bla gene identification by microarrays in phenotypically resistant *Escherichia coli* from young calves in Wallonia, Belgium

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The most frequent resistance mechanism to β-lactam antibiotics is the production of β-lactamase enzymes (BLA) that inactivate the antibiotics. Their actual classification is highly complex [1,2], but 4 groups can be summarised: classical BLA (C), extended-spectrum BLA (ESBL), cephalosporinases (AmpC) and carbapenemases (CPE). According to the results of the disk diffusion assay (DDA) performed at ARSIA, the Regional Veterinary Diagnostics laboratory in Wallonia, Belgium, with 8 β-lactams a fifth class was defined: C associated with a cefoxitin resistance (C_FOX) (Table I).

The aim of this study was to identify the BLA-encoding (*bla*) genes present in *Escherichia* (*E.*) *coli* isolated from young calves with diarrhoea or septicaemia between November 2017 and February 2018 showing different β-lactam resistance phenotypes at the DDA. Of the total of 607 isolates, 94 with different resistance profiles (Table II) were chosen to be tested with the Check-MDR CT103XL® (Check-points BV, Netherlands) microarray [3].

Table I – Resistance phenotypes observed at the disk diffusion assay for the 8 tested β-lactams

Antibiotics	AMX	AMC	XNL	CFQ	CTX	CTC	FOX	MER
C	R	S/R	S	S	S	S	S	S
C_FOX	R	S/R	S	S	S	S	I/R	S
ESBL	R	S/I	R	R	R	S/I	S	S
AmpC	R	R	R	R	R	R	R	S
CPE	R	R	R	R	R	R	R	R

AMX: amoxicillin; AMC: amoxicillin + clavulanic acid; XNL: ceftiofur; CFQ: cefquinome; CTX: cefotaxime; CTC: cefotaxime + clavulanic acid; FOX: cefoxitin; MER: meropenem

Table II – Resistance phenotypes of the 94 bovine isolates tested by microarrays

Disk diffusion assay phenotypes		
C 15	ESBL 40	AmpC 4
C_FOX 20	ESBL+ C 5	AmpC-like 5
	ESBL+ C_FOX 5	
Total of isolates		**94**

A concordance between the detected genes and the phenotypes was observed for 68 (72%) isolates. In isolates with ESBL phenotypes, only different $bla_{\text{CTX-M}}$ genes were detected while in isolates with AmpC phenotypes, the $bla_{\text{CMY II}}$ and bla_{DHA} genes were both identified and in isolates with C phenotypes, only the $bla_{\text{TEM-1}}$ or $bla_{\text{TEM-2}}$ genes were detected. In 9 isolates, the detected *bla* genes did not match with the observed phenotypes. No *bla* genes were detected in 17 isolates including 11 with a C_FOX phenotype. Additional studies by Whole Genome Sequencing are being performed on the isolates with discordant results and specific PCR will subsequently be designed and performed to identify the *bla* genes present in the whole collection of 607 isolates.

References:
[1] R.P. Ambler (1980). *Philos. Trans. R. Soc.* 289:321-33.
[2] K. Bush, G.A. Jacoby (2010). *Antimicrob. Agents Chemother.* 54:969-976.
[3] S.A. Cunningham, S. Vasoo, R. Patel (2016). *J. Clin. Microbiol.* 54:1368-1371.