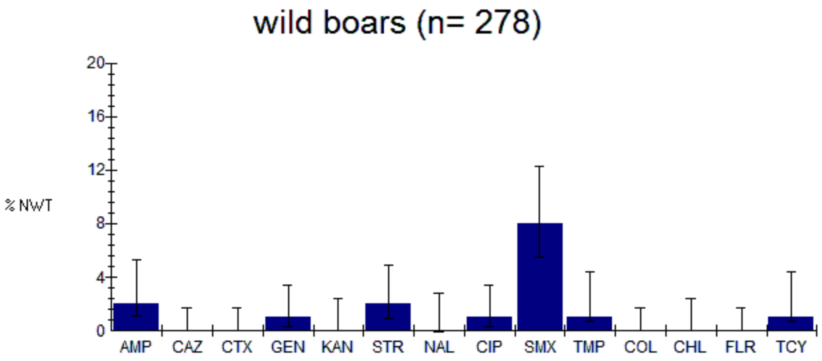
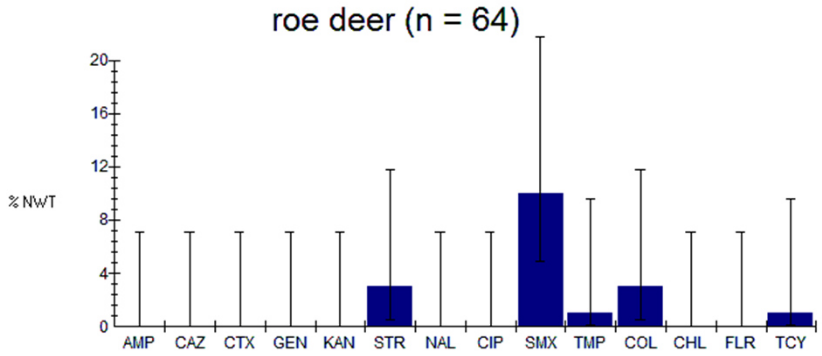
Resistance is present in the wildlife
How farm animals might contribute?





No resistance in *E. coli* from fallow, red deers and bisons

Selection pressure of antimicrobial usage in the agriculture?

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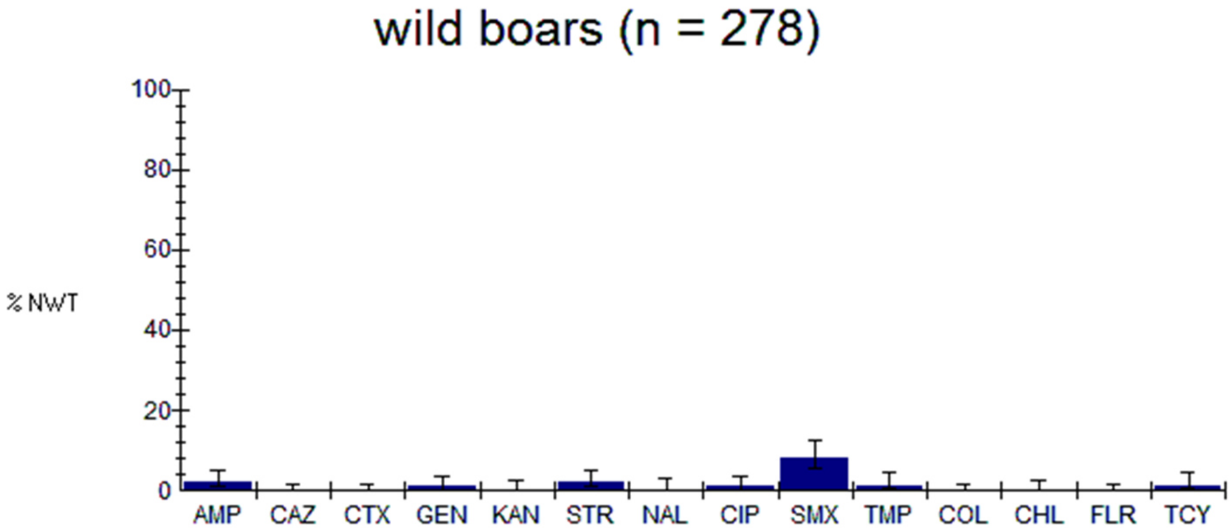
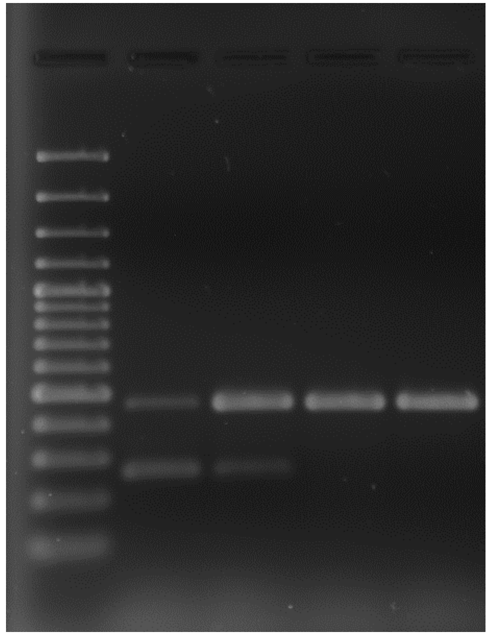
Plasmid-mediated quinolone resistance in *E. coli* isolated from wild boars



No Nal NWT
Two Cip NWT



PCR: *qnrS*

Sequencing: *qnrS1/S3*

Prevalence of cephalosporin resistance in *E. coli* from wildlife

Source (No of samples)		*E. coli*		Cephalosporin-resistant *E. coli*	
		No.	isolation rate	No.	prevalence
fallow deer	24	21	87,5%	**1**	4,2%
wild boar	332	278	83,7%	**9**	**2,7%**
red deer	225	176	78,2%	1	0,4%
roe deer	76	64	84,2%		0,0%
European bison	3	3	100,0%		0,0%
Total	**660**	**542**	**82,1%**	**11**	**1,7%**

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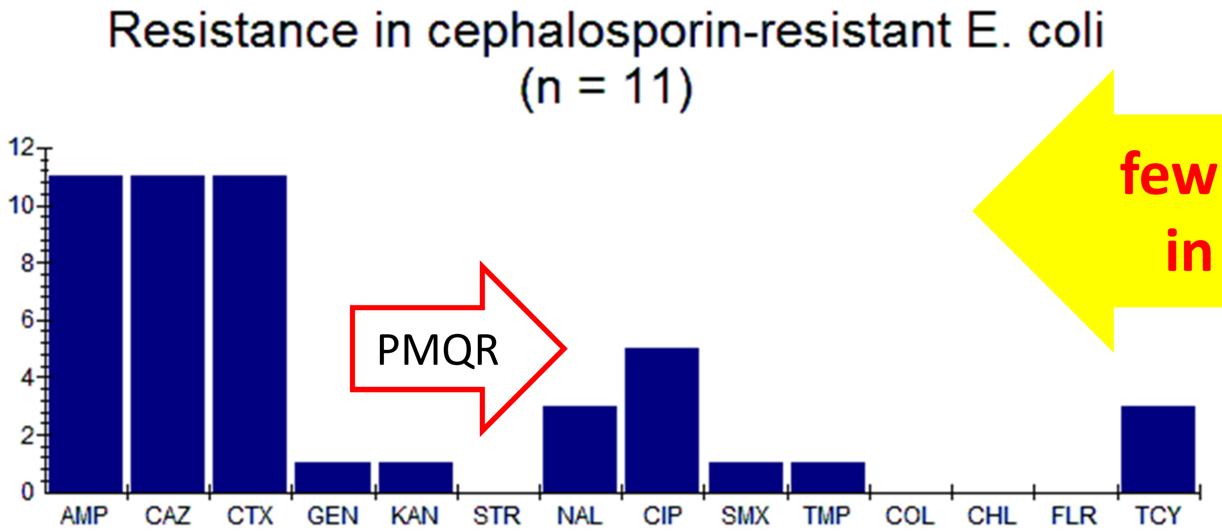
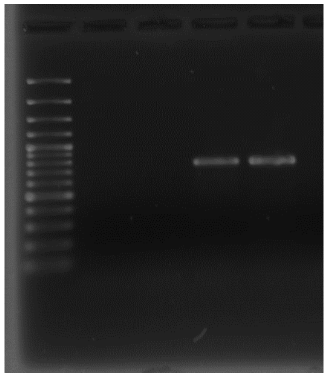
Cephalosporin-resistant pathogenic *Enterobacteriaceae* in humans are multidrug-resistant What about wildlife isolates?



few other resistances in wildlife isolates



Identification of cephalosporin resistance mechanisms (PCR):

- ampC ⇒ *bla*CMY-2 (N = 8)
- ESBL ⇒ *bla*CTX-M (N = 3)

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Cephalosporin resistance background in *E. coli* from wild boars

ID	R-profile	*bla*	Plasmids (kb; S1+B&D)	Inc groups
039B	AmpCtxCazGenKanNalCip	Tem-1, Cmy-2	104; 40; 10,0; 2,5	?
358B	AmpCtxCazSmxTetTmpCip	Ctx-M-15	104, ?, 3,9	IncK, IncY, IncB/O
364B	AmpCtxCazNalCip	Tem-161, Cmy-2	90; 7,0; 5,2; 3,1	IncI1-alpha, IncY
479B	AmpCtxCazCip	Tem-1, Cmy-2	104; 90; 54	IncX1, IncI1-alfa, IncFII
480B	AmpCtxCaz	Cmy-2	90; 80; 60; ?	IncI1-alpha, IncFIC, IncFII
481B	AmpCtxCaz	Ctx-M1/61	140; 85; ?	IncI1-alpha, IncFII
482B	AmpCtxCazTet	Tem-1, Cmy-2	140; 85; ?; 4,8	IncI1-alpha, IncHI1, IncF (FIB), IncFII
483B	AmpCtxCaz	Ctx-M1/61	140; 85; 3,5	?
486B	AmpCtxCaz	Tem-1, Cmy-2	200;100; ?	IncI1-alfa, IncF (FIB), IncFII, IncB/O

food animals: *bla*CTX-M-1; blaCMY-2 Wasyl et al. Microb Drug Resist 2012, 18: 79-82

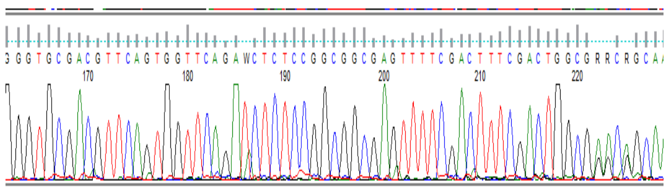
humans: *bla*CTX-M3 Gołębiewski et al. AAC, 2007, 51(11): 3789-95

Transferability of cephalosporin resistance

ID	R-profile	*bla*	Plasmids (kb; S1+B&D)	Inc groups
358B	AmpCtxCazSmxTetTmpCip	Ctx-M-15	104, ?, 3,9	IncK, IncY, IncB/O
…….				
482B	AmpCtxCazTet	Tem-1, Cmy-2	140; 85; ?; 4,8	IncI1-alpha, IncHI1, IncF (FIB), IncFII
Trans 482B	Amp Ctx Caz	Cmy-2	85	IncI1-alpha

PMQR in cephalosporin resistant *E. coli*

ID	R-profile	*bla*
039B	AmpCtxCazGenKanNalCip	Tem-1, Cmy-2
358B	AmpCtxCazSmxTetTmp**Cip**	Ctx-M-15
364B	AmpCtxCazNalCip	Tem-161, Cmy-2
479B	AmpCtxCaz**Cip**	Tem-1, Cmy-2
480B	AmpCtxCaz	Cmy-2
481B	AmpCtxCaz	Ctx-M1/61
482B	AmpCtxCazTet	Tem-1, Cmy-2
483B	AmpCtxCaz	Ctx-M1/61
486B	AmpCtxCaz	Tem-1, Cmy-2



qnrS1/3

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MECHANISMS

Prevalence and Characterization of Quinolone Resistance Mechanisms in Commensal *Escherichia coli* Isolated from Slaughter Animals in Poland, 2009–2012

Dariusz Wasyl

Veterinary Microbiology 171 (2014) 307–314

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Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp.

CrossMark

Dariusz Wasyl *, Andrzej Hoszowski, Magdalena Zając

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Pathogenicity of cephalosporin resistant *E. coli*

ID	R-profile
039B	AmpCtxCazGenKanNalCip
358B	AmpCtxCazSmxTetTmpCip
364B	AmpCtxCazNalCip
479B	AmpCtxCazCip
480B	AmpCtxCaz
481B	AmpCtxCaz
482B	AmpCtxCazTet
483B	AmpCtxCaz
486B	AmpCtxCaz

PCR: pathogenicity markers

- STEC(*stx1/stx2*)
- EPEC (*eae*, *bfp*A)
- EIEC (*ipa*H)
- ETEC (*elt*, *est*)
- EAEC (*agg*R)

Aranda, et al. FEMS Microb. Letters, 2007. 267(2): 145-50

all negative

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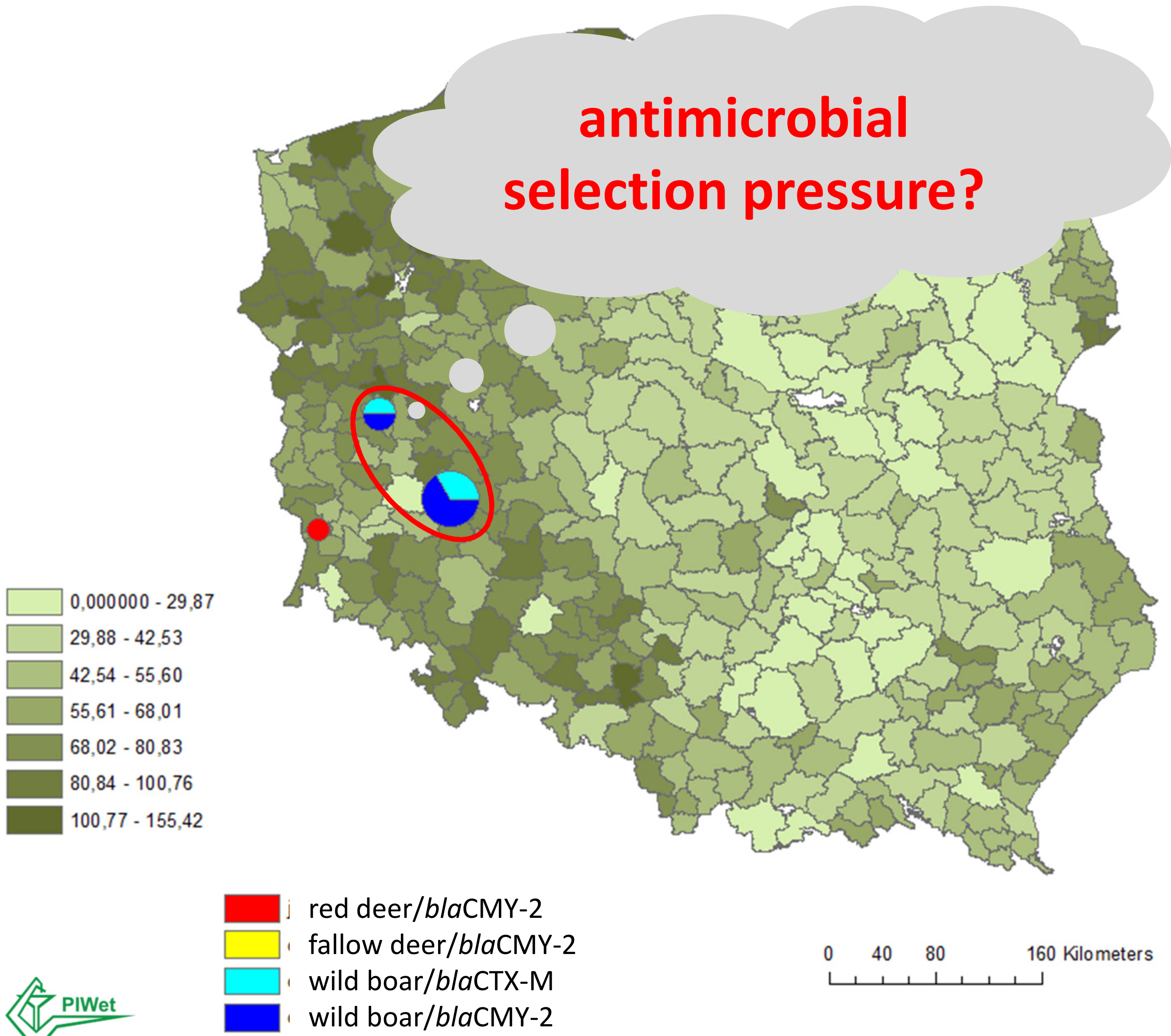
Cephalosporin resistant *E. coli* from wild boars



Two different mechanism in 8 of 11 cephalosporin resistant *E. coli* isolated from, respectively 2 and 6 boars hunted in 13 Nov and 3 Dec 2013, approximately 100 km from each other

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Antimicrobial resistance in other wildlife

Invasive turtle species as a source and vector of animal and human pathogens (NCN project No. 2013/11/B/NZ7/01690)

no *Escherichia coli,*

but

numerous isolates of:

- *Acinetobacter* spp.,
- *Citrobacter* spp.,
- *Pseudomonas* spp.,
- *Shewanella* spp.,
- *Aeromonas* spp.
- …

Antimicrobial resistance in wildlife

Salmonella from

- invasive alien turtles
- European grass snakes (*Natrix natrix*)

resistance is sparse (mainly streptomycin)

Conclusions

- *E. coli* in wild animals (boars) might be considered a reservoir and vector (transferable) of resistance determinants (environmental gene pool), but not the pathogen themselves (no pathogenicity markers found)
- Resistance to cephalosporins and quinolones (PMQR) are presumably of environmental origin – the prevalence might be enhanced by selective pressure of antimicrobial usage in agriculture (i.e. manure/slurry)
- …

Is it safe to hug a boar?

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  - No. NR12-0126-10/2011 (wildlife) and
  - No. 2013/11/B/NZ7/01690 (invasive alien turtles).

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