IMPART: an update of the project

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IMPART (IMproving **P**henotypic **A**ntimicrobial **R**esistance **T**esting**)** includes four topics related to the harmonization of phenotypic methods for detection of antimicrobial resistance: selective isolation and detection of **colistin-resistant *Enterobacteriaceae* (WP1),** selective isolation and detection of **carbapenemase-producing *Enterobacteriaceae* (WP2), setting ECOFFs** for specific pathogen/antibiotic combinations **(WP3**) and development of a standardized **disk diffusion method** for susceptibility testing of ***Clostridium difficile* (WP4).**

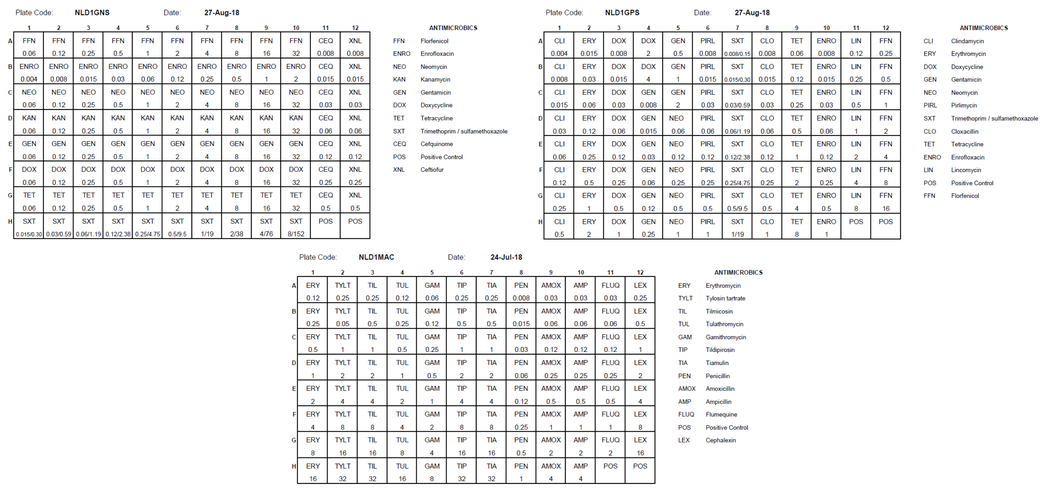


Plate Code: **NLD1GNS** Date: **27-Aug-18**

	1	2	3	4	5	6	7	8	9	10	11	12
A	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.008	0.008
B	ENRO	ENRO	ENRO	ENRO	ENRO	ENRO	ENRO	ENRO	ENRO	ENRO	CEQ	XNL
	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	0.015	0.015
C	NEO	NEO	NEO	NEO	NEO	NEO	NEO	NEO	NEO	NEO	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.03	0.03
D	KAN	KAN	KAN	KAN	KAN	KAN	KAN	KAN	KAN	KAN	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.06	0.06
E	GEN	GEN	GEN	GEN	GEN	GEN	GEN	GEN	GEN	GEN	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.12	0.12
F	DOX	DOX	DOX	DOX	DOX	DOX	DOX	DOX	DOX	DOX	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.25	0.25
G	TET	TET	TET	TET	TET	TET	TET	TET	TET	TET	CEQ	XNL
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	0.5	0.5
H	SXT	SXT	SXT	SXT	SXT	SXT	SXT	SXT	SXT	SXT	POS	POS
	0.015/0.30	0.03/0.59	0.06/1.19	0.12/2.38	0.25/4.75	0.5/9.5	1/19	2/38	4/76	8/152		

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

Plate Code: **NLD1GPS** Date: **27-Aug-18**

	1	2	3	4	5	6	7	8	9	10	11	12
A	CLI	ERY	DOX	DOX	GEN	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.004	0.015	0.008	2	0.5	0.008	0.03/0.15	0.008	0.06	0.008	0.12	0.25
B	CLI	ERY	DOX	DOX	GEN	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.008	0.03	0.015	4	1	0.015	0.015/0.30	0.015	0.12	0.015	0.25	0.5
C	CLI	ERY	DOX	GEN	GEN	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.015	0.06	0.03	0.008	2	0.03	0.03/0.59	0.03	0.25	0.03	0.5	1
D	CLI	ERY	DOX	GEN	NEO	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.03	0.12	0.06	0.015	0.06	0.06	0.06/1.19	0.06	0.5	0.06	1	2
E	CLI	ERY	DOX	GEN	NEO	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.06	0.25	0.12	0.03	0.12	0.12	0.12/.38	0.12	1	0.12	2	4
F	CLI	ERY	DOX	GEN	NEO	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.12	0.5	0.25	0.06	0.25	0.25	0.25/4.75	0.25	2	0.25	4	8
G	CLI	ERY	DOX	GEN	NEO	PIRL	SXT	CLO	TET	ENRO	LIN	FFN
	0.25	1	0.5	0.12	0.5	0.5	0.5/9.5	0.5	4	0.5	8	16
H	CLI	ERY	DOX	GEN	NEO	PIRL	SXT	CLO	TET	ENRO	POS	POS
	0.5	2	1	0.25	1	1	1/19	1	8	1		

ANTIMICROBICS
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

Plate Code: **NLD1MAC** Date: **24-Jul-18**

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	0.12	0.25	0.25	0.12	0.06	0.25	0.25	0.008	0.03	0.03	0.03	0.25
B	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	0.25	0.05	0.5	0.25	0.12	0.5	0.5	0.015	0.06	0.06	0.06	0.5
C	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	0.5	1	1	0.5	0.25	1	1	0.03	0.12	0.12	0.12	1
D	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	1	2	2	1	0.5	2	2	0.06	0.25	0.25	0.25	2
E	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	2	4	4	2	1	4	4	0.12	0.5	0.5	0.5	4
F	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	4	8	8	4	2	8	8	0.25	1	1	1	8
G	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	FLUQ	LEX
	8	16	16	8	4	16	16	0.5	2	2	2	16
H	ERY	TYLT	TIL	TUL	GAM	TIP	TIA	PEN	AMOX	AMP	POS	POS
	16	32	32	16	8	32	32	1	4	4		

ANTIMICROBICS
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

Fig 3 Antimicrobial panels for WP3: NLDGNS for testing Gram-negative bacteria, NLDGPS for testing Gram-positive bacteria and NLD1MAC for testing both Gram-positive and Gram-negative bacteria.

METHODS

To determine the optimal selective culturing method for isolation of colistin-resistant (WP1) and carbapenemase-producing *Enterobacteriaceae* (WP2), two pre-ring trials were organised in 2018. A small number of participants were involved (Anses, NVI, RIVM and WBVR) to test several conditions and most selective media available on the European market as of September 2018 (Fig 1 and Fig 2).

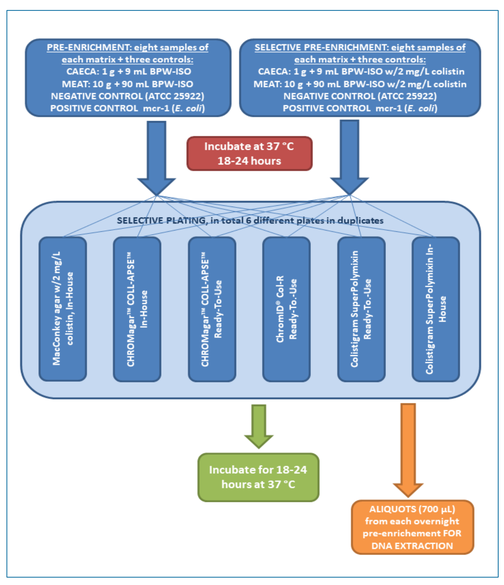


Fig 1 FLOW CHART WP1: colistin-resistant Enterobacteriaceae

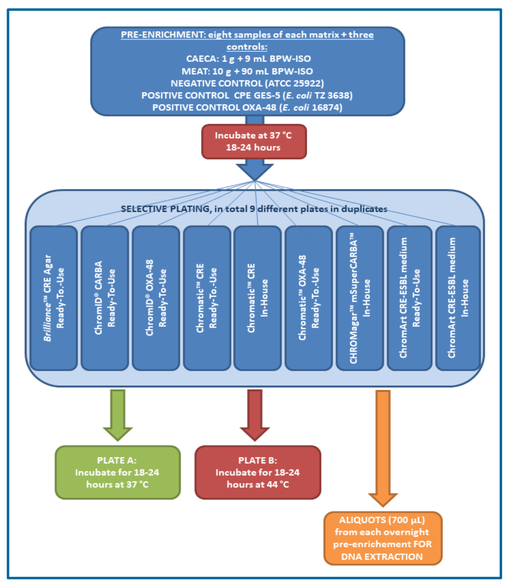


Fig 2 FLOW CHART WP2: carbapenemase-producing Enterobacteriaceae

Within WP3, susceptibility testing of veterinary bacteria will be performed with broth microdilution using panels as described in Fig 3. MIC data will be shared and epidemiological cut-off values (ECOFFs) will be assigned according to EUCAST methods.

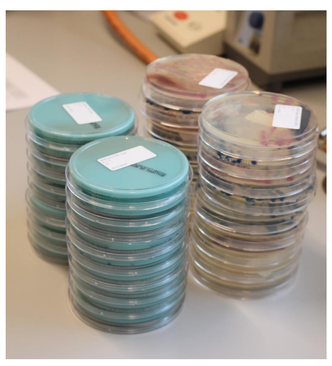
In WP4, *C. difficile* strains were first collected and characterized. A well-characterised, multi-variant *C. difficile* strain collection was then used to test an optimised disk diffusion protocol, analysing different media and conditions.

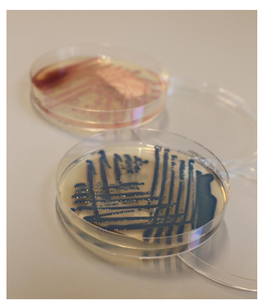
RESULTS

The robustness of the sample preparation protocol was confirmed for ring trials in WP1 and WP2: artificially contaminated samples were stable and homogenous. The three participants were able to recover the expected bacteria, but the different selective media performed unevenly. Nonetheless, the WP1 protocol still requires amendments to eliminate false positive colonies.

For WP3, MIC testing just started to a delay in the delivery of the microtiter plates (Sensititre). We expect to produce > 15.000 MIC-values and establish a significant number of new ECOFFs.

Within WP4, the collection and characterisation of *C. difficile* isolates was completed and an optimized disk diffusion test protocol was developed.





CONCLUSION

The pre-ring trials of WP1 and WP2 delivered essential information to finalize the protocols for the final ring trial to be held in 2019 for all participants.

In WP3, new ECOFFs will be established of antimicrobials for veterinary use.

In WP4, a ring trial will elucidate the interlaboratory reproducibility of the optimized method.

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