Table S1: List of primers and control strains used in the PCR

Antimicrobial resistance factor	Gene	Primer	Amplicon size (bp)	Control strain	Reference
SHV-UP SHV-LO	*bla*$_{SHV}$	for CGCCGGGTTATTCTTATTTGTCGC rev TCTTTCCGATGCCGCCGCCAGTCA	1016	PMON38	(1, 2)
TEM-G TEM-H	*bla*$_{TEM}$	for TTGCTCACCCAGAAACGCTGGTG rev TACGATACGGGAGGGCTTACC	708	ECL3482 or CTX-M15	(1, 2)
CTX-U1 CTX-U2	*bla*$_{CTX-M}$	for ATGTGCAGYACCAGTAARGTKATGGC rev TGGGTRAARTARGTSACCAGAAYCAGCGG	593	CTX-M15	(1, 2)
OXA1-F OXA1-R	*bla*$_{OXA}$	for CGCAAATGGCACCAGATTCAAC rev TCCTGCACCAGTTTTCCCATACAG	464	CTX-M15	(1, 2)
CMY2-A CMY2-B	*bla*$_{CMY}$	for TGATGCAGGAGCAGGCTATTCC rev CTAACGTCATCGGGGATCTGC	323	ECL3482	(1, 2)
uidA for uidA rev	*uidA*	for CCAAAAGCCAGACAGAGT rev GCACAGCACATCAAAGAG	623	ECL7805	(3)

1. Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. 2011. New Delhi metallo-beta-lactamase in Klebsiella pneumoniae and Escherichia coli, Canada. Emerg Infect Dis 17:103-6.
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3. McDaniels AE, Rice EW, Reyes AL, Johnson CH, Haugland RA, Stelma GN, Jr. 1996. Confirmational identification of Escherichia coli, a comparison of genotypic and phenotypic assays for glutamate decarboxylase and beta-D-glucuronidase. Appl Environ Microbiol 62:3350-4.

Table S2: List of antimicrobial disks used for Kirby Bauer assays

Antimicrobial disks	Concentration (μg)
Amoxicillin/Clavulanic acid	30
Ampicillin	10
Azithromycin	15
Cefoxitin	30
Ceftiofur	30
Ceftriaxone	30
Chloramphenicol	30
Ciprofloxacin	5
Gentamicin	10
Nalidixic acid	30
Spectinomycin	100
Streptomycin	10
Sulfisoxazole	250
Tetracycline	30
Trimethoprim–sulfamethoxazole	23,75

Table S3. Proportion of *E. coli* isolates positive for *bla*$_{SHV}$ before and after cessation of *in ovo* administration of ceftiofur and following its substitution with lincomycin-spectinomycin in indicator and potential ESBL/AmpC-producer collections from newly hatched, broiler and breeder birds from Canada

Sample origin	Before or after ceftiofur cessation in broiler	*In ovo* administration of:	No of isolates tested in indicator collection	No of isolates positive for *bla*$_{SHV}$	No of isolates tested in ceftriaxone-enriched collection	No isolates positive for *bla*$_{SHV}$
Meconium of newly hatched chicks	Before (2014)	Ceftiofur	96	0	57	0
	After (2015)	Nothing	69	0	40	0
	After (2015)	Lincomycin Spectinomycin	80	0	47	0
Pooled feces of broilers	Before (2014	Ceftiofur	100	2	60	0
	After (2015)	Nothing	70	0	42	0
	After (2015)	Lincomycin Spectinomycin	79	0	48	1
Pooled feces of breeders	Before (2014)	Ceftiofur	109	1	66	0
	Before (2015)	Ceftiofur	119	0	72	1

Table S4. Percentage of multidrug resistance [a] by antimicrobial class [b] and antimicrobials [c] in all the potential ESBL/AmpC-producing isolate collection in association with resistance gene combination

Combination of β-lactamase	No of isolates	FLQ		CPS		MAC	PEN	PEN/I	AMG			CPM	FOL		PHE	TET
		NA	CIP	TIO	CRO	AZM	AMP	AMC	GEN	SPT	STR	FOX	SXT	SSS	CHL	TET
bla$_{CMY-2}$ only	269	3,3	0,4	98,1	98,1	2,23	100	98,9 [d]	43,1	32,3	68,8	98,9 [d]	19,7	60,2 [d]	19,3	59,4 [d]
bla$_{CTX-M}$ only	15	0	0	100	100	0	100	26,7 [e]	20	13,3	73,3	13,3 [e]	33,3	86,7 [e]	0 [d]	100 [e]
bla$_{CMY-2}$ and *bla*$_{CTX-M}$	5	0	0	100	100	0	100	100 [d]	60	60	100	100 [d]	40	80	40 [e]	80

[a] Tested by Kirby Bauer

[b] Antimicrobial class: (FLQ) Fluoroquinolones; (CPS) Cephalosporines; (MAC) Macrolides; (PEN) Penicillins; (PEN/I) Penicillins+ β-Lactamases inhibitors; (AMG) Aminoglycosides; (CPM) Cephamycins; (FOL) Folate inhibitors; (PHE) Phenicols; (TET) Tetracyclines.

[c] Antimicrobials: (NA) Nalidixic acid; (CIP) Ciprofloxacin; (TIO) Ceftiofur; (CRO) Ceftriaxone; (AZM) Azithromycin; (AMP) Ampicillin; (AMC) Amoxicillin/clavulanic acid; (GEN) Gentamicin; (SPT) Spectinomycin; (STR) Streptomycin; (FOX) Cefoxitin; (SXT) Trimethoprim-sulphamethoxazole; (SSS) Sulfisoxazole; (CHL) Chloramphenicol; (TET) Tetracycline.

[de] Letters c and d in superscript in the same column of antimicrobial indicate significantly different results

Table S5: Effect of ceftiofur cessation and the substitution with lincomycin-spectinomycin on the proportion of antimicrobial [a] phenotypic co-resistance profiles in samples from newly hatched, broiler and breeder birds with *E. coli* of the ceftriaxone enriched collection

Phenotypic combinations	Administration *in ovo* of:							
	Ceftiofur				No antimicrobial		Lincomycin spectinomycin	
	2014			2015	2015		2015	
	Breeder (n=22)	Meconium (n=22)	Broiler feces(n=22)	Breeder (n=24)	Meconium (n=14)	Broiler feces(n=14)	Meconium (n=16)	Broiler feces(n=16)
(TIO CRO) [b] AMP AMC FOX	4	4		3	1	2		
(TIO CRO) AM AMC FOX SSS		1			2	2		
Other combinations [c] with 6 antimicrobial n-sus [d]		1	1	3				
(TIO CRO) AMP AMC STR FOX TET	7	1	1	3	1			
Other combinations with 7 antimicrobial n-sus	1	1	2		1			
(TIO CRO) AMP AMC STR FOX SSS TET	1	1						
Other combinations with 8 antimicrobial n-sus			1	3		1	1	2
(TIO CRO) AMP AMC STR FOX (SXT SSS) TET		1	4	2	1		2	
Other combinations with 9 antimicrobial n-sus			5	1	2	2	1	
(TIO CRO) AMP AMC (GEN STR) FOX SSS CHL TET		1			1	1		
(TIO CRO) AMP AMC (GEN SPT STR) FOX SSS TET	2	5		3	2	4	3	2
Other combinations with 10 antimicrobial n-sus		1	1	1		1		3
(TIO CRO) AMP AMC (GEN SPT STR) FOX SSS CHL TET	3	2		4			4	2
(TIO CRO) AMP AMC (GEN STR) FOX (SXT SSS) TET SPT							2	2
Other combinations, 11 antimicrobial n-sus	2		1			1	1	1
(TIO CRO) AMP AMC (GEN SPT STR) FOX (SXT SSS) CHL TET			1	1	3		2	4
Other combinations with 12 antimicrobial n-sus	2		1					
Other combinations with 13-14 antimicrobial n-sus			2					

[a] Antimicrobials: (NA) Nalidixic acid; (CIP) Ciprofloxacin; (TIO) Ceftiofur; (CRO) Ceftriaxone; (AZM) Azithromycin; (AMP) Ampicillin; (AMC) Amoxicillin/clavulanic acid; (GEN) Gentamicin; (SPT) Spectinomycin; (STR) Streptomycin; (FOX) Cefoxitin; (SXT) Trimethoprim-sulphamethoxazole; (SSS) Sulfisoxazole; (CHL) Chloramphenicol; (TET) Tetracycline.

[b] Antimicrobials in the same parentheses are from the same antimicrobial class.

[c] Other combinations are combinations which did not repeat or are less common.

[d] n-sus: non-susceptibility.