AMR IN THE PORK CHAIN

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Monitoring of antimicrobial susceptibility of E. coli and Salmonella from pigs in the Netherlands, 2016-2018

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GD Animal Health (AH) monitors antimicrobial susceptibility (AMS) of pathogens from different animal species. Previously, AMS testing was performed by agar diffusion using tablets; in 2012 GD AH switched to broth-microdilution and minimal inhibitory concentrations (MICs) are being determined since.
The objective of the present study was to analyse the *in vitro* AMS of *E. coli* (ECO) and *Salmonella* isolates originating from clinical submissions and post-mortem examinations from pigs, between January 2016 and December 2018.

MICs of in total 18 antimicrobials were assessed , MIC_{50} and MIC_{90} values were determined (results shown for ECO) and MICs were interpreted as susceptible, intermediate and resistant using CLSI veterinary breakpoints (when available).
ECO isolates (n=905) showed relatively high levels of resistance to the (according to the Dutch Pig Formulary) 1st choice antimicrobials tetracycline and trimethoprim/sulfamethoxazole (≥54%) and the 2nd choice antimicrobials spectinomycin and ampicillin (indicator of amoxicillin) (≥42%). ECO were well susceptible to the 2nd choice antimicrobials apramycin, gentamicin, amoxicillin-clavulanic acid, flumequine, colistin (≤2% resistance) and neomycin (≤9% resistance). Also for the 3rd choice antimicrobial enrofloxacin resistance was very low (≤1%) (see Table 1 and Table 2 for more details).
Dilution series applied for each individual antibiotic are marked green and red; green refers to the 'susceptible' and red to the 'resistant' range (where applicable, 'resistant' includes both

Table 1: MIC distribution (%) for enteropathogenic ECO isolates (n=270) originating from pigs submitted for post-mortem examination at GD AH and faecal samples submitted to the laboratory of GD AH, 2018

Antibiotic	Enteropathogenic E. coli (n=270)											
	MIC-values (µg/mL)											
	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amoxicillin/Clavulanic acida	0.0	0.4	9.6	26.7	25.6	36.3	1.5	0.0	0.0			
Ampicillin	0.0	0.0	11.5	23.3	8.5	0.7	0.0	0.0	55.9			
Apramycin						95.9	3.0	1.1	0.0			
Cefepime			98.9	0.4	0.0	0.4	0.0	0.4	0.0			
Colistin		86.3	10.0	1.1	0.7	1.1	0.4	0.4				
Cefotaxime			99.3	0.0	0.0	0.7						
Enrofloxacin	96.3	3.3	0.4	0.0	0.0							
Florfenicol				4.1	48.1	35.2	12.6					
Flumequine				93.7	4.8	1.5	0.0	0.0				
Gentamicin				98.5	0.7	0.4	0.4					
Neomycin					93.3	0.0	0.4	6.3				
Sulfamethoxazole									14.1	1.9	0.7	83.3
Spectinomycin						0.4	1.9	35.6	20.0	8.5	33.7	
Streptomycin				27.4	9.3	4.1	6.3	8.1	13.3	31.5		
Tetracycline	0.0	1.5	36.3	7.8	0.0	0.4	0.7	53.3				
Tiamulin	0.0	0.0	0.0	0.0	0.0	0.4	0.4	3.0	96.3			
Tilmicosin	0.0	0.0	0.0	0.0	0.0	0.0	0.4	12.6	87.0			
Trimethoprim	0.0	38.5	1.1	0.4	0.0	0.0	0.0	60.0				
Trimethoprim-Sulfamethoxazoleb	39.3	0.0	0.7	0.4	0.0	59.6						
Tylosin	0.0	0.0	0.0	0.0	0.0	100.0						

'intermediate susceptible' and 'resistant'). To the right of the dilution ranges shown in green and red, percentages of isolates with a MIC value higher than the highest concentration of the dilution range are mentioned in red. The percentage of isolates mentioned at the lowest concentration of a dilution range, refers to isolates with a MIC value equal to or lower than the lowest concentration evaluated in the specific dilution range. In bold the antibiotics mentioned in the Dutch treatment Formulary for Pigs for enteropathogenic ECO infections are shown.

[a] *Only the concentration of amoxicillin, tested in a 2:1 ratio (amoxicillin : clavulanic acid), is mentioned;*

[b] *Only the concentration of trimethoprim, tested in a 1 :19 ratio (trimethoprim : sulfamethoxazole) is mentioned.*

Similar results were found for *Salmonella* Typhimurium (STY; n=47) and other group B *Salmonella* isolates (SGB; n=101): increased levels of resistance to trimethoprim/sulfamethoxazole (≥28% of STY, ≥13% of SGB isolates), high levels of resistance to tetracycline (≥46% of STY, ≥63% of SGB isolates) and high levels of resistance to the 2nd choice antimicrobial amoxicillin (ampicillin is tested) (≥54% of STY, ≥73% of SGB isolates). For the 2nd choice antimicrobials apramycin, flumequine, neomycin, amoxicillin-clavulanic acid the percentage of resistant isolates was low (0-3%). No STY or SGB isolates tested resistant to enrofloxacin.

Among ECO, STY and SGB from pigs, high levels of resistance to the 1st choice antimicrobials are found, whereas emergence of resistance to 2nd and 3rd choice antimicrobials appears to be (very) limited. Hence, also resistance against antimicrobials of high interest for human health (colistin) is (very) low. Interpretation of MICs for ECO and *Salmonella* is strongly hampered by the lack of CLSI-defined clinical veterinary breakpoints. More veterinary breakpoints are needed to overcome this problem and to conduct a clinically reliable monitoring of AMS.

Table 2: MIC50 and MIC90, and percentage susceptible, intermediate and resistant for enteropathogenic ECO isolates from post-mortem examination at GD AH and faecal samples submitted to the laboratory of GD AH, 2018, 2017 en 2016

	E. coli (n=270), 2018			**E. coli (n=339), 2017**			**E. coli (n=296), 2016**		
Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	R (%)	MIC50 (µg/mL)	MIC90 (µg/mL)	R (%)	MIC50 (µg/mL)	MIC90 (µg/mL)	R (%)
Amoxicillin/Clavulanic acida	4	8	0	4	8	0.3	4	8	0.0
Ampicillin	>32	>32	55.9	>32	>32	60.2	>32	>32	58.8
Apramycin	≤8	≤8	1.1	≤8	≤8	0.0	≤8	≤8	0.0
Cefepime	≤1	≤1	0.4	≤1	≤1	0.9	≤1	≤1	0.3
Colistin	≤0.5	1	1.9	≤0.5	≤0.5	1.5	≤0.5	≤0.5	2.4
Cefotaxime	≤1	≤1	0.7	≤1	≤1	0.9	≤1	≤1	0.7
Enrofloxacin	≤0.25	≤0.25	0	≤0.25	≤0.25	0.3	≤0.25	≤0.25	0.0
Florfenicol	4	>8	47.8	4	8	48.4	4	8	38.5
Flumequine	≤2	≤2	0	≤2	≤2	1.5	≤2	≤2	0.3
Gentamicin	≤2	≤2	0.4	≤2	≤2	0.0	≤2	≤2	0.0
Neomycin	≤4	≤4	6.3	≤4	≤4	8.6	≤4	≤4	6.8
Sulfamethoxazole	>256	>256	83.3	>256	>256	76.1	>256	>256	74.0
Spectinomycin	64	>128	42.2	64	>128	49.9	64	>128	42.9
Streptomycin	32	>64	53	32	>64	56.6	64	>64	57.6
Tetracycline	>16	>16	54.1	>16	>16	66.1	>16	>16	69.9
Tiamulin	>32	>32	99.3	>32	>32	99.7	>32	>32	98.3
Tilmicosin	>32	>32	99.6	>32	>32	99.1	>32	>32	98.6
Trimethoprim	>16	>16	60	>16	>16	65.2	>16	>16	64.2
Trimethoprim-Sulfamethoxazoleb	>4	>4	59.6	>4	>4	64.6	>4	>4	63.9
Tylosin	>4	>4	Rint	>4	>4	Rint	>4	>4	Rint