Multidrug resistant clusters in commensal E. coli from livestock

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Introduction & objectives

To combat the threat of antimicrobial resistance (AMR), policy makers need a quick overview of evolution of AMR in animal reservoirs to further develop and implement policy. For that reason, livestock is monitored by testing indicator organisms such as commensal indicator E. coli. To inform policy, the quantitative interpretation of such monitoring data (often vast and complex) should be improved. There is a need for outcome indicators in tested microorganisms that summarise AMR for multiple antimicrobial classes, and at the same time preferably quantify mutual dependencies of AMR to different antimicrobial classes in multidrug resistant isolates.

Quantifying multidrug resistance (MDR) is necessary to prioritise specific problems in animal reservoirs as public health threats. To develop outcome indicators of AMR monitoring, we performed a model-based cluster analysis on a dataset of minimum inhibitory concentrations (MIC) for 10 antimicrobials of commensal E. coli isolates (N=12,986) derived from four animal species (broilers, pigs, veal calves, and dairy cows) in Dutch AMR monitoring, 2007-2018.

Results

1) Cluster composition: mean resistant proportion of isolates (N=12,986) for the 10 antimicrobials per cluster in the four clusters from this analysis

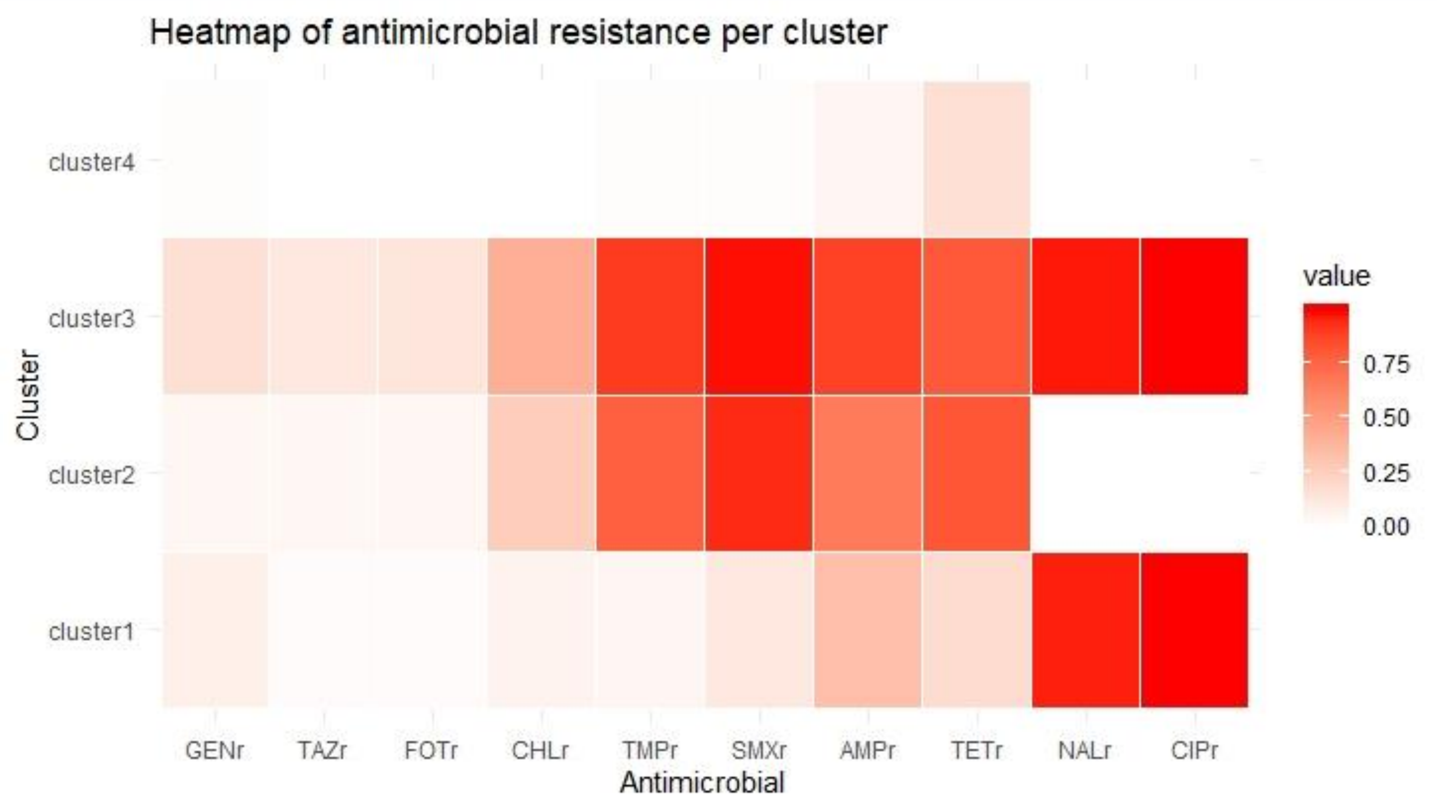

Table 1. Results of model-based clustering, showing the mean resistant proportion per cluster in commensal E. coli isolates (N=12,986) of broilers, dairy cows, slaughter pigs and veal calves from the Netherlands, 2007-2018

	GENr[a]	TAZr	FOTr	CHLr	TMPr	SMXr	AMPr	TETr	NALr	CIPr
Cluster 1	0.08[b]	0.02	0.02	0.06	0.05	0.12	0.33	0.18	0.96	0.99
Cluster 2	0.04	0.04	0.04	0.26	0.77	0.94	0.66	0.81	0	0
Cluster 3	0.16	0.12	0.13	0.41	0.90	0.98	0.88	0.80	0.97	0.99
Cluster 4	0.01	0	0	0	0.01	0.01	0.05	0.16	0	0

[a]GEN = gentamicin, TAZ = ceftazidime, FOT = cefotaxime, CHL = chloramphenicol, TMP = trimethoprim, SMX = sulfamethoxazole, TET ... AMP = ampicillin, NAL = nalidixic acid, CIP = ciprofloxacin.
[b] Mean proportion of resistant isolates per cluster for each of the ten tested antimicrobials

2) Proportion of isolates in the four clusters (1-4) of multidrug resistance in commensal E. coli isolates (N=12,986) of Dutch broilers, dairy cows, slaughter pigs and veal calves, 2007-2018

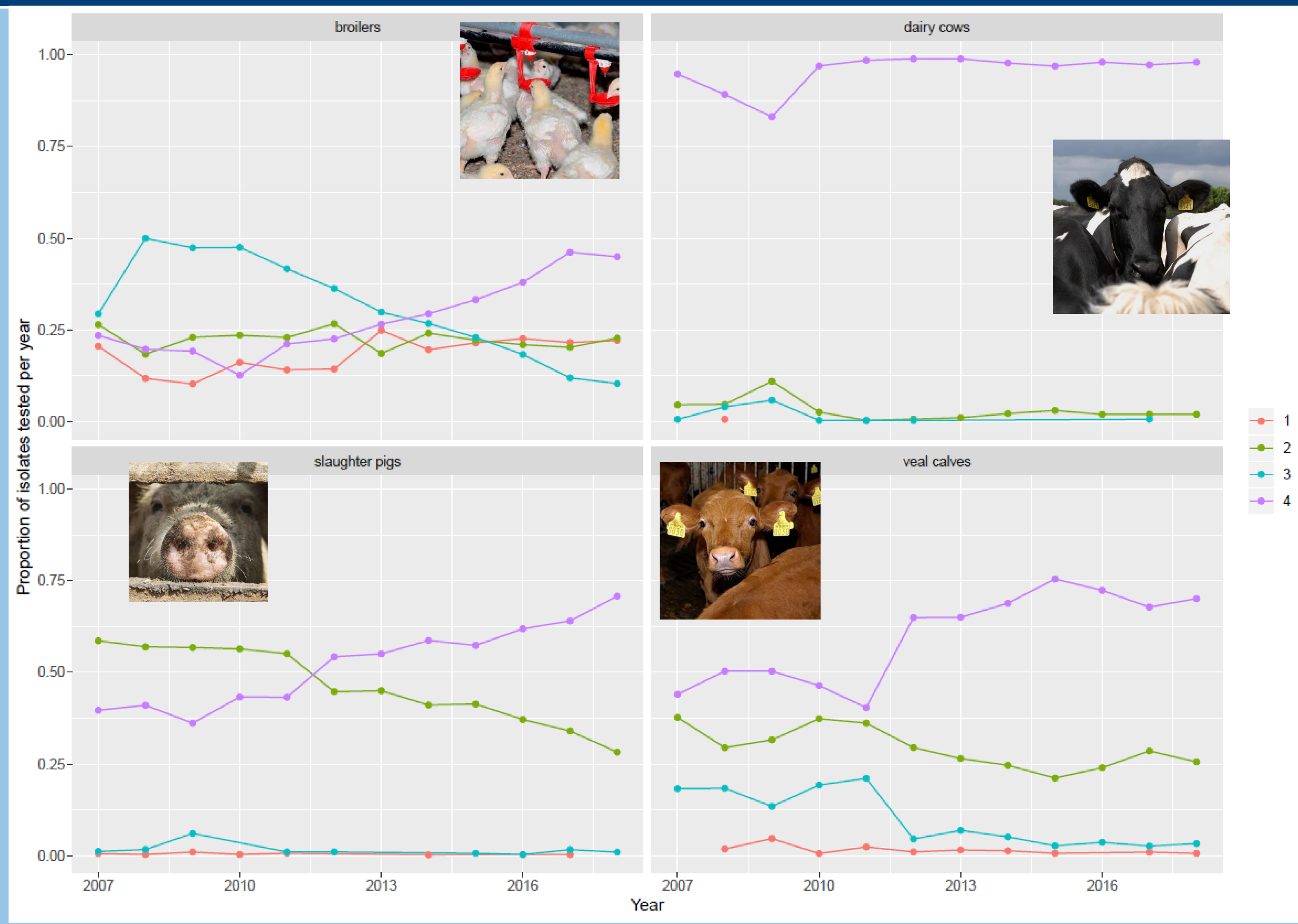

Table 2. Comparison of clusters with outcome indicators defined by EFSA (2)

Animal species	Indicator	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total[a]
Broilers (n=3,602)	Pan-S[b]	0.00	0.00	0.00	0.20	0.20
	>=3[c]	0.05	0.19	0.31	0.00	0.55
	CIP-R[d]	0.18	0.00	0.31	0.00	0.49
Dairy cows (n=2,958)	Pan-S	0.00	0.00	0.00	0.95	0.95
	>=3	0.00	0.02	0.01	0.00	0.02
	CIP-R	0.00	0.00	0.01	0.00	0.01
Slaughter pigs (n=3,491)	Pan-S	0.00	0.00	0.00	0.33	0.33
	>=3	0.00	0.36	0.01	0.00	0.38
	CIP-R	0.003	0.00	0.01	0.00	0.01
Veal calves (n=2,935)	Pan-S	0.00	0.00	0.00	0.46	0.46
	>=3	0.01	0.24	0.08	0.00	0.33
	CIP-R	0.01	0.00	0.08	0.00	0.09
Total (n=12,986)	Pan-S	0.00	0.00	0.00	1.00	0.46
	>=3	0.05	0.21	0.11	0.00	0.33
	CIP-R	0.05	0.00	0.11	0.00	0.16

[a] Total proportion of isolates from this animal species and from the total number of isolates, belonging to this outcome indicator; [b] Proportion of pan-susceptible isolates per animal species per cluster; [c] Proportion of isolates resistant to three or more classes per animal species per cluster; [d] Proportion of ciprofloxacin resistant isolates per animal species per cluster

Methods

The data used for this analysis were Minimum Inhibitory Concentrations (MIC) of 12,986 bacterial isolates, all being randomly isolated commensal indicator E. coli isolates from faecal or caecal samples of livestock as prescribed by EU-legislation: 3,602 from broiler chickens, 2,958 from dairy cows, 3,491 from slaughter pigs, and 2,935 from veal calves. All isolates were collected in the Dutch national monitoring program for AMR in livestock, from 2007 to 2018. Details of data collection and antimicrobial susceptibility testing in this monitoring program were described extensively by Hesp et al, 2019 (1).

MIC of 12,986 E. coli isolates were recoded to binary variables and model-based clustering using Bayesian statistics was applied. The output clusters are entirely data derived and not based on heuristic choices (seemingly arbitrary), as have to be made in hierarchical clustering. We used the Flexmix package in R for model-based clustering (stepFlexmix function with 1000 repetitions) to identify the most likely number of clusters in the data, based on integrated completed likelihood criterion (ICL).

Conclusions

- **Model-based clustering can be used to identify the clusters necessary to describe MDR**
- **More than one cluster/indicator is necessary to describe MDR in commensal E. coli in livestock**
- **Regarding mutual dependencies of resistance to more than one antimicrobial per isolate, 201 unique resistant combinations were found out of 1024 possible combinations. The presence of these combinations differ much per animal species. This determines the composition of the four clusters found with model-based clustering**
- **In the comparison between the clusters and EFSA outcome indicators we show potential use of these multidrug resistant clusters as monitoring outcome indicators**
- **However, this cluster analysis should first be tested on an international dataset with more variety to test if it is robust**

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References

1. Hesp A, Veldman K, van der Goot J, Mevius D, van Schaik G. Monitoring antimicrobial resistance trends in commensal Escherichia coli from livestock, the Netherlands, 1998 to 2016. Eurosurveillance. 2019;24(25):1800438.
2. ECDC, EFSA and EMA Joint Scientific Opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food-producing animals. EFSA Journal. 2017;15(10):05017.