BIOR

PĀRTIKAS DROŠĪBAS, DZĪVNIEKU VESELĪBAS
UN VIDES ZINĀTNISKAIS INSTITŪTS

ANTIMIKROBIĀLĀ REZISTENCE PĀRTIKAS ĶĒDĒ UN VIDĒ

Aivars Bērziņš, Dr.med.vet., Ph.D.

Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta «BIOR» direktors
LLU Veterinārmedicīnas fakultātes profesors
Eiropas Pārtikas nekaitīguma iestādes (EFSA) Valdes loceklis

AMR monitorings/ uzraudzība un pētniecība Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūtā «BIOR»

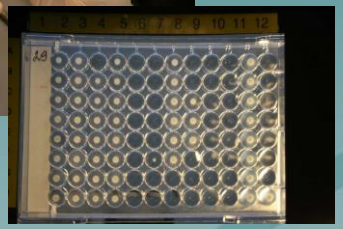
…20-30’ie

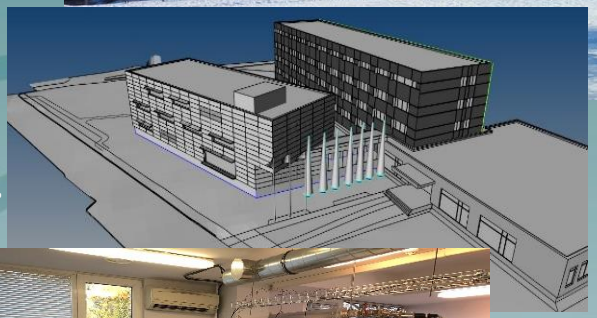
40-80’ie

90’ie-2004

2004-2006

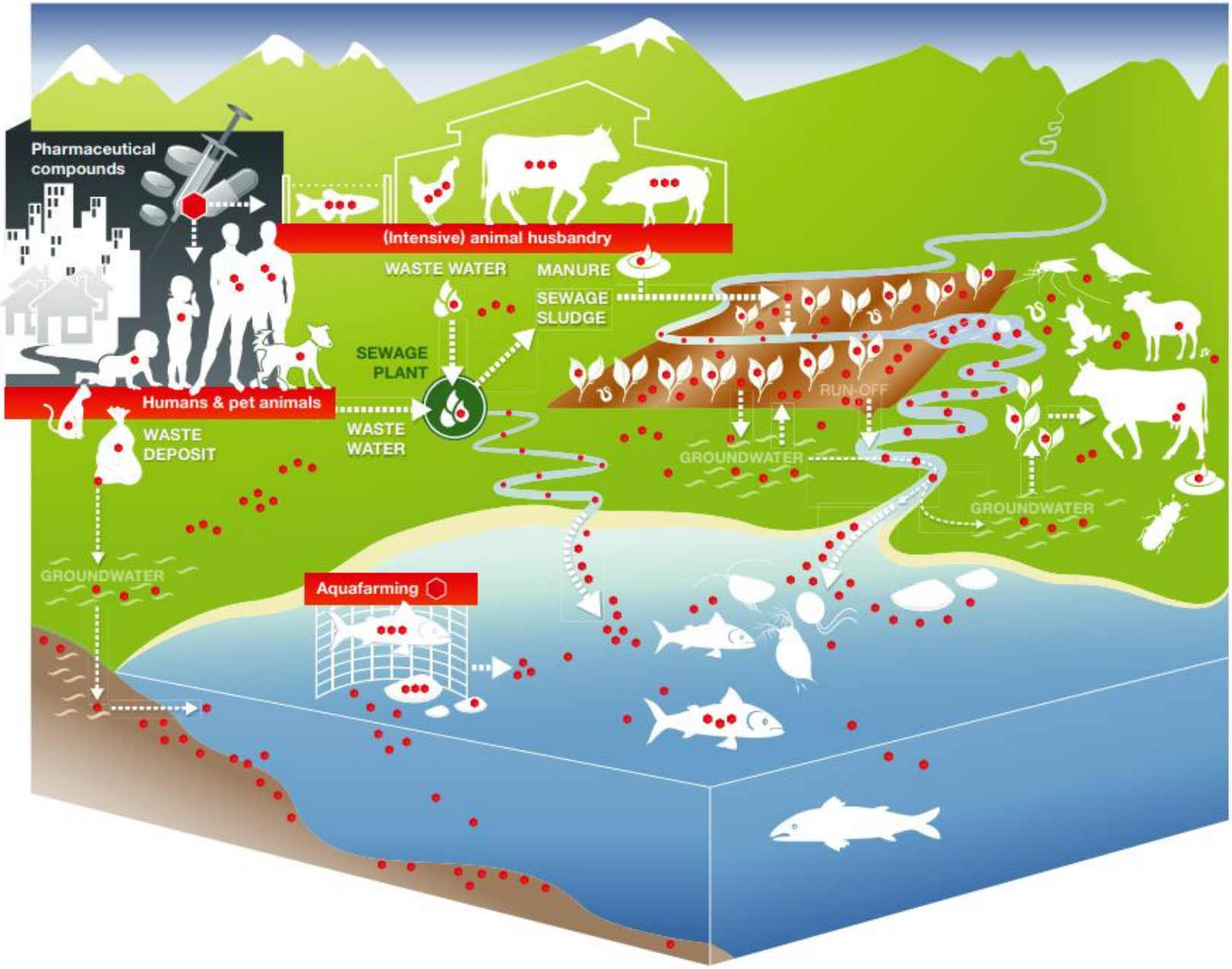
2006-2009





2010- …

AMR UN AB ATLIEKVIELAS APKĀRTĒJĀ VIDĒ



Berkner S. et al. EMBO Reports, 2014

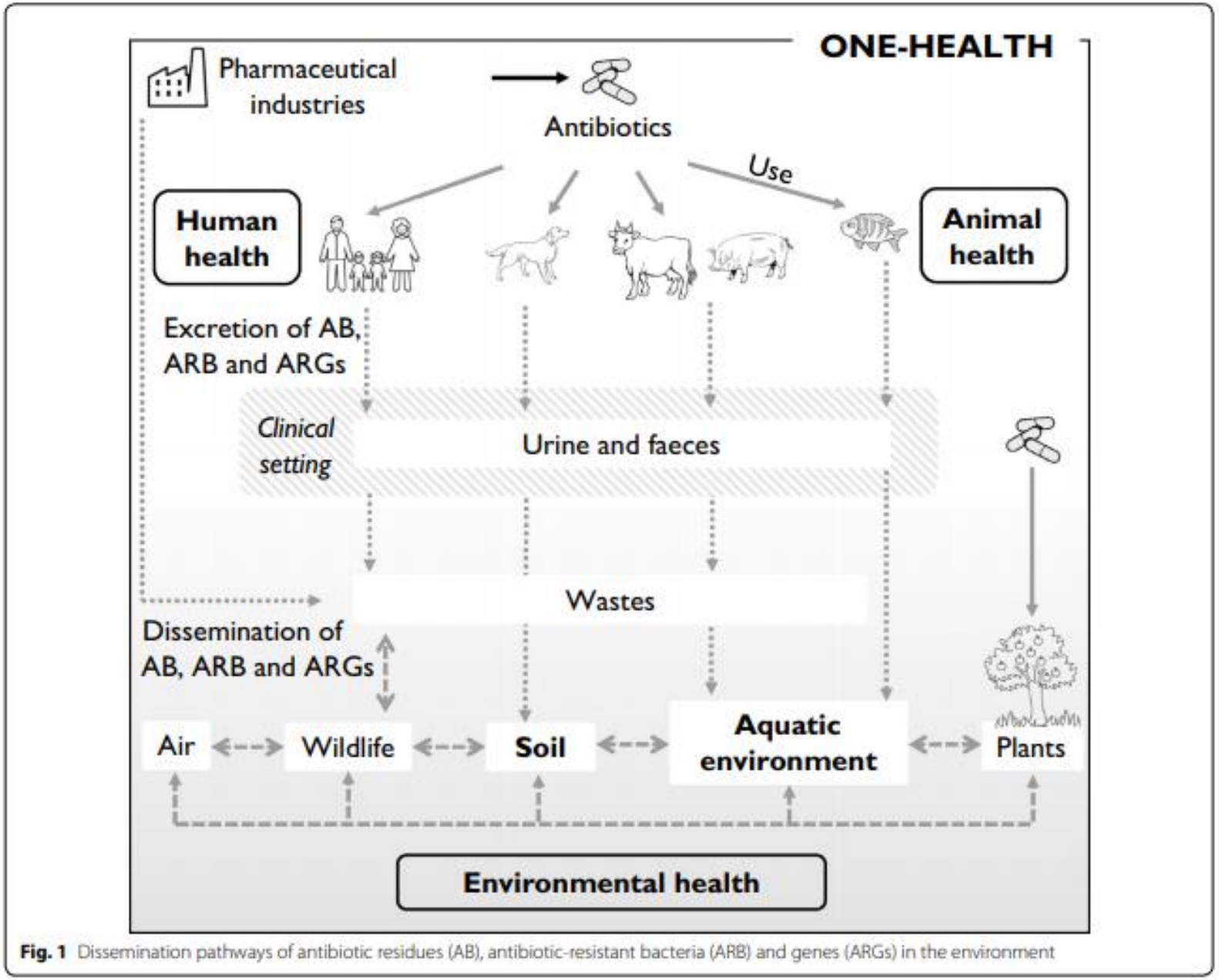
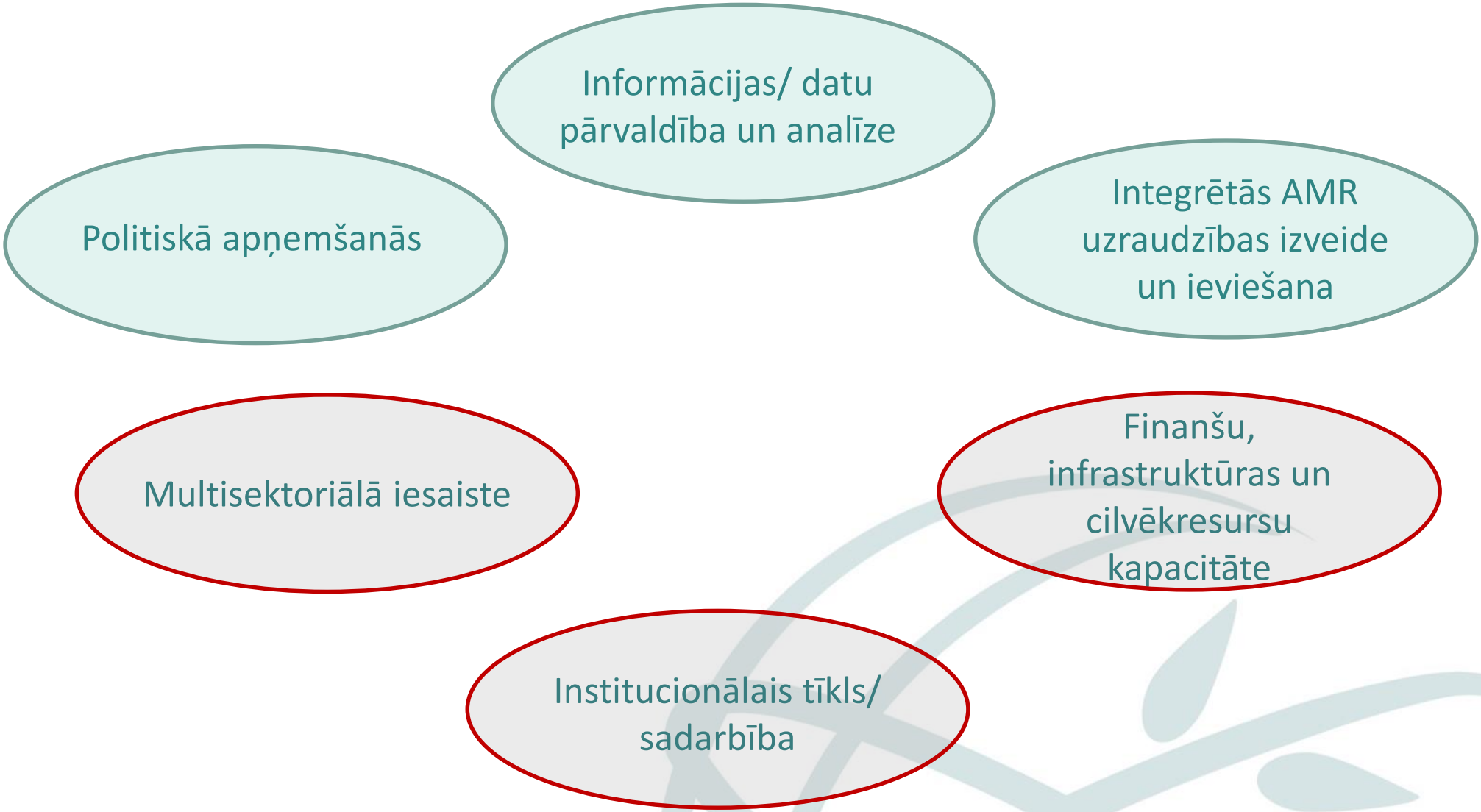


Fig. 1 Dissemination pathways of antibiotic residues (AB), antibiotic-resistant bacteria (ARB) and genes (ARGs) in the environment

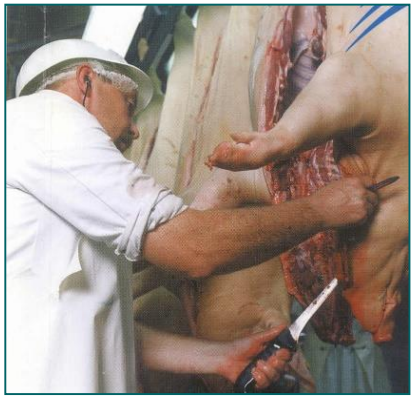
Goulas et al., *Environmental Evidence,* 2018

AMR INTEGRĒTĀ UZRAUDZĪBA = VIENAS VESELĪBAS UZRAUDZĪBA



AMR INTEGRĒTĀ UZRAUDZĪBA PĀRTIKAS ĶĒDĒ

- Antimikrobiālā rezistence (EK, EFSA, LV u.c.)
- Antibakteriālo līdzekļu patēriņš (LV, EK u.c.)



30.11.2018

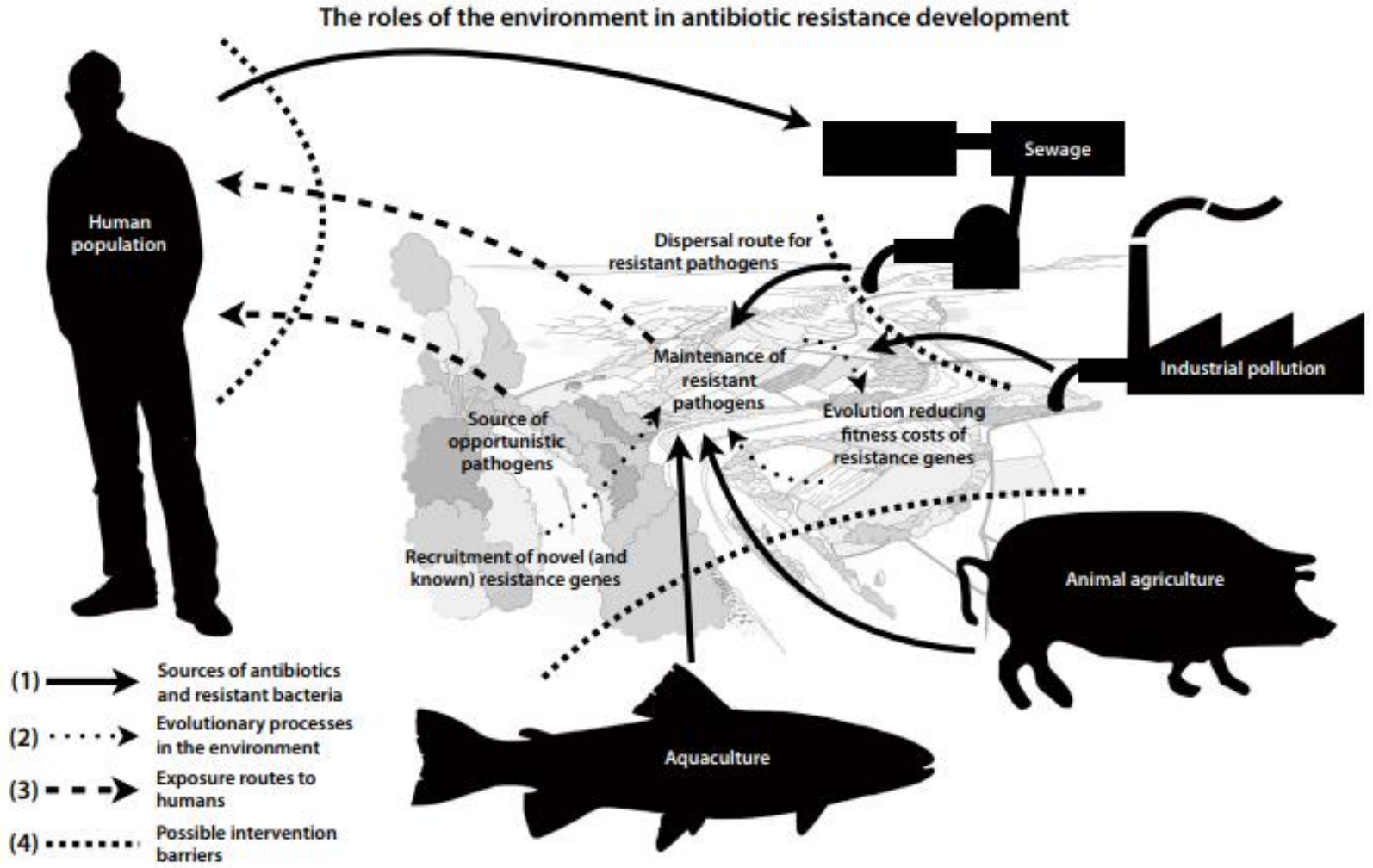


Fig. 1. Overview of the environmental processes influencing the development and spread of antibiotic resistance and how they relate to the knowledge gaps presented in this article. Silhouettes represent common sources of antibiotics and resistance genes to the environment. The human silhouette on the left also represents the human microbiome as a recipient of resistance genes and resistant bacteria from the environment. Arrows correspond to the first three broad categories of knowledge gaps, while the dashed lines show possible points of intervention (related to the fourth, broad knowledge gap). The illustration is adapted from Bengtsson-Palme et al. (2018a), FEMS Microbiol. Rev. doi: https://doi.org/10.1093/femsre/fux05, distributed under the CC-BY-NC license. (https://creativecommons.org/licenses/by-nc/4.0/).

Larsson D.G.J. Et al., *Environmental International,* 2018

Trends in Microbiology January 2014, Vol. 22, No. 1

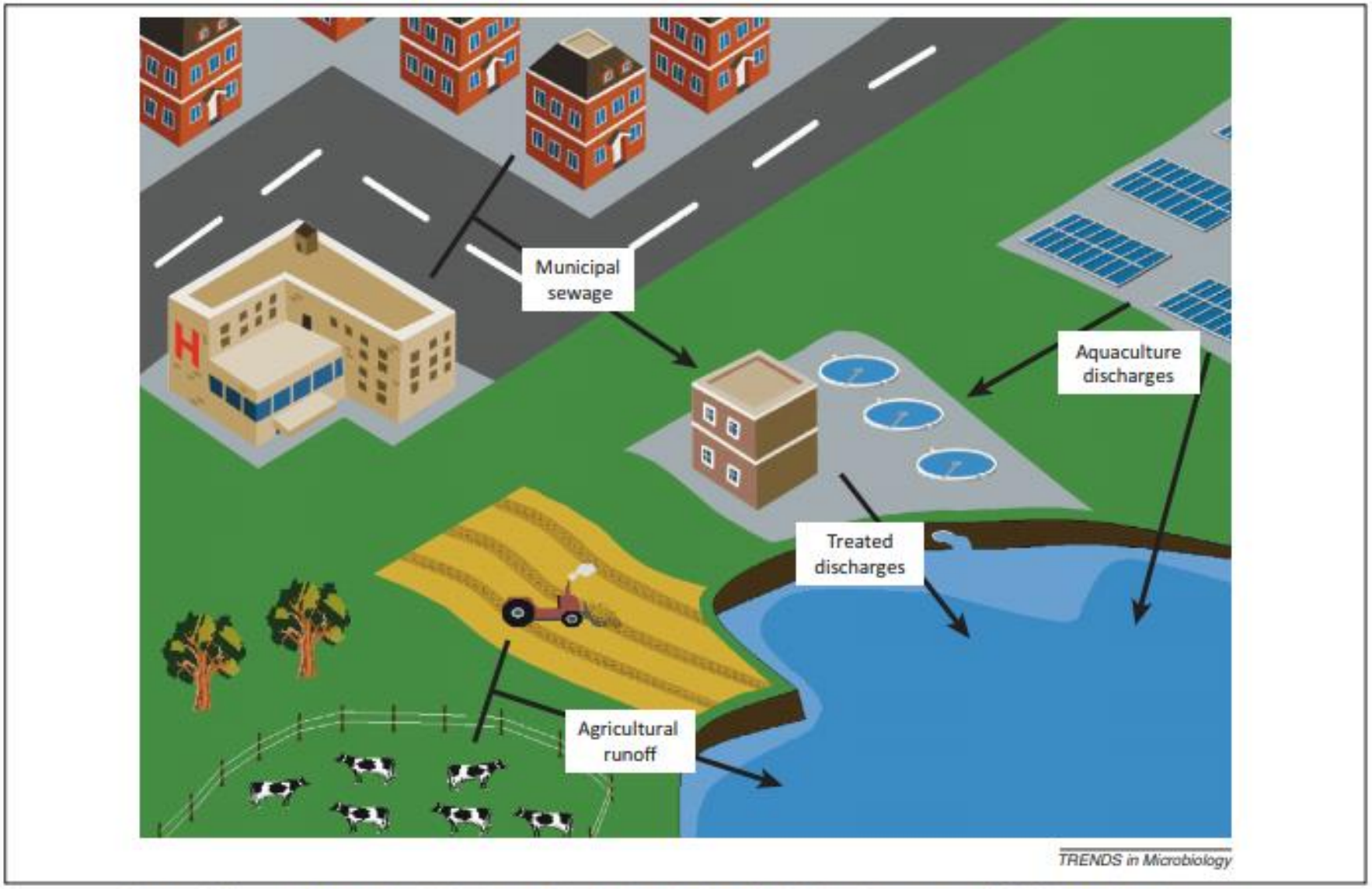


Figure 1. Different anthropogenic activities that result in the dissemination of antibiotic resistance genes (ARGs) in aquatic environments.

Marti E. et al., *Trends in Microbiology,* 2014

Determination of pharmaceutical residues and assessment of their removal efficiency at the Daugavgriva municipal wastewater treatment plant in Riga, Latvia

I. Reinholds, O. Muter, I. Pugajeva, J. Rusko, I. Perkons and V. Bartkevics

ABSTRACT

Pharmaceutical products (PPs) belong to emerging contaminants that may accumulate along with other chemical pollutants in wastewaters (WWs) entering industrial and/or urban wastewater treatment plants (WWTPs). In the present study, the technique of ultra-high-performance liquid chromatography coupled to Orbitrap high-resolution mass spectrometry (Orbitrap-HRMS) was applied for the analysis of 24 multi-class PPs in WW samples collected at different technological stages of Daugavgriva WWTP located in Riga, Latvia. Caffeine and acetaminophen levels in the range of 7,570–11,403 ng/L and 810–1,883 ng/L, respectively, were the predominant compounds among 19 PPs determined in the WW. The results indicate that aerobic digestion in biological ponds was insufficiently effective to degrade most of the PPs (reduction efficiency <0–50.0%) with the exception of four PPs that showed degradation efficiency varying from 55.0 to 99.9%. Tests of short-term chemical and enzymatic hydrolysis for PP degradation in WW samples were performed, and the results reflected the complexity of different degradation mechanisms and physicochemical transformations of PPs. The toxicological studies of WW impact on *Daphnia magna* indicated gradual reduction of the total toxicity through the treatment stages at the WWTP.

Key words | biological treatment, hydrolysis, Orbitrap-HRMS, pharmaceuticals, toxicology studies, wastewater treatment plant

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INTRODUCTION

Recently, global concerns have been raised about the wastewater (WW) treatment efficiency at wastewater treatment plants (WWTPs) that discharge partially purified water into surface waters. Besides the typical environmental pollutants (pesticides, persistent organic compounds, plasticizers, heavy metals, etc.), WWs contain residues of pharmaceutical products (PPs) and their metabolites generated by the pharmaceutical industry (Ma *et al.* 2016), livestock farms (Sim *et al.* 2013), hospitals (Carraro *et al.* 2016), and other economic and social sources. PPs belong to emerging contaminants, most of which have undefined tolerable levels in water, and potential impacts of their presence in the environment on human health are less investigated (Gadipelly *et al.* 2014). Antibiotics, cytostatics, and hormonally active compounds are the most notable PPs, which may cause adverse effects on organisms, promote bacterial resistance to antibiotics used in veterinary and human medicine, cause hormonal disturbances and present ot human health risks associated with prolonge contaminated water or consumption of aqua (Gavrilescu *et al.* 2015).

Some PPs, especially antibiotics (e.g., ma fonamides, fluoroquinolones), undergo limite in WWTPs, thus raising issues of their potent and human health hazards (Watkinson *et al.* 2 ger *et al.* (2009) have indicated an increase levels during the WW treatment stages, attribu solution of pharmaceuticals from solid wa faeces, bile, and other biogenic matter and t ation into forms free from metabolites due reactions with β-glucuronidase.

The latest technologies of industrial a WWTPs include the combined use of seve methods such as chemical oxidation by a

doi: 10.2166/wst.2016.528

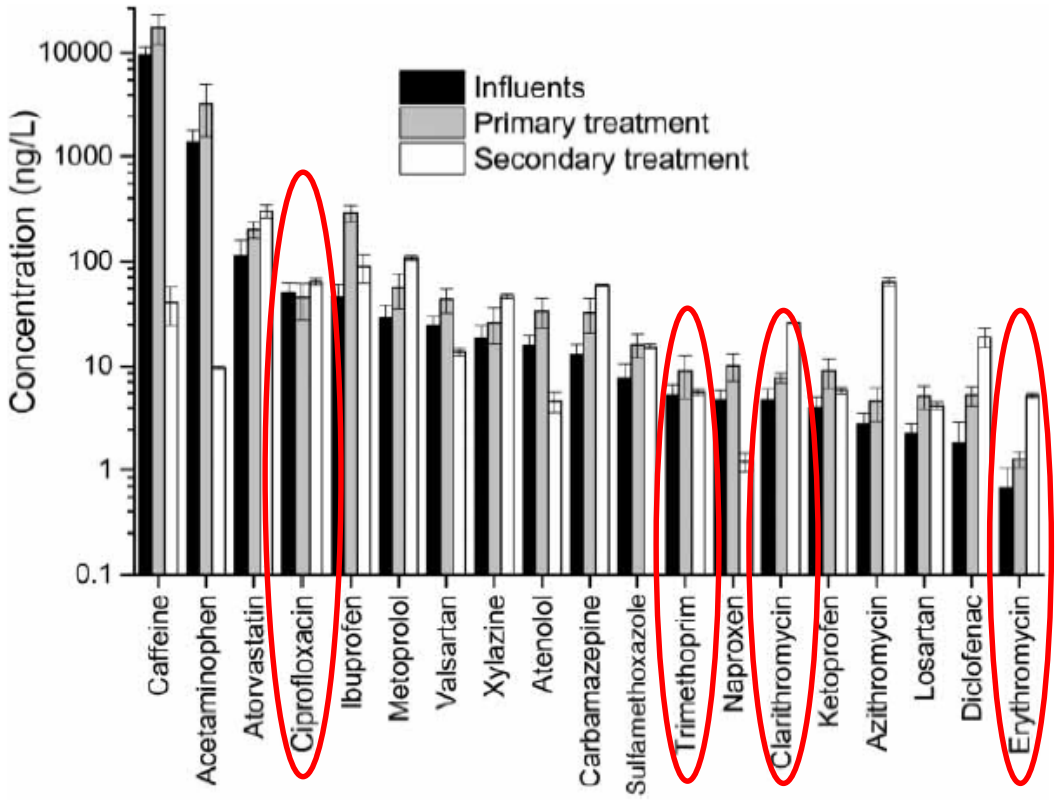


Figure 3 | Differences of medium PP concentration levels in WW samples collected before and after the primary (sedimentation) and the secondary (biological pond basins) treatment stages.

					
Pētījumā iekļauti	Teļi <1 g.v.	Cūkas	Broileri	Pārtikas produkti	Cilvēki
Pētījums veikts	2015.g.	2015.g. 2017.g.	2014.g. 2016.g.	2015.g. (c, l/l gala) 2016.g. putnu gaļa	2015.g.
Paraugu skaits	n=180	n=150 n=149	n=147 n=100	n=300 (c,l/l gaļa) n= 25x5 (putnu gaļa)	n=434
Parauga veids	fekālijas no taisnās zarnas	aklo zarnu saturs	aklo zarnu saturs	cūkgaļa l/l gaļa, putnu gaļa	fēces
Paraugu ņemšana	19 piena ražošanas saimniecībās	41 kautuvēs no 79 dažādām saimniecībām	kautuvēs 2 putnu fabrikās	tirdzniecības vietās	2 slimnīcās
Paraugi ņemti	projekts	ES monitorings	ES monitorings	ES monitorings	projekts
Izmeklējumi veikti:					
EK/ZM monitoringā ietvaros		✓ Ind.E.coli ✓Enz.E.coli	✓ Ind.E.coli (t.sk. ESBL, AmpC) ✓Campylobacter	✓ Enz. E.coli	
VPP AgroBioRes projektā Ietvaros	**✓ Ind.E.coli ✓Ind. Enterokoki ✓Enz.E.coli ✓Zoonotiskie aģ. ✓AMR genotips**	**✓ Ind.Enterokoki ✓ Zoonotiskie aģ. ✓AMR genotips**	**✓ Ind.Enterokoki ✓AMR genotips**	**✓ Zoonotiskie aģ. ✓AMR genotips ✓C.jejuni**	**✓ Campylobacter ✓ Ind. E.coli ✓ AMR genotips**

PRODUKTĪVIE DZĪVNIEKI UN SVAIGA GAĻA – PATOGĒNU SASTOPAMĪBA (%),2014.-2017.G.

				
paraugu skaits (n)	**n=180**	**n=150 +149**	**n=147+100**	**n=150 (c),150 (l/l), 25x5(p)**
Enzīmproducējošā E.coli	11,1 %	2015.g.=**48,7%** 2017.g.=**46,3%**	*2014.g.= **29,9**% 2016.g.= **91%**	8,6 % (c) 8,0 % (l/l) **91,6% (p)**
Salmonella	0,0 %	25,3 %	NT	3,3 % (c) 1,3 % (l/l)
Campylobacter	16,1%	**77,3** %	**63,3** %	0,0 % (c) 0,0 % (l/l)
Yersinia enterocolitica	0,0 %	8,7 %	NT	**25,3** % (c) **18,0** % (l/l)
Listeria monocytogenes	0,5 %	NT	NT	**24,6** % (c) **21,3** % (l/l)
Pseudomonas aeruginosa	14,4 %	21,3 %	NT	NT

Apzīmējumi: NT- nav testēts; c-cūkgaļa; l/l – liellopu gaļa; p – putnu gaļa

Enzīmproducējošā E.coli sastopamība (%) Latvijā, 2014.-2017.g.

11%

teļi (n=180)

49%

46%

cūkas (n=150+149)

30%

91%

broileri (n=147+100)

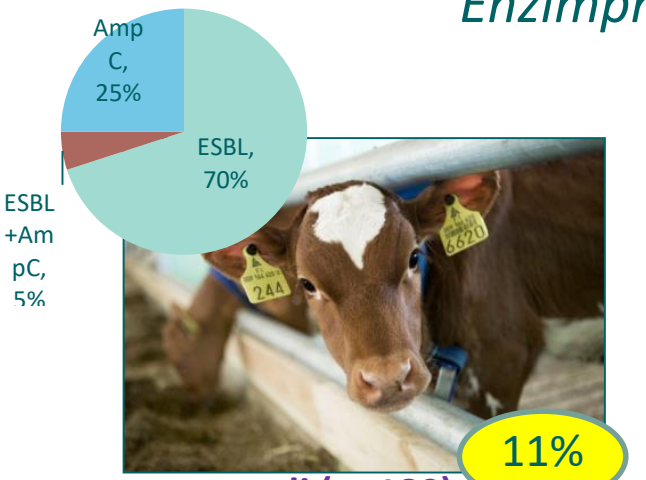
8%

liellopu gaļa (n=150)

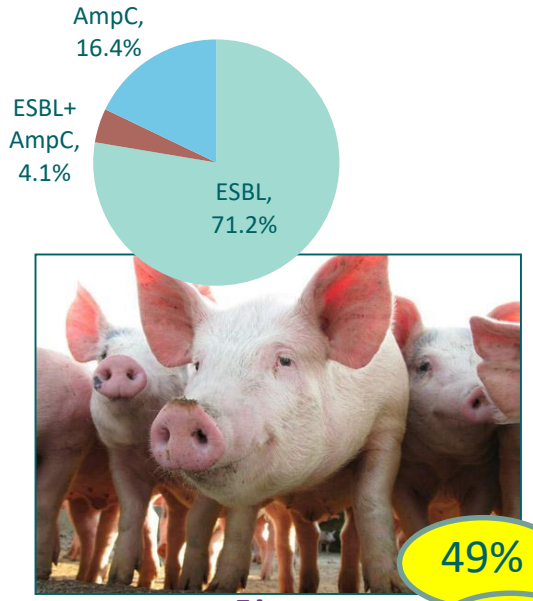
9%

cūkgaļa (n=150)

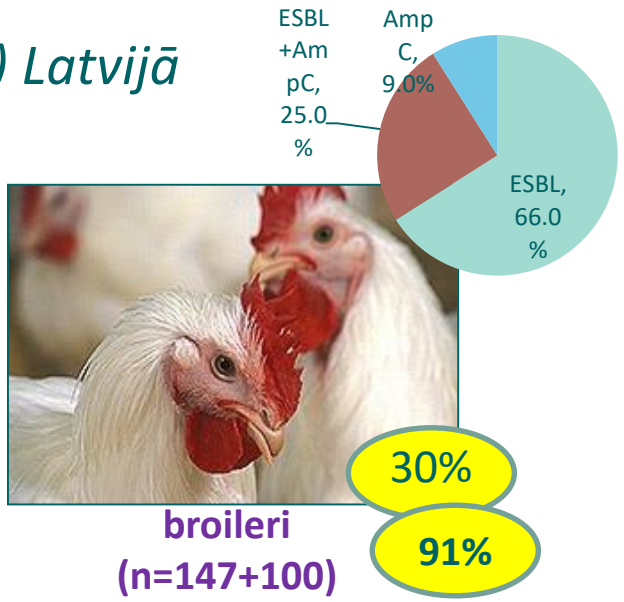
Enzīmproducējošā E.coli sastopamība (%) Latvijā



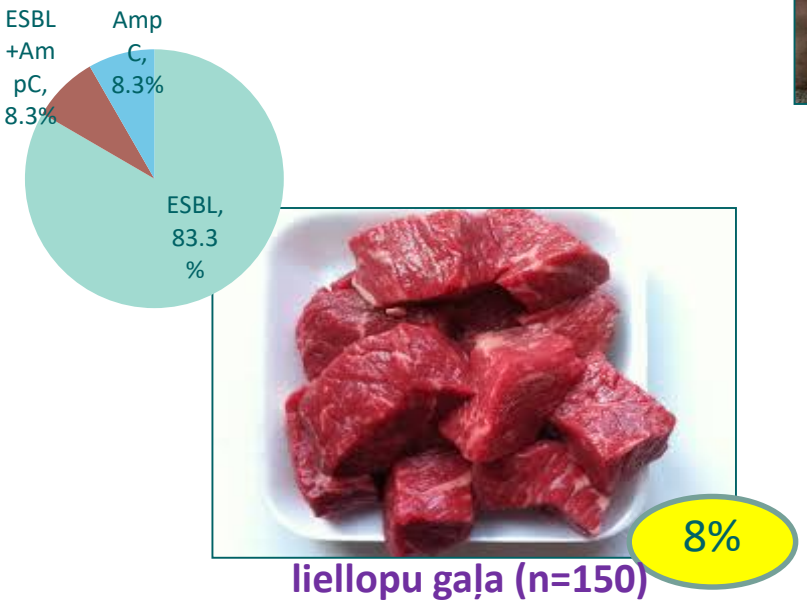
teļi (n=180)



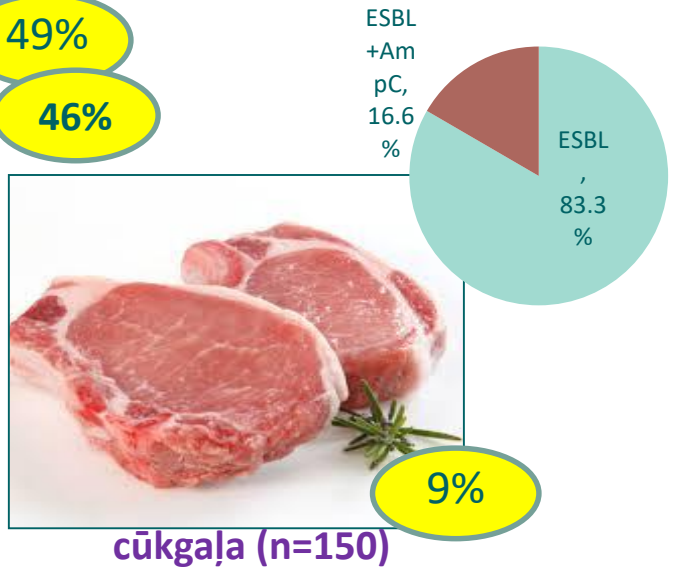
cūkas (n=150+149)



broileri (n=147+100)



liellopu gaļa (n=150)



cūkgaļa (n=150)

ESBL (paplašināta spektra βeta-laktamāze) ir biežāk sastopamais *Enzīmproducējošo E.coli* fenotips

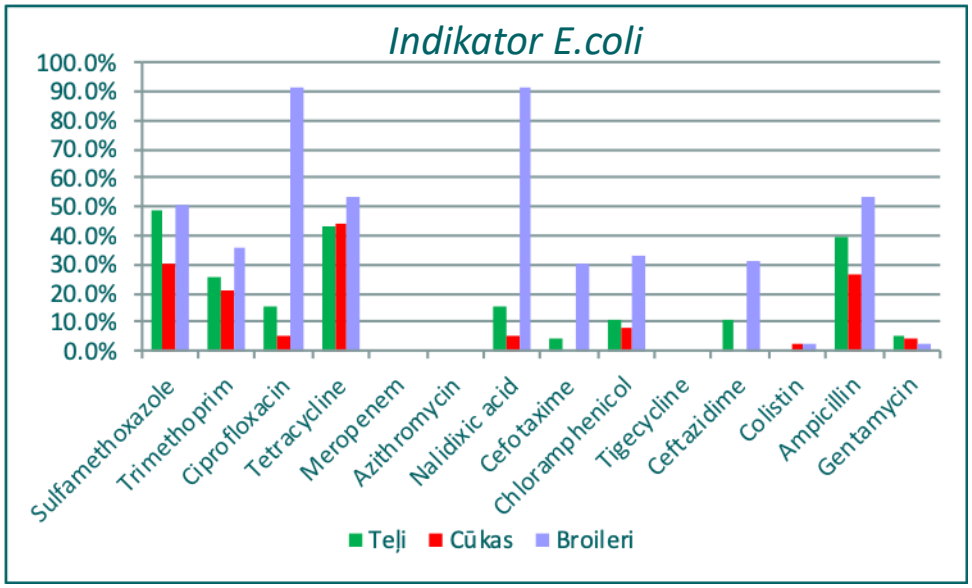
Enzīmproducējošā E.coli -

Biežāk sastopamie rezistences genotipi (TEM, CTX M I, CMY II)

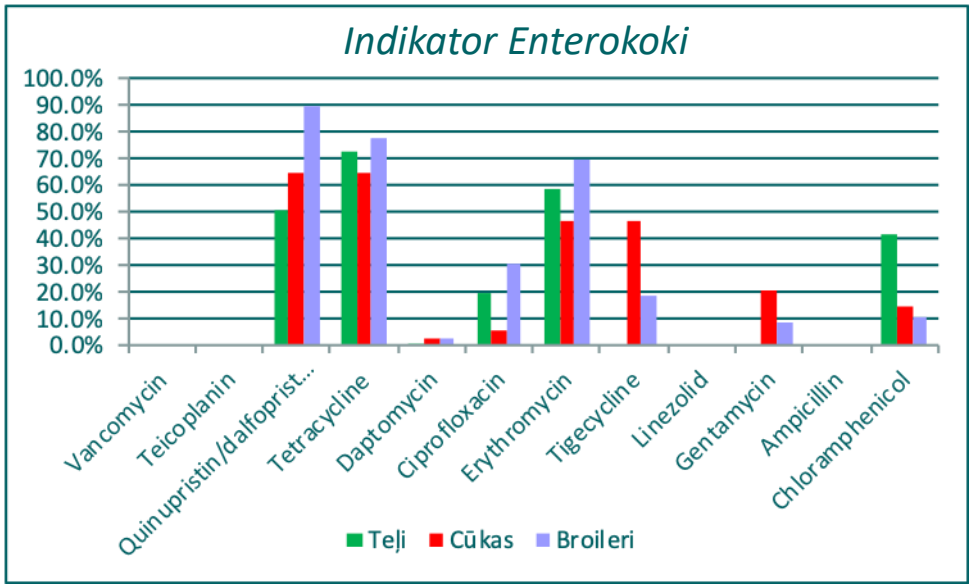
Nr. p/k	BIOR Nr.	Parauga veids	Dzīvnieks	Gads	Rezistences tipi							
					CTX M IV	TEM	OXA	SHV	CMY II	CTX M I	CTX M II	DHA
1	26901/3	fekālijas	teļš	2015						+/-		
2	26901/5	fekālijas	teļš	2015						+		
3	26901/7	fekālijas	teļš	2015		+				+		
4	26901/10	fekālijas	teļš	2015						+/-		
5	26902/3	fekālijas	teļš	2015						+		
6	26902/8	fekālijas	teļš	2015							+	
7	26902/9	fekālijas	teļš	2015						+		
8	26902/10	fekālijas	teļš	2015						+		
9	26903/2	fekālijas	teļš	2015		+						
10	26903/7	fekālijas	teļš	2015					+	+		
11	26903/8	fekālijas	teļš	2015		+			+	+		
12	26903/10	fekālijas	teļš	2015					+			
13	26903/16	fekālijas	teļš	2015						+		
14	26903/18	fekālijas	teļš	2015						+		
15	26903/20	fekālijas	teļš	2015						+		
16	32216/3	fekālijas	teļš	2015						+		
17	32218/2	fekālijas	teļš	2015		+						
18	32218/3	fekālijas	teļš	2015		+						
19	26901/4	fekālijas	teļš	2015		+				+		
20	26901/6	fekālijas	teļš	2015		+				+		
21	31890	svaiga gaļa	cūkgaļas šķiņķis	2015						+		
22	65694	svaiga gaļa	cūkas muskulis	2015		+						
23	85426	svaiga gaļa	cūkgaļa	2015		+			+			
24	62697	svaiga gaļa	liellopu stilbs	2015		+				+		
25	86350	svaiga gaļa	cūkgaļas kakla karbonāde	2015		+				+		
26	45425	svaiga gaļa	cūkgaļas šķiņķis	2015		+/-				+		
27	37567	svaiga gaļa	liellopu kotlešu gaļa	2015		+				+		
28	45850	svaiga gaļa	liellopu gaļa	2015					+			
29	56859	svaiga gaļa	cūkgaļas fileja	2015		+				+		
30	33202	svaiga gaļa	cūkgaļas kakla karbonāde	2015						+		
31	61762	svaiga gaļa	liellopu gaļa	2015						+		
32	63867	svaiga gaļa	cūkgaļa	2015					+			

Informācijas avots: J.Trofimova "ESBL un kolistīnrezistentās *E.coli* no cūkām, teļiem, dzīvnieku izcelsmes pārtikas produktiem - genotipa raksturojums" (VPP AgroBiores seminārs, 22.03.2016., ZI BIOR, Rīga)

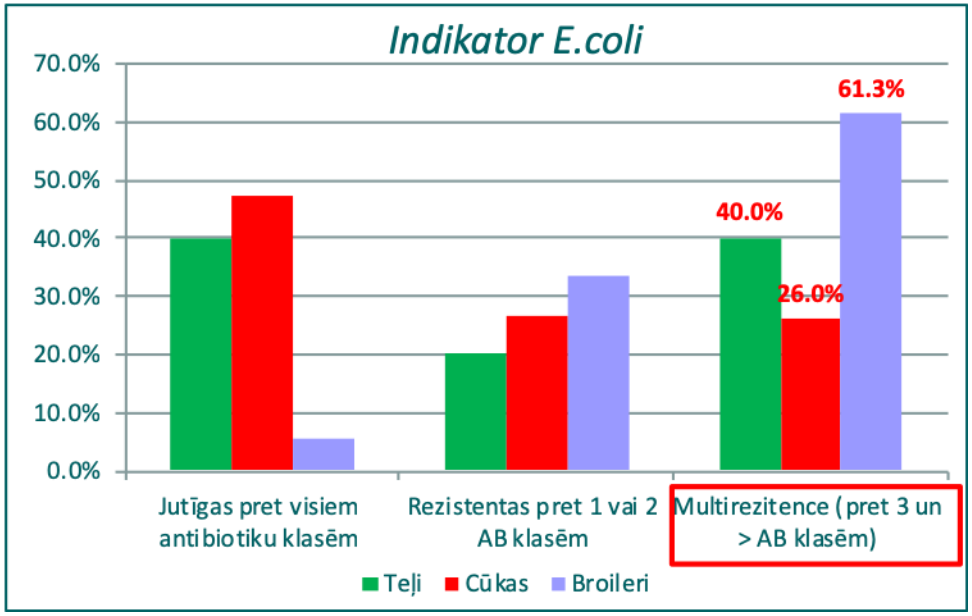
Indikatorbaktēriju rezistence un multirezistence (%), 2014.-2015.g.

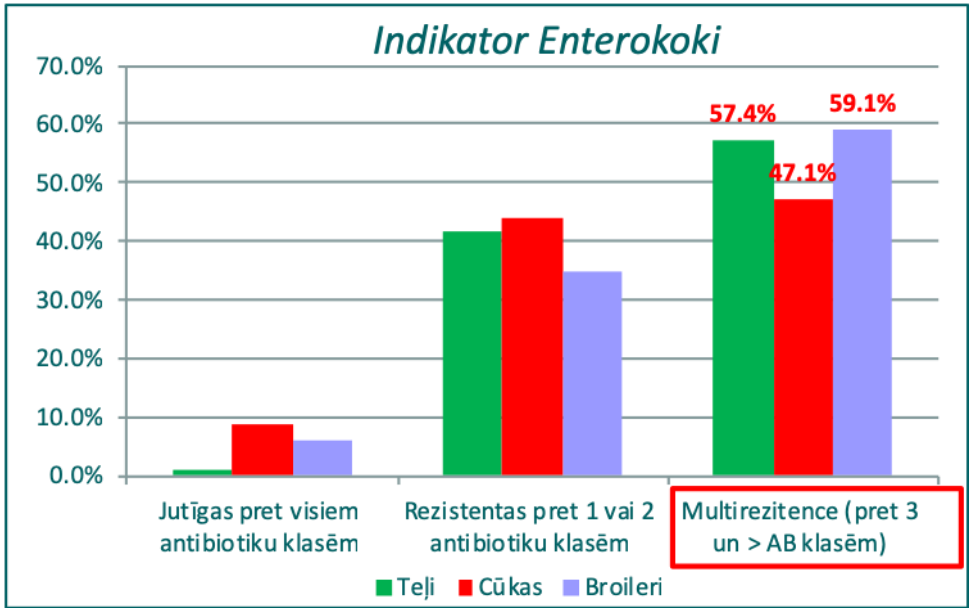


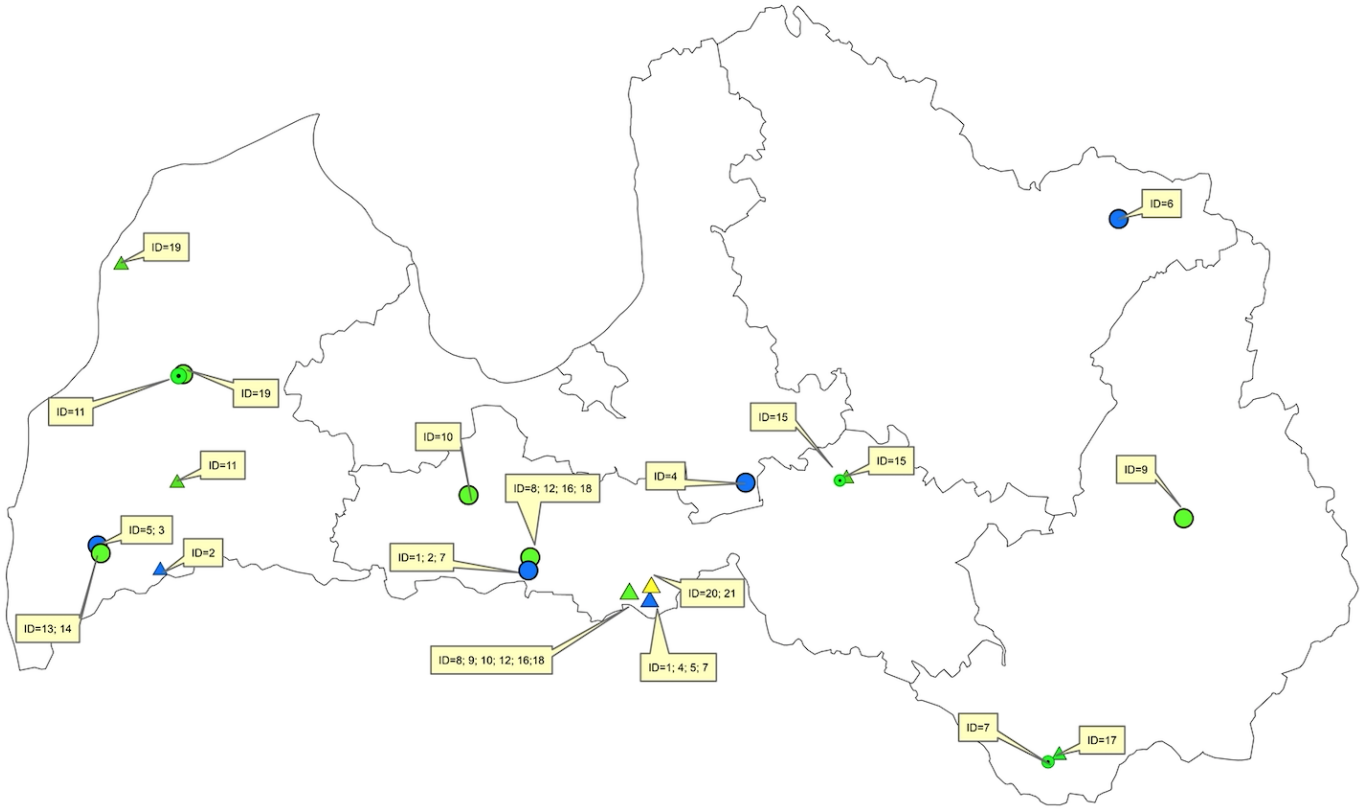
Visām sugām ir novērota augsta rezistence pret sulfonamīdiem, tetraciklīniem un ampicilīniem, broileriem – ļoti augsta rezistence pret kvinoloniem



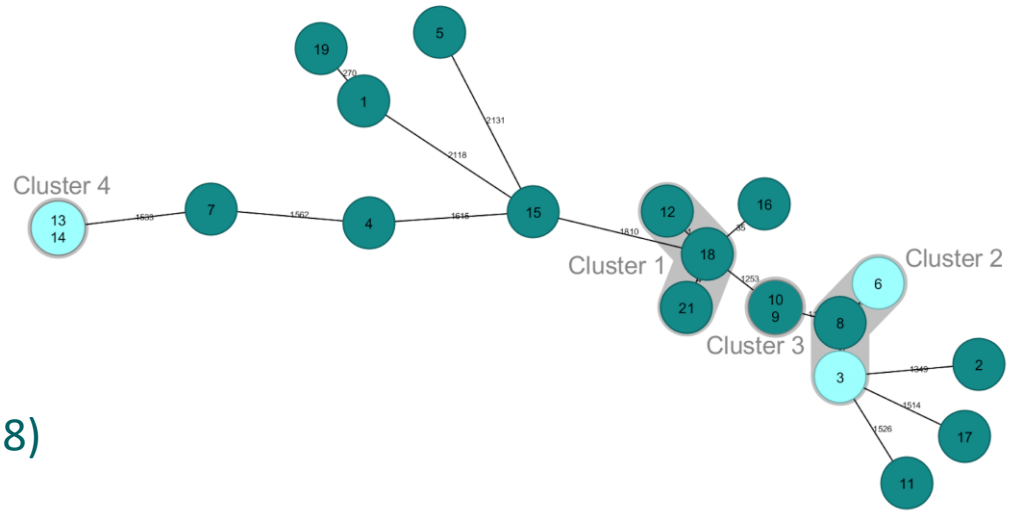
Visām sugām ir novērota augsta rezistence pret tetraciklīniem, makrolīdiem (erytromycin) un streptogramīniem (quinupristin/dalfopristin), cūkām – pret tigecycline, teļiem – pret chloramphenicol







Kontaminācija ar kolistīna rezistento *E.coli* pārtikas ķēdē: dzīvnieku novietne-kautuve-gaļas pārstrāde…



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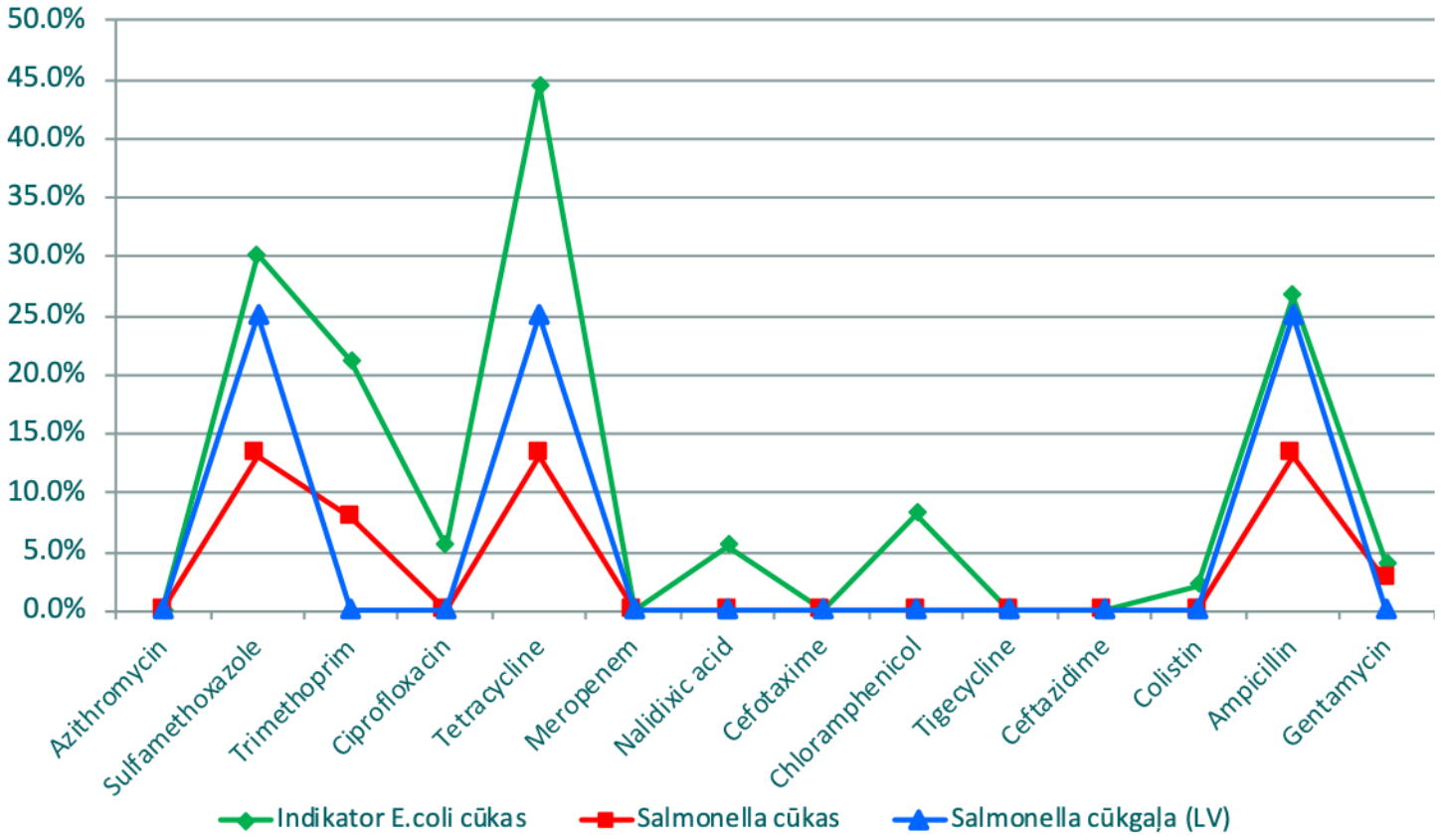
(Bergšpica et al., nepublicēti dati, 2018)

Salmonellas:

- *Salmonellu* sastopamība: dzīvnieku populācijā (…0- 25%), gaļas produkti (…0-3%)
- Biežāk sastopamie salmonellu serotipi cūkām un cūkgaļā: *S.Derby* un *S.Typhimurium*
- *Antimikrobiāla rezistence*: Ir novērota augsta rezistence pret sulfonamīdiem, tetraciklīniem un ampicilīniem



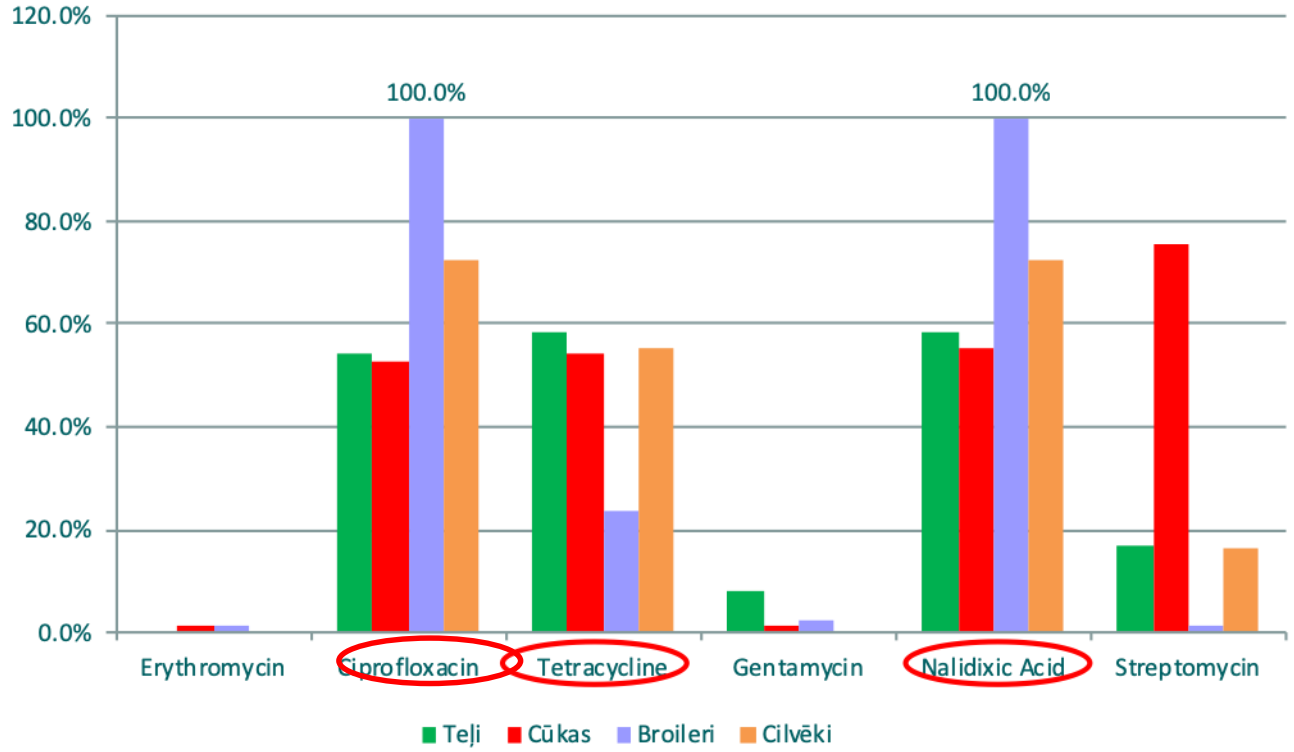
Attēls: Indikator E.coli un Salmonella rezistences salīdzinājums (%) cūkām 2015.g.

Kampilobaktērijas:

- *Kampilobaktēriju sastopamība populācijā*: ….0…70%

- *Biežāk sastopamās kampilobaktēriju sugas*: broileriem, teļiem, cilvēkiem - *C.jejuni*, cūkām - *C.coli*

- *Antimikrobiāla rezistence*:
  - ✓ visām sugām tika konstatēts augsts rezistences līmenis pret tetraciklīniem un kvinoloniem (ciprofloxacin, nalidixic acid); cūku populācijā (*C.coli*) – ļoti augsts rezistences līmenis pret aminoglikozīdiem (streptomycin)
  - ✓ broileru populācijā ir konstatēta 100 % rezistence pret kvinoloniem



Rezistences līmenis (pēc EFSA):
Zems >1% - 10%
Vidējs >10% - 20%
Augsts >20% - 50%
Ļoti augsts >50% - 70%
Ekstremāli augsts >70%

Attēls: Kampilobaktēriju rezistences salīdzinājums (%) 2015.g.

ORIGINAL ARTICLE

Antimicrobial Resistance Profiles of *Campylobacter* spp. Isolated from Broiler Chicken Meat of Estonian, Latvian and Lithuanian Origin at Estonian Retail Level and from Patients with Severe Enteric Infections in Estonia

M. Mäesaar[1,2], T. Kramarenko[1,2], K. Meremäe[2], J. Sõgel[3], M. Lillenberg[2], L. Häkkinen[1], M. Ivanova[4,5], K. Kovalenko[6], A. Hörman[7], M.-L. Hänninen[8] and M. Roasto[2]

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Impacts

- Our antimicrobial susceptibility results indicate that the use of antimicrobial agents, particularly fluoroquinolones, in Estonian broiler chicken production has been reduced during recent years which can be associated with the policies in restrictive use of antimicrobials implemented by the European Commission in 2006.
- Resistances to one or more antimicrobials occurred significantly less frequently in the products of Estonian origin than in the products of Latvian and Lithuanian origin available at Estonian retail.
- It was found that problems caused by the inappropriate use of antimicrobials extend beyond the country in which a food originates; therefore, the origin of broiler chicken meat may pose different risks for human population.

Keywords:
broiler chicken meat; *Campylobacter*; human isolates; antimicrobial resistance; Baltic countries

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Summary

The resistance patterns of *Campylobacter* meat originating either from Estonia, L were determined. Additionally, in collab Estonian hospitals, antimicrobial suscep *Campylobacter* isolates from patients wi tions. The isolates were identified at the sp tively, 88.8% of the isolates were *C. jejun* *Campylobacter* isolates of broiler chicken minimal inhibitory concentrations (MIC MIC[TH] method (National Veterinary Ins six antimicrobials. Resistance to one or m (63.3%) of *Campylobacter* broiler chicke human-origin isolates. Large proportion were resistant to ciprofloxacin (60.2%). more unrelated antimicrobials) was detected in five (5.1%) *C. jejuni* isolates. Among the human isolates, 20 (71.4%) were resistant to fluoroquinolones, and two (7.1%) *C. jejuni* isolates exhibited multidrug resistance. The chicken meat isolates of Estonian origin were the most susceptible. However, a high proportion

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High occurrence of *Campylobacter* spp. in Latvian broiler chicken pro

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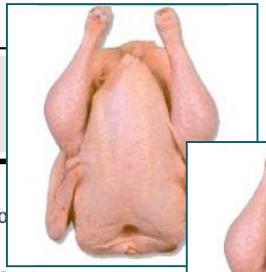
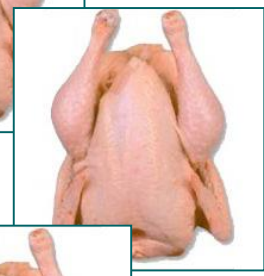
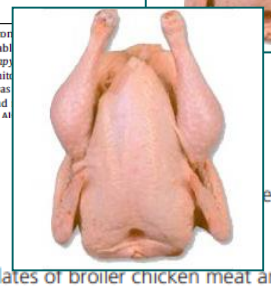
ABSTRACT

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp., most cor
jejuni and *C. coli*. However, no official data for human campylobacteriosis in Latvia is availabl
Commission Decision 2007/516/EC the *Campy*
was no continuous monit
(the present study was
ughterhouse and
therhouses. Al







50-60%
Campylobacter spp.
poz.

Antibiotic Resistance of Baltic Origin Ca

esaar et al.

Table 3. Number and proportion of antimicrobial resistant *Campylobacter jejuni* and *Campylobacter coli* isolates of broiler chicken meat and human origin

	Broiler chicken meat isolates				
	Estonian	Latvian	Lithuanian	All	Human isolates
Antimicrobial	No (%)	No (%)	No (%)	No (%)	No (%)
Erythromycin	0 (0.0)	0 (0.0)	1 (2.2)	1 (1.0)	0 (0.0)
Ciprofloxacin	6 (16.7)	14 (87.5)	39 (84.8)	59 (60.2)	19 (67.9)
Tetracycline	4 (11.1)	1 (6.3)	9 (19.6)	14 (14.3)	12 (42.9)
Streptomycin	1 (2.8)	0 (0.0)	7 (15.2)	8 (8.2)	3 (10.7)
Gentamicin	1 (2.8)	0 (0.0)	1 (2.2)	2 (2.0)	0 (0.0)
Nalidixic acid	7 (19.4)	14 (87.5)	37 (80.4)	58 (59.2)	19 (67.9)
Sensitive to all six	29 (80.6)	2 (12.5)	5 (10.9)	36 (36.7)	8 (28.6)
Resistant to one or more	7 (19.4)	14 (87.5)	41 (89.1)	62 (63.3)	20 (71.4)
Multidrug resistant[a]	1 (2.8)	0 (0.0)	4 (8.7)	5 (5.1)	2 (7.1)
Total No[b]	36	16	46	98	28

[a]Multidrug resistant defined as resistant to three or more unrelated antimicrobials.
[b]Total no. of strains does not equal the sum of rows, because some strains are multidrug resistant.

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High occurrence rates of enrofloxacin and ciprofloxacin residues in retail poultry meat revealed by an ultra-sensitive mass-spectrometric method, and antimicrobial resistance to fluoroquinolones in *Campylobacter* spp

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ABSTRACT

An ultra-sensitive mass spectrometric confirmation and quantification method for the determination of selected fluoroquinolones—enrofloxacin and its main metabolite ciprofloxacin—was developed and validated in poultry meat samples. The achieved limits of quantification were 1 ng kg^{-1} for enrofloxacin and 10 ng kg^{-1} for ciprofloxacin. The analysis of 40 retail poultry samples originating from Estonia, Latvia, Lithuania, Poland and France revealed that 93% of samples contained residues of enrofloxacin in the range from 3.3 to 1126 ng kg^{-1}. Previous studies have shown high levels of antimicrobial resistance to fluoroquinolones, particularly in *Campylobacter* spp. and various faecal indicators isolated from broiler meat. Consequently, the revealed widespread usage of fluoroquinolones in the poultry industry may result in the further emergence of antimicrobial resistance of *Campylobacter* in the food chain.

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KEYWORDS
Fluoroquinolone; enrofloxacin; poultry meat; UHPLC-MS/MS; antimicrobial resistance

Introduction

Enrofloxacin is a fluoroquinolone antimicrobial drug with a broad spectrum of activities used for the treatment of bacterial infections of respiratory and intestinal tracts in poultry. Metabolic studies of enrofloxacin have identified ciprofloxacin as the main metabolite formed via the *N*-de-ethylation process of the ethyl group linked to the piperazine ring, with concentrations below 10% those of enrofloxacin in the case of poultry muscle, but with a higher tendency of metabolism in poultry liver tissues (Morales-Gutierrez et al. 2015). Enrofloxacin is authorised only for veterinary use; however, ciprofloxacin is intended for use in human medicine and

EU. The maximum permitted limits (MRLs) fo
antibiotics in foodstuffs of animal origin were estab
lished by the Commission Regulation (EU) No 37
2010. In broilers, the defined MRL is 100 μg kg^{-1} fo
muscle tissues, calculated as a sum of enrofloxacir
and ciprofloxacin concentrations. The exception i
animals producing eggs for human consumption, fo
which enrofloxacin cannot be used (Europear
Commission 2010). Meanwhile, the US Food anc
Drug Administration (FDA) suspended an approva
of the use of enrofloxacin in poultry in 2005, due t
the emerging antimicrobial resistance in variou
foodborne pathogens, including *Campylobacter* anc
Salmonella. These foodborne pathogens and som
strains of *Escherichia coli*, which are resistant o

ORIGINAL ARTICLE

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www.aaem.pl

Prevalence and antimicrobial resistance of *Salmonella* in meat and meat products in Latvia

Margarita Terentjeva[1], Jeļena Avsejenko[2], Madara Streikiša[2], Andra Utināne[2], Kaspars Kovaļenko[1], Aivars Bērziņš[1,2]

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Terentjeva M, Avsejenko J, Streikiša M, Utināne A, Kovaļenko K, Bērziņš A. Prevalence and antimicrobial resistance of *Salmonella* in meat and meat products in Latvia. Ann Agric Environ Med. 2017; 24(2): 317–321.

Abstract

Introduction and objective. *Salmonella* is a foodborne pathogen which causes gastrointestinal illness in consumers, and exhibits resistance to antimicrobials of veterinary and clinical significance. The aim of this study is to detect the prevalence and antimicrobial resistance of *Salmonella* isolates from meat in Latvia.

Materials and method. A total of 3,152 samples of raw and ready-to-eat (RTE) meats were collected during the official control and in-house control procedures in 2015. Samples were tested in accordance with ISO 6579:2002. All *S.* Typhimurium, *S.* Enteritidis and other isolates recovered from the official control samples (*S.* Derby, *S.* Give) were tested for antimicrobial resistance. The minimum inhibitory concentration (MIC) values were investigated in line with the requirements of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Results. The prevalence of *Salmonella* was 0.8% (25/3152). The highest prevalence (1.5%) of *Salmonella* was found in minced meat and meat preparations (7/481), while the lowest (0%) in frozen meat and meat preparations (0/349) and RTE meats (0/364). The most common serovars were *S.* Typhimurium (36%, 9/25) and *S.* Derby (32%, 8/25). In total, 62% (13/21) of *Salmonella* isolates were resistant to at least one antimicrobial agent. Altogether, 40% (8/20) of isolates were resistant to sulfamethoxazole, 25% (5/20) to nalidixic acid, ciprofloxacin, ampicillin and 20% (4/20) to tetracycline. All isolates were susceptible to ceftazidime, cefotaxime, meropenem, azithromycin and tigecycline. *S.* Typhimurium exhibited antimicrobial resistance more often (87.5%) than other serovars.

Conclusion. The study shows that the presence of *Salmonella* in meat, together with the high prevalence of resistant strains, is a significant public health related issue in Latvia.

Key words
Salmonella, pork, poultry, minced meat preparations, antimicrobial resistance, Latvia

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Type: research-article

Original Article

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AU1 ▶

Prevalence and Antimicrobial Resistance of *Escherichia coli*, *Enterococcus* spp. and the Major Foodborne Pathogens in Calves in Latvia

AU2 ▶

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Abstract

The aim of the present study was to detect the prevalence and antimicrobial resistance of fecal indicators and major foodborne pathogens in feces of calves and to identify the factors associated with increased prevalence of resistant bacteria on farms. Altogether, 180 rectal swabs were collected from 18 farms in Latvia. Samples were investigated to detect the prevalence and antimicrobial resistance of *Escherichia coli*, *Enterococcus* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp., *Staphylococcus aureus*, and *Campylobacter* spp. Among all, 64% (74/110) of commensal *E. coli*, 100% (78/78) *Enterococcus faecalis* and 96% (22/23) *Enterococcus faecium* isolates were resistant at least to one antibiotic. The prevalence of extended-spectrum β-lactamase (ESBL)/AmpC-producing *E. coli* were 11.1% (20/180) with $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{CMY} genes identified. *Campylobacter jejuni* (12.8%, 23/180) and *Campylobacter coli* (2.8%, 5/180) were the most resistant to tetracycline (61%, 14/23; 100%, 5/5) and fluoroquinolones (61%, 14/23; 100%, 5/5). Prevalence of *L. monocytogenes* was 0.6% (1/180) and *S. aureus* 1.7% (3/180). All samples were *Salmonella* and *Y. enterocolitica* negative. Farm size, bought calves, contact with other calves, and antimicrobial treatment of cows were associated with increased prevalence of resistant *E. coli* and *Enterococcus* spp. Despite the low overall usage of antimicrobials in Latvia, the high rates of antimicrobial resistance in fecal indicators and *Campylobacter*, in addition to the high prevalence of ESBL-producing *E. coli*, highlights the necessity for the prudent use of antimicrobials in dairy farms in Latvia.

Keywords: ESBL/AmpC-producing *Escherichia coli*, cephalosporins, *Campylobacter*, bought calves

Introduction

THE ANTIMICROBIAL RESISTANCE is a threat affecting public health worldwide (WHO, 2015). The limited efficiency and, hence, the availability of antimicrobials for application in clinical cases, alongside with the increased health care costs may complicate the patient treatment and care effectiveness. Therefore, the efforts are being made to address the ongoing increase in the antimicrobial resistance both in human and veterinary medicine (Eliopoulos *et al.*, 2003; Marshall and Levy, 2011).

Misuse of antimicrobials in human medicine and animal production has been recognized as the main factor in the development of antimicrobial resistance (Marshall and Levy, 2011). Inappropriate application of antimicrobials in animal production may lead to a distribution of resistant microorganisms, which could be transferred from animal to human through the contaminated environment, direct contact, or contaminated foods (Van Boeckel *et al.*, 2015). The commensal microbiota could serve as a reservoir of resistance genes with the transfer of those to pathogens facilitating a spread of antimicrobial resistance. The widespread occurrence of antimicrobial resistance in commensal bacteria and in foodborne pathogens and clinical isolates has been reported (Kaesbohrer *et al.*, 2012; Kramarenko *et al.*, 2016; Terentjeva *et al.*, 2017).

The occurrence of extended-spectrum β-lactamase (ESBL) *Escherichia coli* in animals and food chain is a growing area of concern. ESBL/AmpC-producing *E. coli* is characterized by resistance to penicillins, second and third generation of

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Research Note

Prevalence and Antimicrobial Resistance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in Slaughter Pigs in Latvia

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ABSTRACT

The prevalence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* was determined in slaughter pigs from 47 farms in Latvia. Tonsils from 404 pigs representing farms from four geographical areas (Kurzeme, Latgale, Vidzeme, and Zemgale) were collected at slaughter in 2007 and 2008. The prevalences of *Y. enterocolitica* and *Y. pseudotuberculosis* were 35 and 3%, respectively. All *Y. enterocolitica* isolates belonged to bioserotype 4/O:3. *Y. enterocolitica* was recovered from 35 of 47 pig farms, and *Y. pseudotuberculosis* was found on 6 farms. The prevalence of *Yersinia* was highest in Latgale (90%) followed by Kurzeme (39%), Zemgale (33%), and Vidzeme (21%). *Y. enterocolitica* 4/O:3 was sensitive to amoxicillin–clavulanic acid, aztreonam, cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim, and trimethoprim-sulfamethoxazole and resistant to ampicillin, cephalothin, erythromycin, streptomycin, sulfamethoxazole, and tetracycline. *Y. pseudotuberculosis* exhibited resistance to erythromycin and sulfamethoxazole but not to the other antimicrobial agents tested. The results of this study are a valuable starting point for monitoring the prevalence and antimicrobial resistance of *Yersinia* in pigs in Latvia.

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are foodborne pathogens that cause human yersiniosis *(6, 23)*. Pork and edible offal have been found contaminated with enteropathogenic *Yersinia (10, 12)*, and *Y. enterocolitica* and *Y. pseudotuberculosis* have been isolated from pig tonsils. These data indicate that human pathogenic yersiniae have a strong link to pigs and that pigs may serve as an important source of these pathogens *(1, 7, 9, 16, 22)*. Because enteropathogenic *Yersinia* are present in swine herds *(5, 9, 11, 14)*, information regarding the prevalence of the pathogens is relevant to development of adequate measures to minimize or control the pathogens at the farm level. Data collection on the prevalence of enteropathogenic *Yersinia* is not mandatory, and information about the prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in Baltic countries, including Latvia, is limited to a report by Martínez et al. *(17)*.

A baseline study to determine the prevalence of enteropathogenic *Yersinia* in the food chain, including pig farms, was initiated in Latvia. Antimicrobial resistance testing was included in this study because the emergence of resistance in strains of certain foodborne pathogens has been reported *(26)*. The aim of the present study was to determine the prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in pigs at slaughter in Latvia and to determine the antimicrobial susceptibility of these isolates.

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MATERIALS AND METHODS

Sampling. A total of 404 pig tonsils were collected from five slaughterhouses in Latvia between June 2007 and April 2008. Samples originated from pigs from four major Latvian geographical areas: Kurzeme ($n = 142$), Latgale ($n = 50$), Vidzeme ($n = 128$), and Zemgale ($n = 84$). Slaughterhouses were located in Zemgale (slaughterhouses A and B), Vidzeme (slaughterhouse C), and Kurzeme (slaughterhouses D and F). Altogether, 47 pig farms were sampled, and 2 to 10 samples were collected from each farm.

Tonsils were detached from the mass of removed organs after evisceration in slaughterhouses A, B, and D and from the pig head in slaughterhouses C and F. All samples were collected from the first batch of slaughtered pigs to exclude the possibility of cross-contamination from pigs from other farms.

Individual tonsils were placed in sterile sampling bags and transported to the laboratory on ice within 2 h of collection. Samples were tested immediately after arrival at the laboratory or were stored at −20°C when testing was not initiated in the first 12 h. Ten grams of tonsil was added to 90 ml of peptone mannitol bile salt broth (PMB), homogenized, and used for bacteriological testing.

Bacteriological testing. *Yersinia* was cultured by direct plating, selective enrichment, and cold enrichment. For direct plating, 100 μl of tonsil homogenate was plated on a cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid, Basingstoke, UK) plate after 1 h of resuscitation. For selective enrichment, 9 ml of irgasan–ticarcillin–potassium chlorate (ITC) broth (Fluka, Buchs, Switzerland) was inoculated with 1 ml of homogenate and incubated at 25°C for 48 h. A 100-μl sample of enriched ITC broth suspension was plated onto CIN agar and incubated at 30°C for 24 to 48 h. For

PĒTNIECĪBAS IZAICINĀJUMI ATTIECĪBĀ UZ AMR PĀRTIKAS ĶĒDĒ UN VIDĒ

- ✓ Dažādu antibiotiku un rezistento baktēriju avoti apkārtējā vidē
- ✓ Vides loma un antropogēno faktoru loma AMR attīstībā
- ✓ Apkārtējās vides rezistento baktēriju ietekme uz cilvēku un dzīvnieku veselību
- ✓ Dažādu tehnoloģisko, sociālo, ekonomisko pasākumu ieviešana AMR mazināšanā/ ierobežošanā (nacionālie AMR ierobežošanas rīcības plāni?!)

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A One Health approach to antimicrobial resistance surveillance: is there a business case for it?

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Antimicrobial resistance
AMR
Surveillance
One Health
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Economics

ABSTRACT

Antimicrobial resistanc
Health approach. Resist
driver of this resistance
presented to include all
are described, highlight
nomic and scientific evi
of integrated surveillan
interventions and outco
A One Health assessmen
impacts on animal heal
sumer confidence in saf
to realise and are deper
ble, such as improved s
identification, should als
ecosystem health and re
social capital-building,
© 2

1. Introduction and background

In 2001, the World Health Organization (WHO) described anti
microbial resistance (AMR)[1] as a global problem requiring a globa
response [1], but until recently it has failed to gain the urgent at
tention it deserves. Economic evidence is used as a tool to prioritis
policy decisions and it has been argued that previous estimates o
the health cost of AMR have been too negligible [2] and they hav
failed to consider the wider impacts on health care [3]. However
recently more alarming estimates of AMR have appeared in the lit
erature. The European Commission claimed that costs attributed t
resistant bacterial infections amounted to €1.5 billion annually [4
Healthcare systems in the USA estimate the additional cost o

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[1] Although by definition AMR refers to resistance of all microbes including bacteria, viruses, parasites and fungi, this paper and the majority of the literature use the term with a bias towards bacterial resistance.

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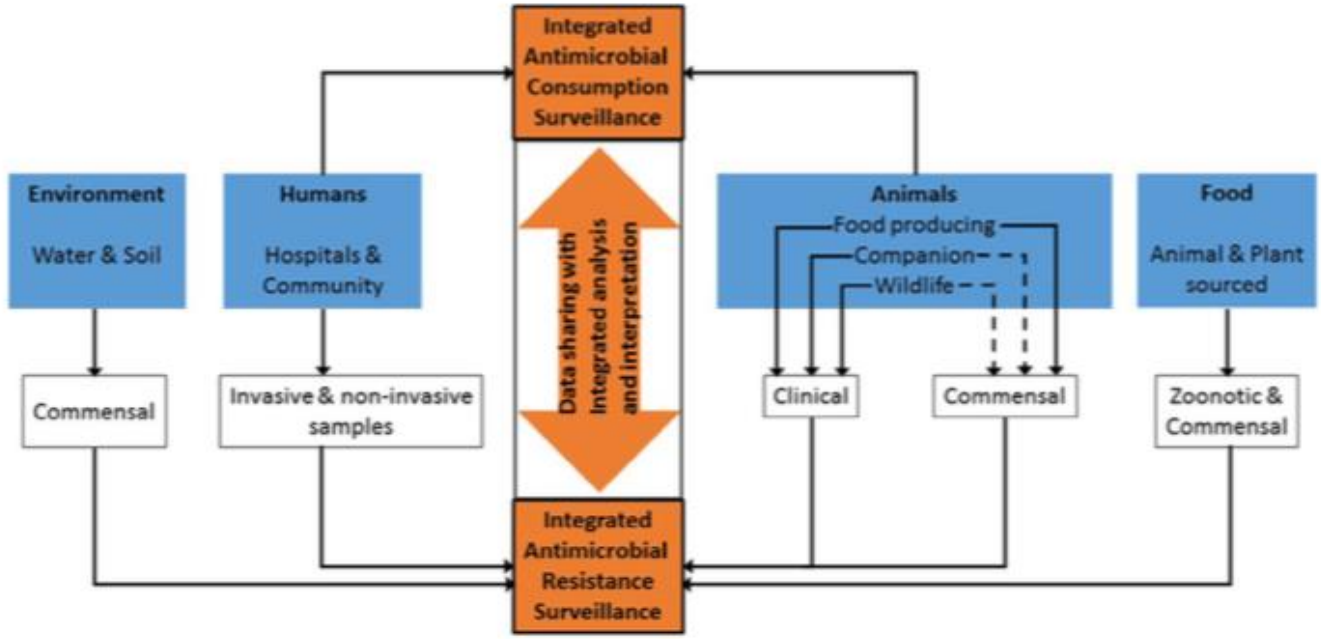

Fig. 1. An interconnected and integrated One Health surveillance framework that puts at its centre antimicrobial resistance and antimicrobial consumption

fulfilment) with improved international collaboration and capacity [11]. One Health, however, also refers to a broad, systems-based approach to complex problems [12]. It is therefore suited to AMR surveillance because it considers the underlying structural factors

BIOR

PĀRTIKAS DROŠĪBAS, DZĪVNIEKU VESELĪBAS
UN VIDES ZINĀTNISKAIS INSTITŪTS

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