Susceptibility testing of veterinary pathogenic bacteria as a first step in setting new epidemiological cut-off values (ECOFFs)

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Setting of epidemiological cut-off values (ECOFFs) supports the process of defining animal specific clinical breakpoints for veterinary antimicrobials and improves harmonization in monitoring of resistance in animal pathogens. The aim of this project was to determine missing ECOFFs for antimicrobials for veterinary use by performing susceptibility testing of animal pathogens from strain collections of partner institutes using standardised antimicrobial panels.

METHODS

Nine partner institutes performed AST of veterinary pathogenic bacteria by broth microdilution according to international guidelines (ISO, CLSI) with three different antimicrobial panels using commercial plates (Sensititre©). The first antimicrobial panel (NLD1GPS) was designed with 12 different antimicrobials for testing Gram-positive bacteria, the second panel (NLD1GNS) with ten different antimicrobials was intended for testing Gram-negative bacteria and the third panel (NLD1MAC) with mainly macrolides and penicillins was intended for testing both Gram-positive and Gram-negative bacteria. All panels comprised long concentration ranges to determine minimum inhibitory concentrations (MICs).

Fig 1a Panel NLD1GNS

FFN 0.06	FFN 0.12	FFN 0.25	FFN 0.5	FFN 1	FFN 2	FFN 4	FFN 8	FFN 16	FFN 32	CEQ 0.008	XNL 0.008
ENRO 0.004	ENRO 0.008	ENRO 0.015	ENRO 0.03	ENRO 0.06	ENRO 0.12	ENRO 0.25	ENRO 0.5	ENRO 1	ENRO 2	CEQ 0.015	XNL 0.015
NEO 0.06	NEO 0.12	NEO 0.25	NEO 0.5	NEO 1	NEO 2	NEO 4	NEO 8	NEO 16	NEO 32	CEQ 0.03	XNL 0.03
KAN 0.06	KAN 0.12	KAN 0.25	KAN 0.5	KAN 1	KAN 2	KAN 4	KAN 8	KAN 16	KAN 32	CEQ 0.06	XNL 0.06
GEN 0.06	GEN 0.12	GEN 0.25	GEN 0.5	GEN 1	GEN 2	GEN 4	GEN 8	GEN 16	GEN 32	CEQ 0.12	XNL 0.12
DOX 0.06	DOX 0.12	DOX 0.25	DOX 0.5	DOX 1	DOX 2	DOX 4	DOX 8	DOX 16	DOX 32	CEQ 0.25	XNL 0.25
TET 0.06	TET 0.12	TET 0.25	TET 0.5	TET 1	TET 2	TET 4	TET 8	TET 16	TET 32	CEQ 0.5	XNL 0.5
SXT 0.015/0.3	SXT 0.03/0.6	SXT 0.06/1.2	SXT 0.12/2.4	SXT 0.25/4.75	SXT 0.5/9.5	SXT 1/19	SXT 2/38	SXT 4/76	SXT 8/152	POS	POS

FFN	Florfenicol
ENRO	Enrofloxacin
NEO	Neomycin
KAN	Kanamycin
GEN	Gentamicin
DOX	Doxycycline
TET	Tetracycline
SXT	Trimethoprim / sulfamethoxazole
CEQ	Cefquinome
POS	Positive Control
XNL	Ceftiofur

Fig 1b Panel NLD1GPS

CLI 0.004	ERY 0.015	DOX 0.008	DOX 2	GEN 0.5	PIRL 0.008	SXT 0.008/0.15	CLO 0.008	TET 0.06	ENRO 0.008	LIN 0.12	FFN 0.25
CLI 0.008	ERY 0.03	DOX 0.015	DOX 4	GEN 1	PIRL 0.015	SXT 0.015/0.3	CLO 0.015	TET 0.12	ENRO 0.015	LIN 0.25	FFN 0.5
CLI 0.015	ERY 0.06	DOX 0.03	GEN 0.008	GEN 2	PIRL 0.03	SXT 0.03/0.6	CLO 0.03	TET 0.25	ENRO 0.03	LIN 0.5	FFN 1
CLI 0.03	ERY 0.12	DOX 0.06	GEN 0.015	NEO 0.06	PIRL 0.06	SXT 0.06/1.2	CLO 0.06	TET 0.5	ENRO 0.06	LIN 1	FFN 2
CLI 0.06	ERY 0.25	DOX 0.12	GEN 0.03	NEO 0.12	PIRL 0.12	SXT 0.12/2.4	CLO 0.12	TET 1	ENRO 0.12	LIN 2	FFN 4
CLI 0.12	ERY 0.5	DOX 0.25	GEN 0.06	NEO 0.25	PIRL 0.25	SXT 0.25/4.8	CLO 0.25	TET 2	ENRO 0.25	LIN 4	FFN 8
CLI 0.25	ERY 1	DOX 0.5	GEN 0.12	NEO 0.5	PIRL 0.5	SXT 0.5/9.5	CLO 0.5	TET 4	ENRO 0.5	LIN 8	FFN 16
CLI 0.5	ERY 2	DOX 1	GEN 0.25	NEO 1	PIRL 1	SXT 1/19	CLO 1	TET 8	ENRO 1	POS	POS

CLI	Clindamycin
ERY	Erythromycin
DOX	Doxycycline
GEN	Gentamicin
NEO	Neomycin
PIRL	Pirlimycin
SXT	Trimethoprim / sulfamethoxazole
CLO	Cloxacillin
TET	Tetracycline
ENRO	Enrofloxacin
LIN	Lincomycin
POS	Positive Control
FFN	Florfenicol

Fig 1c Panel NLD1MAC

ERY 0.12	TYLT 0.25	TIL 0.25	TUL 0.12	GAM 0.06	TIP 0.25	TIA 0.25	PEN 0.008	AMOX 0.03	AMP 0.03	FLUQ 0.03	LEX 0.25
ERY 0.25	TYLT 0.5	TIL 0.5	TUL 0.25	GAM 0.12	TIP 0.5	TIA 0.5	PEN 0.015	AMOX 0.06	AMP 0.06	FLUQ 0.06	LEX 0.5
ERY 0.5	TYLT 1	TIL 1	TUL 0.5	GAM 0.25	TIP 1	TIA 1	PEN 0.03	AMOX 0.12	AMP 0.12	FLUQ 0.12	LEX 1
ERY 1	TYLT 2	TIL 2	TUL 1	GAM 0.5	TIP 2	TIA 2	PEN 0.06	AMOX 0.25	AMP 0.25	FLUQ 0.25	LEX 2
ERY 2	TYLT 4	TIL 4	TUL 2	GAM 1	TIP 4	TIA 4	PEN 0.12	AMOX 0.5	AMP 0.5	FLUQ 0.5	LEX 4
ERY 4	TYLT 8	TIL 8	TUL 4	GAM 2	TIP 8	TIA 8	PEN 0.25	AMOX 1	AMP 1	FLUQ 1	LEX 8
ERY 8	TYLT 16	TIL 16	TUL 8	GAM 4	TIP 16	TIA 16	PEN 0.5	AMOX 2	AMP 2	FLUQ 2	LEX 16
ERY 16	TYLT 32	TIL 32	TUL 16	GAM 8	TIP 32	TIA 32	PEN 1	AMOX 4	AMP 4	POS	POS

ERY	Erythromycin
TYLT	Tylosin tartrate
TIL	Tilmicosin
TUL	Tulathromycin
GAM	Gamithromycin
TIP	Tildipirosin
TIA	Tiamulin
PEN	Penicillin
AMOX	Amoxicillin
AMP	Ampicillin
FLUQ	Flumequine
POS	Positive Control
LEX	Cephalexin

Phenicols, Lincosamides, Aminoglycosides, Beta-lactams, Fluoroquinolones, Penicillins, Tetracyclines, Macrolides-Pleurmotulins, Sulfonamides

RESULTS

NLD1GNS	No isolates tested	No MIC-values	No distributions/ antibiotic	No distributions/ bacterial species
M. haemolytica	290	2900	7	70
P. multocida	308	3080	7	70
A. pleuropneumoniae	113	1130	4	40
P. aeruginosa	121	1210	4	40
K. pneumoniae	210	2100	3	30
B. bronchiseptica	80	800	1	10
H. parasuis	47	470	1	10
K. oxytoca	27	270	1	10
K. variicola	22	220	1	10
total	**1218**	**12180**	**29**	**290**

NLD1GPS	No isolates tested	No MIC-values	No distributions/ antibiotic	No distributions/ bacterial species
S. pseudintermedius	504	6048	10	120
S. hyicus	166	1992	6	72
S. dysgalactiae	174	2088	6	72
S. suis	250	3000	6	72
S. uberis	159	1908	6	72
S. agalactiae	135	1620	5	60
S. canis	60	720	2	24
S. equisimilis	57	684	2	24
S. equi subsp. zooepidemicus	50	600	2	24
S. equi subsp. equi	58	696	2	24
total	**1613**	**19356**	**47**	**564**

NLD1MAC	No isolates tested	No MIC-values	No distributions/ antibiotic	No distributions/ bacterial species
S. pseudintermedius	455	5460	10	120
M. haemolytica	244	2928	7	84
P. multocida	258	3096	7	84
S. hyicus	105	1260	4	48
A. pleuropneumoniae	57	684	3	36
B. bronchiseptica	80	960	2	24
H. parasuis	47	564	2	24
S. suis	64	768	2	24
P. aeruginosa	32	384	1	12
total	**1342**	**16104**	**38**	**456**

- number of distributions: ≥ 5 (sufficient to set ECOFFs)
- number of distributions: 3 or 4
- number of distributions: ≤ 2

Joined susceptibility testing of a collection of animal bacterial pathogens generated a sufficient number of MIC-distributions for setting ECOFFs of several bacteria-antimicrobial agent combinations.

REFERENCES

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ACKNOWLEDGEMENTS

This project was co funded by the Dutch Ministry of Agriculture, Nature and Food Quality.

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This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.