Microbial targets and indicators (resistance markers), and some other topics

Prof. Jaap Wagenaar DVM, PhD, Dipl ECVM

Division of Infectious Diseases and Immunology,

Faculty of Veterinary Medicine, Utrecht University, Utrecht - NL

Wageningen Bioveterinary Research, Lelystad - NL

j.wagenaar@uu.nl

Universiteit Utrecht

World Organisation for Animal Health
Founded as OIE

World Health Organization

WAGENINGEN UNIVERSITY & RESEARCH

Outline

- Priority microbial targets and indicators
- When sequencing?
- Examples of (Integrated) Surveillance-based interventions
    - colistin - Indonesia,
    - ESBL in the Netherlands

- Colistin (Indonesia)
- ESBL-producing *E. coli (the Netherlands)*

What

Table 6. Microbial targets for One Health integrated surveillance by evidence for transmission of AMR among sectors

Sectors and Interface (see Section 4.2/ Table 1 for the basis for prioritization)	**Interface of interest**	**Proposed populations for sampling**	**Specimen to be collected from sampled populations**	**Microbial Targets**	**ARGs targets**	**Key microorganism–drug combination**	**Antimicrobial agents to be monitored**
Sectors: human, animal, environment **(Tricycle)**	Human–animal–environment	Humans: People with bloodstream infections Healthy people, including pregnant women Food-producing animals: Chickens Environment: River sites: upstream and downstream Wastewater: community and wet markets	Humans in hospital: Blood cultures Health-care facilities: Stools or faecal swab Food-producing animals: chicken caeca Environment: rivers: surface water Wastewater: human and animal	*E. coli*	ESBL genes Optional: carbapenemase-encoding genes; other classes of resistance genes depending on country context (e.g. *gyrAB/parCE* mutations and *qnr* acquisition)	*E. coli* – third-generation cephalosporins; Optional: carbapenems, fluoroquinolones, others	Cefotaxime/ceftri-axone, ceftazidime Optional: meropenem, cipro-floxacin, enroflox-acin, tetracyclines

Universiteit Utrecht

World Organisation for Animal Health
Founded as OIE

World Health Organization

WAGENINGEN
UNIVERSITY & RESEARCH

Sectors and interface (see Section 4.2/ Table 1 for the basis for prioritization)	Interface of interest	Proposed populations for sampling	Specimen to be collected from sampled populations	Microbial Targets	ARGs targets	Key microorganism–drug combination	Antimicrobial agents to be monitored
Sectors: Humans and food animals **(CIPARS)**	Animal-derived products; direct contact with animals and/or their waste	Infected humans Healthy food-producing animals Animal-derived food products (e.g. fresh meat) obtained from retail stores	Humans: faeces/stool samples Healthy animals: faeces/ stool samples Faecal material from fresh faecal pats, boot swabs collected on a farm and/or caecum at a slaughterhouse. Fresh meat, milk, eggs, fish, vegetables or processed foods obtained at processing and/or point of sale	Invasive non-typhoidal *Salmonella spp.* (non-typhoidal), *Campylobacter* Methicillin-resistant *S. aureus* Commensal bacteria: *E. coli*, *Enterococcus faecium* and *E. faecalis*	Fluoroquinolone-resistant genes (*gyrA/E* and *parCE* mutations and *qnr* acquisition) Genes encoding β-lactamases and ESBL Carbapenemase-encoding genes *mecA* gene Other classes of resistance genes to be selected, depending on the country	*E. coli*, third- and fourth-generation cephalosporins, carbapenems, fluoroquinolones Non-typhoidal *Salmonella*– fluoroquinolones, third/fourth generation cephalosporins MRSA *Campylobacter*-macrolides, fluoroquinolones	Third- and fourth-generation cephalosporins Carbapenems Fluoroquinolones Macrolides Tetracyclines Methicillin and other β-lactams Trimethoprim sulfamethoxazole
Sectors: Food animals (aquatic) and food derived from animals **(WOAH Aquatic Animal Health Code)**	Animal-derived products	Aquatic animals Animal-derived food products obtained from retail stores	Animals: e.g, fish, crustaceans, molluscs Food: seafood products to be consumed raw or undercooked	Foodborne pathogens: *Salmonella* spp.; *Vibrio parahaemolyticus*; *Listeria monocytogenes*	Not stated	Not stated	Major antimicrobial classes used to treat disease in aquatic animals
Sectors: Food animals (terrestrial) and food derived from animals **(WOAH Terrestrial Animal Health Code)**	Animal-derived products at different steps of the food chain (slaughterhouse, packaging, retail) Animal-related environment Animal feed	Healthy terrestrial animals Animal feed Animal-derived foods (at different stages of the food chain: slaughterhouse; packaging facility; retail)	Animals: faeces, caeca, carcass Food product: e.g. meat, milk, eggs Animal feed Animal-related environment: e.g. slurry, manure	Foodborne bacteria: *Campylobacter* spp.; *Salmonella* spp. Commensal bacteria: *E. coli*; *Enterococcus* spp.	Not stated	Not stated	Colistin, fluoroquinolones, third and fourth-generation cephalosporins, phosphonic acid derivatives.

- When sequencing?

Communication, reporting

- JIACRA report (Zoltan)

Approved: 26 January 2024
DOI: 10.2903/j.efsa.2024.8589

SCIENTIFIC REPORT

Antimicrobial consumption and resistance in bacteria from humans and food-producing animals

Fourth joint inter-agency report on integrated analysis of antimicrobial agent consumption and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the EU/EEA

JIACRA IV – 2019–2021

European Centre for Disease Prevention and Control (ECDC) | European Food Safety Authority (EFSA) | European Medicines Agency (EMA)

Correspondence:
ECDC: arhai@ecdc.europa.eu
EFSA: zoonoses@efsa.europa.eu
EMA: https://www.ema.europa.eu/en/about-us/contacts/send-question-european-medicines-agency

Abstract
The fourth joint inter-agency report on integrated analysis of antimicrobial consumption (AMC) and the occurrence of antimicrobial resistance (AMR) in bacteria from humans and food-producing animals (JIACRA) addressed data obtained by the Agencies' EU-wide surveillance networks for 2019–2021. The analysis also sought to identify whether significant trends in AMR and AMC were concomitant over 2014–2021. AMC in both human and animal sectors, expressed in mg/kg of estimated biomass, was compared at country and European level. In 2021, the total AMC was assessed at 125.0 mg/kg of biomass for humans (28 EU/EEA countries, range 44.3–160.1) and 92.6 mg/kg of biomass for food-producing animals (29 EU/EEA countries, range 2.5–296.5). Between 2014 and 2021, total AMC in food-pro-

SIMPLIFIED SUMMARY

21 Feb 2024

Fourth joint inter-agency report on integrated analysis of antimicrobial consumption and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the European Union (JIACRA IV – 2019–2021)

Background

- Antimicrobial resistance (AMR) is a major global threat to human and animal health.
- The use and misuse of antimicrobials in humans and in food-producing animals are major drivers of AMR. Addressing AMR requires a coordinated effort from the human and animal sectors across the globe.
- Antimicrobial-resistant bacterial infections are a serious health problem in Europe, causing over 35,000 deaths annually. This is comparable to the combined impact of influenza, tuberculosis and HIV/AIDS. Recent data show that antimicrobial-resistant bacteria are causing a growing number of infections and deaths in humans, particularly in healthcare settings.
- In accordance with *the European One Health Action Plan against Antimicrobial Resistance*, the European Commission (EC) tasked the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA), to gather data on the link between antimicrobial consumption (AMC) and AMR in humans and food-producing animals.

World Health Organization

What if your data are (too) alarming or have a potential negative effect on trade?

Some more examples

Colistin

Highest prioritized Critically Important Antimicrobial – WHO-MIA-list

COINCIDE in Indonesia

Objective: To understand colistin resistance in humans and animals <u>from a policy, behavioral, epidemiological and molecular perspectives</u>

Pre-ban

Post-ban

2020

Colistin ban in animals
(Policy as an intervention)

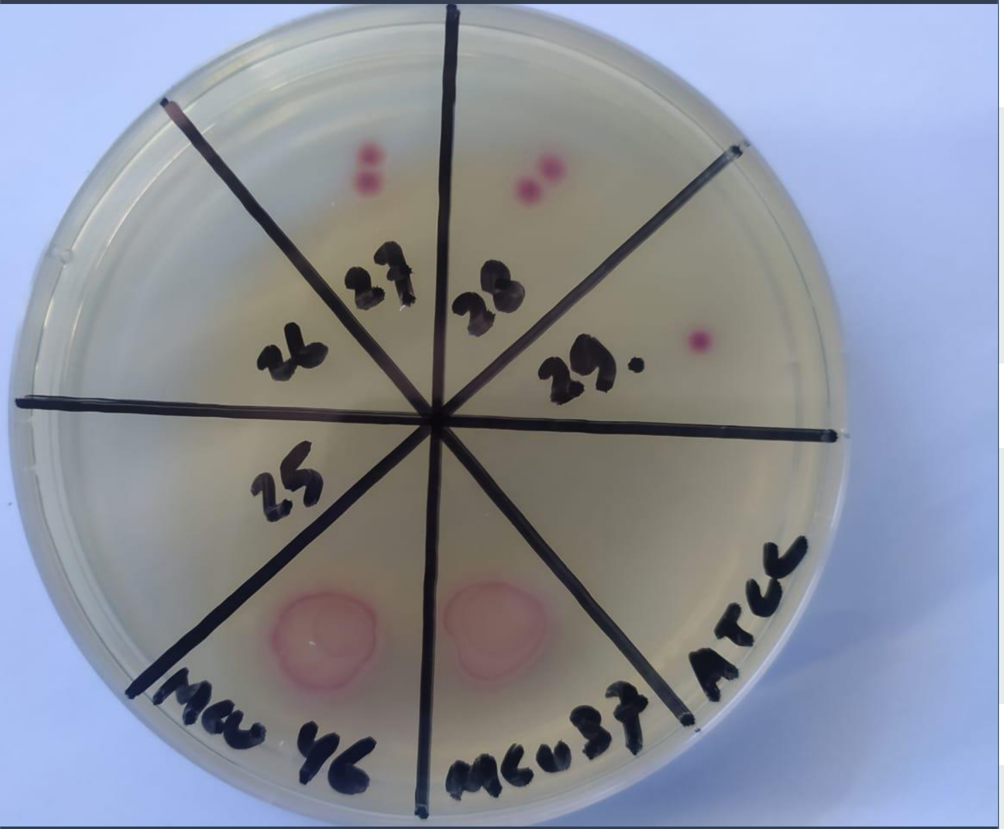
Escherichia coli
as an indicator organism

Research question

What is the impact of the colistin ban in livestock on colistin resistance in humans and poultry in Indonesia?

1. Colistin resistance in humans
2. Colistin resistance in broilers and layers
3. Transmission between chicken and humans

Microbiology
Phenotype vs genotype



Prevalence of phenotypic colistin-resistant *E. coli*

COINCIDE

Group	Test	Pre-ban		Post-ban	
1 Humans in community	CHROMagar	15/423	(3.5%)	13/671	(1.9%)
	MIC	3/423	(1.2%)	0/671	(0%)
	mcr	2/423	(0.8%)	0/671	(0%)
2 Layer chickens	CHROMagar	10/398	(2.5%)	29/900	(3.2%)
	MIC	1/398	(0.25%)	0/900	(0%)
	mcr	1/398	(0.25%)	0/900	(0%)
3 Layer farmers	CHROMagar	0/48	(0%)	5/127	(3.9%)
	MIC	0/48	(0%)	0/127	(0%)
	mcr	0/48	(0%)	0/127	(0%)

Legend: CHROMagar (blue), MIC (green), *mcr* (yellow)

Take-home messages

COINCIDE

COINCIDE: Impact of reducing colistin use on colistin resistance in humans and poultry in Indonesia

1. Phenotypic colistin resistance is low in Central Java

2. Next step is to investigate molecular epidemiology and validate phenotypic-genotypic association

3. COINCIDE expands the initial colistin ban in the animal production sector into **an integrative multi-sectoral One Health intervention**

Intermezzo: *In ovo* vaccination

Fig. 5.

In ovo vaccination machine: focus on the injection system. Courtesy of CEVA Animal Health.

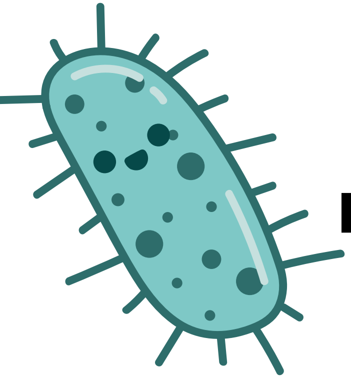
ceftiofur

gentamicin

injection itself (19,20,21). A common feature to keep in mind is that injecting through the egg chamber increases subsequent risks of bacterial and, even more, fungal contamination in case of poor air hygiene in the hatchery. *Aspergillus* contaminations were indeed commonly described at the beginning of implementation of *in ovo* vaccination in hatcheries (22). Therefore, a very strict control of air quality at the hatchery is a prerequisite to any installation of *in ovo* machines.

E-Trike

Extended Tricyle project Indonesia for extended-spectrum beta-lactamase positive *Klebsiella pneumoniae* and *Escherichia coli*



Fleming Fund Fellowship Collaborative Project

Patients with bloodstream infection

Cipto Mangunkusumo Hospital

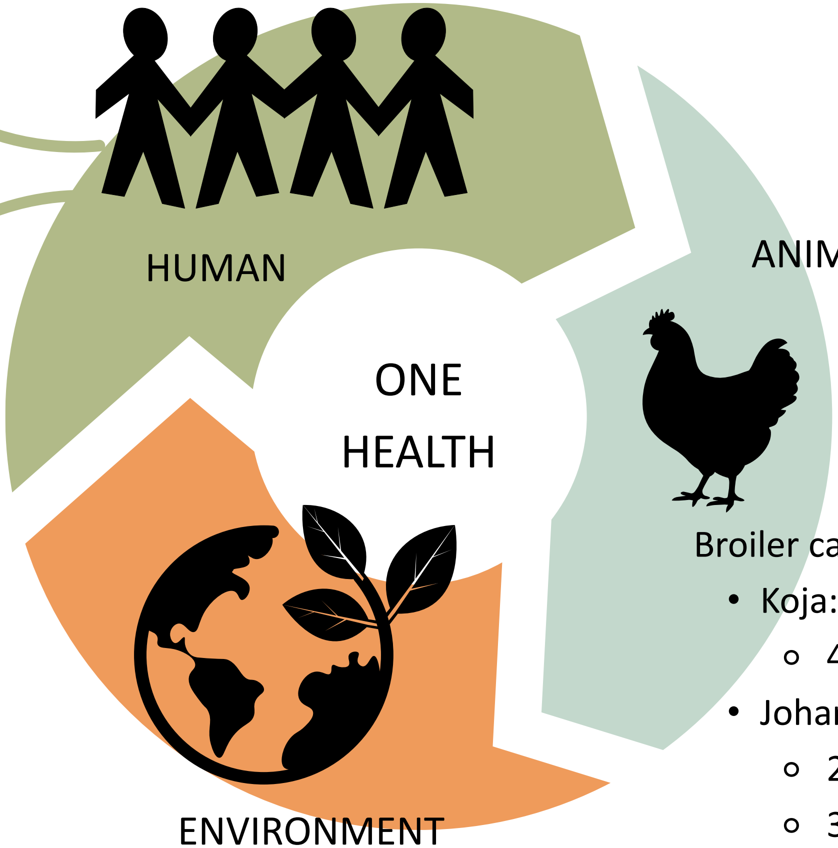
Persahabatan Hospital

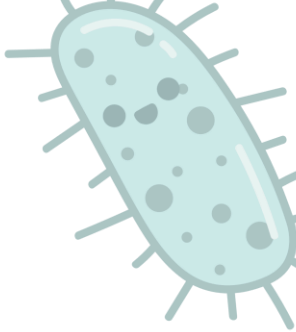
Pregnant women (rectal & throat swabs)

PHC Pulo Gadung

PHC Johar Baru

PHC Koja





Water samples from rivers:

- 2 upstream
- 2 near slaugtherhouse
- 1 hospital effluent
- 1 communal
- 2 downstream

Broiler caeca:

- Koja:
  - 4 wet markets
- Johar Baru:
  - 2 wet markets
  - 3 poultry collectors/shelters
- Pulo Gadung:
  - 1 wet market
  - 7 sites poultry shelters
  - 7 sites poultry slaughterhouses

Drivers for ESBL producing *E. coli*

3rd and 4th generation cephalosporines are Highest Prioritized Critically Important Antimicrobials (MIA-list of WHO)

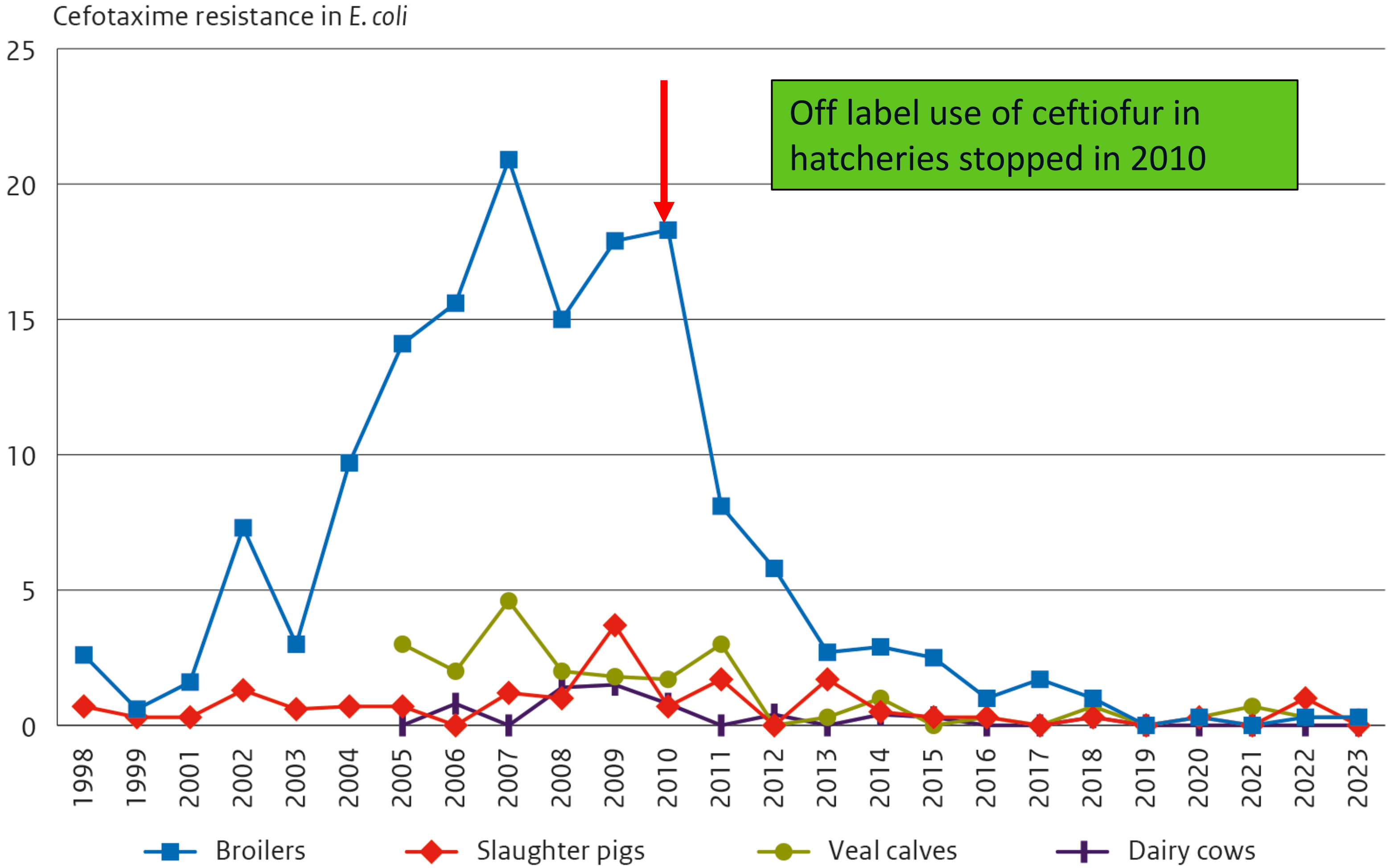
Risk factors: antimicrobial use in dairy and ESBL

- DD/DY based on data from vets in 2011
  - Total AMU
  - oral AMU in calves
  - Dry cow therapy
  - 1st, 2nd, 3rd choice antimicrobials **OR = 4.2**
  - 3rd, 4th generation cephalosporines **OR = 4.6**
  - all cephalosporines **OR = 2.8**
  - Other classes of antimicrobials
  - mastitis injectors
  - mastitis injectors with 3rd, 4th generatie cephalosporines **OR = 6.9**
  - mastitis injectors with penicillins
  - mastitis injectors with aminopenicillins

Thanks to prof. Theo Lam

abres Verantwoord medicijngebruik
GD
Universiteit Utrecht
World Organisation for Animal Health Founded as OIE
World Health Organization
WAGENINGEN UNIVERSITY & RESEARCH

Cefotaxime resistance of indicator *E. coli* (non-selective culturing)



Thanks to Kees Veldman, WBVR

Reduction of antimicrobial use in animal production: the Dutch approach

Disclaimer

Prof. Jaap Wagenaar DVM, PhD
David Speksnijder DVM, PhD

Department of Infectious Diseases and Immunology,
Faculty of Veterinary Medicine, Utrecht University, Utrecht - NL

Zoonoses and Public Health

ORIGINAL ARTICLE

Reduction of Veterinary Antimicrobial Use in the Netherlands. The Dutch Success Model

D. C. Speksnijder[1,2], D. J. Mevius[1,3], C. J. M. Bruschke[4] and J. A. Wagenaar[1,3]

Universiteit Utrecht

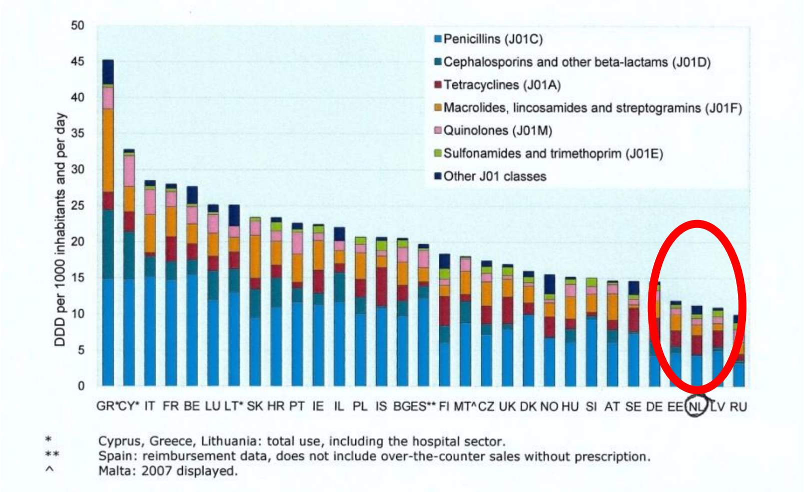
Background Dutch situation

- All antimicrobials are on prescription only
- Antimicrobial Growth Promotors are banned in the EU since 2006
- AMR/AMU in humans is for decades a well-controlled issue
- AMR/AMU in animals was forced to improve considerably around 2007
- AMR surveillance (since 1999) mandatory for EU-member states

- 2nd exporter of agricultural products in the world!

Triggers for reduction policy: publications in 2007

Low in humans



High in animals

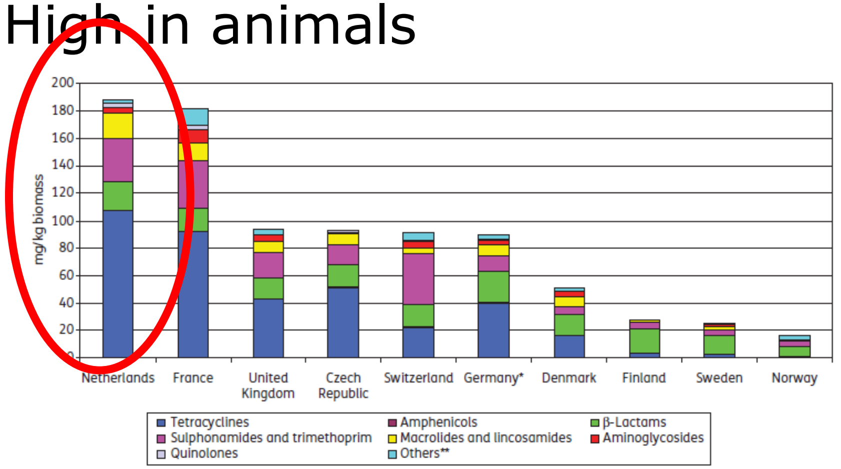


Figure 1. Amounts, in mg, of veterinary antibacterial agents sold in 2007 per kg biomass of pig meat, poultry meat and cattle meat produced plus estimated live weight of dairy cattle. *2005 data. **The substances included vary from country to country.

Strong appeal for a more responsible and restrictive application of antibiotics in animal production

Triggers for reduction policy in the Netherlands

- Publications about high AMU in animals and low AMU in humans
- Emergence of resistant bacteria in livestock and their risk for transmission to humans (e.g. Livestock Associated-MRSA, ESBL-producing *E. coli*)
- Growing concern about human health implications of livestock production in the Netherlands
- Primary motivation in public health, following the precautionary principle

Key elements of reduction policy in NL

- **Mandatory reduction targets**: -20% in 2011; -50% in 2013; -70% in 2015
- **Regulation and enforcement** on preventive use and on use of highest prioritized Critically Important Antimicrobials for Human Medicine (CIA-list) – 3rd/4th gen cephalosporines and fluoroquinolones
- **Transparency**: all antimicrobial use for each farm registered in mandatory central databases (surveillance of AMU)
- **Benchmarking and defining AMU-targets for sectors** by the independent Veterinary Medicines Institute (SDa)
- **Self-regulation** by well organised animal sectors
- **1—1 relationship** between farmer and veterinarian (farm health and farm treatment plan)

Universiteit Utrecht

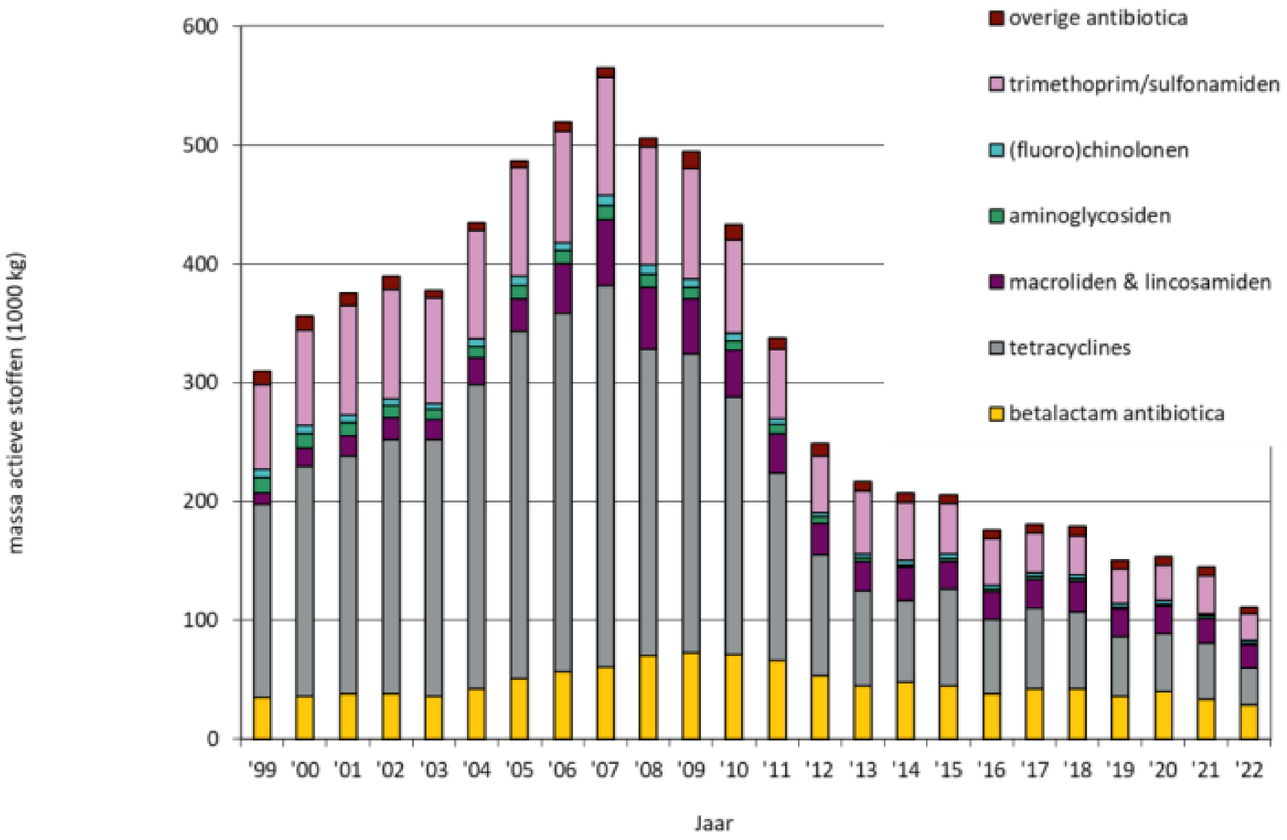
World Organisation for Animal Health
Founded as OIE

World Health Organization

SDa

WAGENINGEN UNIVERSITY & RESEARCH

Antimicrobial use in animals in the Netherlands (2009-2022)



Antimicrobial Veterinary Medical Product (AVMPs) sales (2009-2022) in kg 1000 (Source FIDIN)

- 77% reduction (2022 to reference year 2009)
- Fluoroquinolones and 3rd/4th-gen cefalosporines hardly used

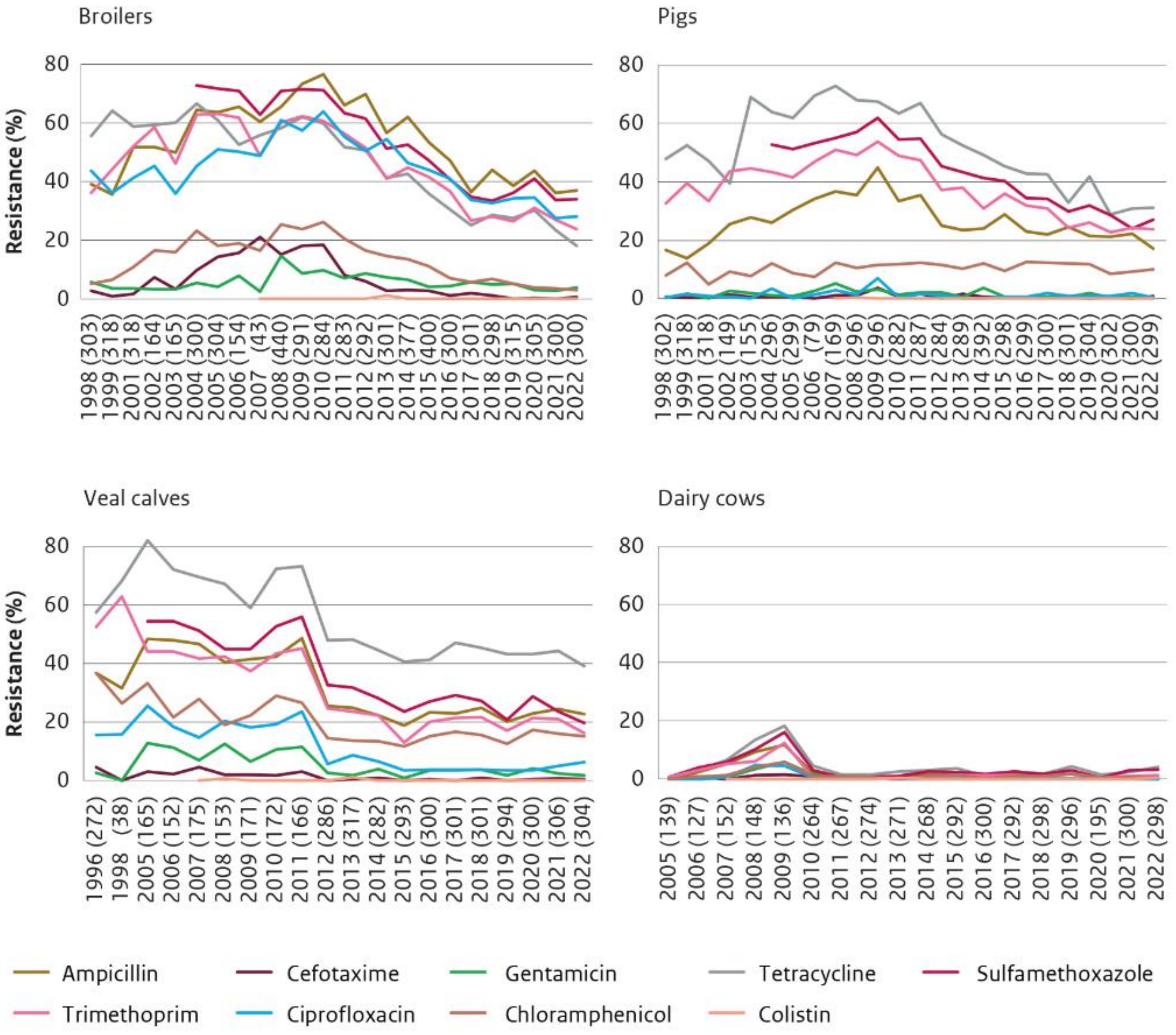
Universiteit Utrecht

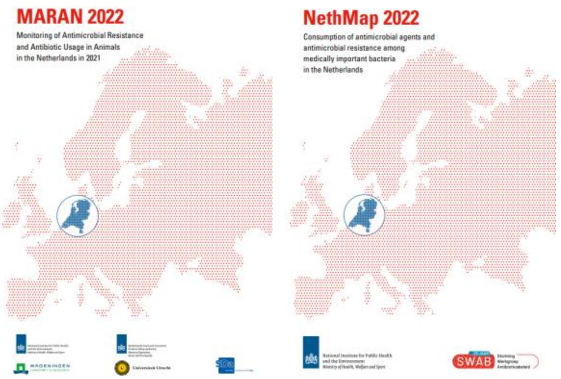
World Organisation for Animal Health
Founded as OIE

World Health Organization

WAGENINGEN UNIVERSITY & RESEARCH

Figure Eco01 Trends in proportion of resistance (%) of *E. coli* isolated from broilers, slaughter pigs, veal calves and dairy cattle in the Netherlands from 1998-2022





RESEARCH

Monitoring antimicrobial resistance trends in commensal *Escherichia coli* from livestock, the Netherlands, 1998 to 2016

Ayla Hesp[1,2], Kees Veldman[1], Jeanet van der Goot[1], Dik Mevius[1,2], Gerdien van Schaik[3,4]
1. Department of Bacteriology and Epidemiology, Wageningen Bioveterinary Research, Lelystad, the Netherlands
2. Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
3. GD Animal Health, Deventer, the Netherlands
4. Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
Correspondence: Ayla Hesp (ayla.hesp@wur.nl)

Universiteit Utrecht

World Organisation for Animal Health
Founded as OIE

World Health Organization

Association between AMU and AMR: ESTABLISH-project

Does the reduction in usage of antibiotics in animals have a positive effect on the level of resistance in human pathogens?

Based on AMU en AMR data from 2008 - 2019

Salmo: positive correlation AMU animal – AMR human BUT…
E. coli: no correlation AMU animal – AMR human
Campy: negative correlation AMU animal – AMR human

One Health 19 (2024) 100844

Contents lists available at ScienceDirect

One Health

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

ELSEVIER

Temporal association of antimicrobial use in livestock with antimicrobial resistance in non-typhoid *Salmonella* human infections in the Netherlands, 2008–2019

Linda E. Chanamé Pinedo[a,b,*], Anouk P. Meijs[a], Huifang Deng[b], Sabine C. de Greeff[a], Engeline van Duijkeren[a], Cindy M. Dierikx[a], Kees T. Veldman[c], Pim Sanders[d], Maaike J.C. van den Beld[a], Bart Wullings[e], Eelco Franz[a], Roan Pijnacker[a], Lapo Mughini-Gras[a,b]

Epidemiology and Infection

www.cambridge.org/hyg

Original Paper

Cite this article: Deng H, Chanamé Pinedo LE, Meijs AP, Sanders P, Veldman KT, Brouwer MSM, Wieke A-vK, Wullings B, ISIS-AR Study Group, van den Beld MJC, de Greeff SC, Dierikx CM, van Duijkeren E, Franz E, Mughini-Gras L and Pijnacker R (2024). Reducing antimicrobial use in livestock alone may be not sufficient to reduce antimicrobial resistance among human *Campylobacter* infections: an ecological study in the Netherlands. *Epidemiology and Infection*, **152**, e148, 1–8
https://doi.org/10.1017/S0950268824001511

Reducing antimicrobial use in livestock alone may be not sufficient to reduce antimicrobial resistance among human *Campylobacter* infections: an ecological study in the Netherlands

Huifang Deng[1], Linda E. Chanamé Pinedo[2], Anouk P. Meijs[2], Pim Sanders[3], Kees T. Veldman[4], Michael S. M. Brouwer[4], Altorf-vander Kuil Wieke[2], Bart Wullings[5], ISIS-AR Study Group, Maaike J. C. van den Beld[2], Sabine C. de Greeff[2], Cindy M. Dierikx[2], Engeline van Duijkeren[2], Eelco Franz[2], Lapo Mughini-Gras[1,2] and Roan Pijnacker[2]

[1]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands; [2]Centre for Infectious Disease

J Antimicrob Chemother. 2024 Oct 1;79(10):2622-2632. doi: 10.1093/jac/dkae268.

Association between antimicrobial usage in livestock and antimicrobial resistance in Escherichia coli isolates from human urinary tract infections in the Netherlands, 2009-2020

Anouk P Meijs[1], Linda E Chanamé-Pinedo[1 2], Huifang Deng[2], Kees T Veldman[3], Michael S M Brouwer[3], Maaike J C van den Beld[1], Cindy M Dierikx[1], Pim Sanders[4], Bart Wullings[5], Sabine C de Greeff[1], Engeline van Duijkeren[1], Eelco Franz[1], Roan Pijnacker[1], Lapo Mughini-Gras[1 2]; ISIS-AR study group

WAGENINGEN UNIVERSITY & RESEARCH

echt

World Organisation for Animal Health
Founded as OIE

World Health Organization

Thanks to Kees Veldman, WBVR

Trends in Campylobacter-AMR from broilers, chicken meat and human patients

Figure C01 Trends in resistance (%) of *Campylobacter jejuni* isolated from broilers and chicken meat in the Netherlands

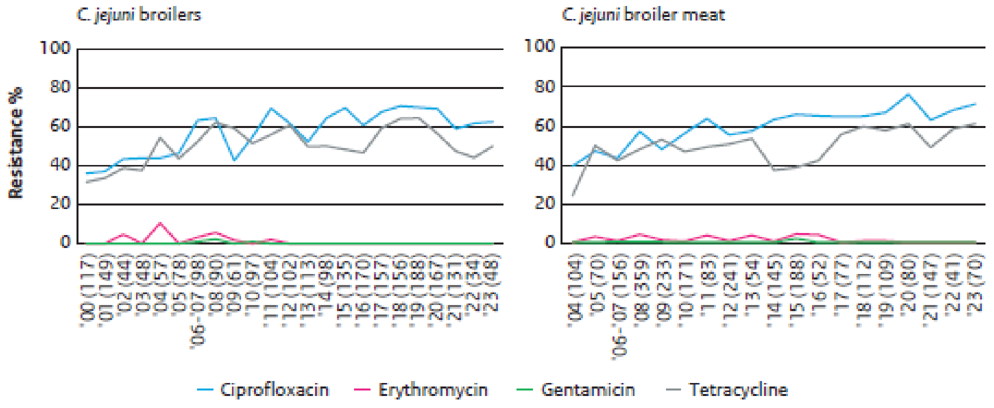


Figure C02 Trends in resistance of *Campylobacter coli* isolated from broilers and chicken meat in the Netherlands

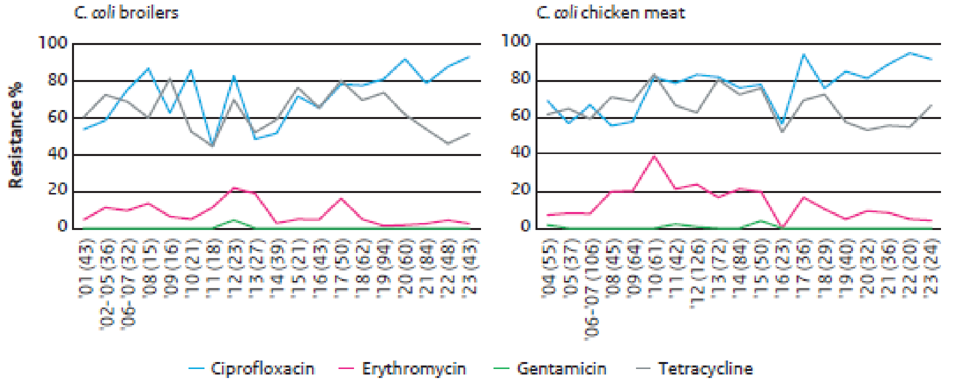
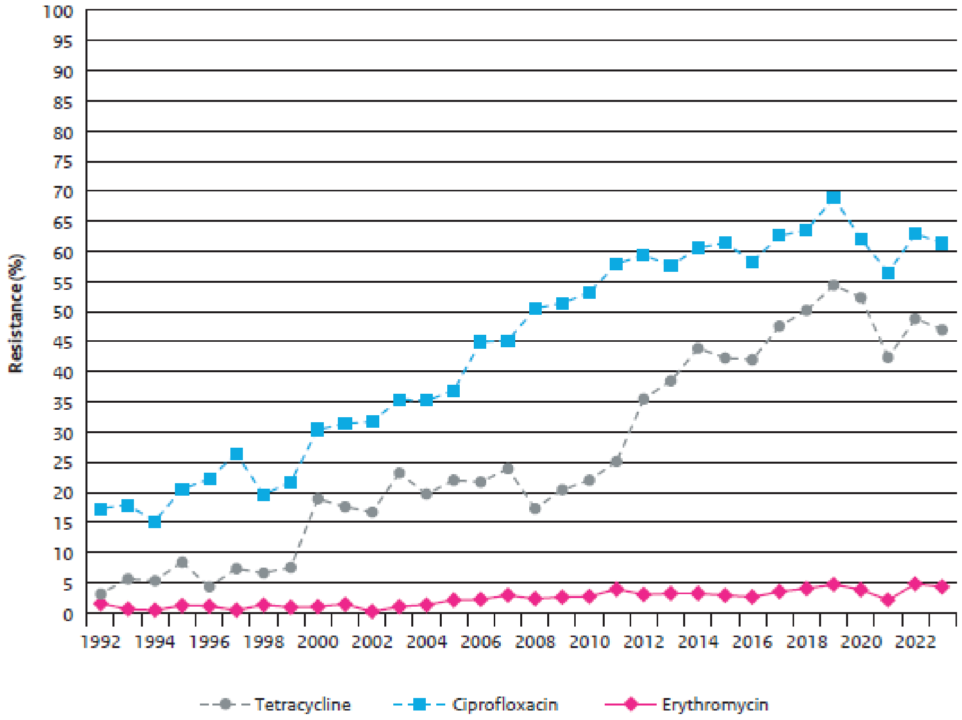


Figure C03 Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2023



World Health Organization

Thanks to Kees Veldman, WBVR

UNIVERSITY & RESEARCH

Effect on LA-MRSA in pigs?

- 99% of pigs still positive at slaughter…..

Conclusions

- When bacterial species differ between sectors, genes are an alternative for Integrated Surveillance

- Be transparent in communication!

Universiteit Utrecht

World Organisation for Animal Health
Founded as OIE

World Health Organization

WAGENINGEN
UNIVERSITY & RESEARCH