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First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland☆

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ABSTRACT

The herd prevalence of third-generation cephalosporin-resistant *Escherichia coli* (3GC-R-*Ec*) was determined for broilers (25.0% [95% confidence interval (CI) 17.6–33.7%]), pigs (3.3% [(95% CI 0.4–11.5%]), and cattle (3.9% [95% CI 0.5–13.5%]), using a sampling strategy that was representative of the livestock population slaughtered in Switzerland between October 2010 and April 2011. The 3GC-R-*Ec* isolates were characterized by the measurement of the MICs of various antibiotics, microarray analyses, analytical isoelectric focusing, polymerase chain reaction and DNA sequencing for *bla* genes, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing. CMY-2 ($n = 12$), CTX-M-1 ($n = 11$), SHV-12 ($n = 5$), TEM-52 ($n = 3$), CTX-M-15 ($n = 2$), and CTX-M-3 ($n = 1$) producers were found. The majority of CMY-2 producers fell into 1 PFGE cluster, which predominantly contained ST61, whereas the CTX-M types were carried by heterogeneous clones of *E. coli*, as shown by the numerous PFGE profiles and STs that were found. This is the first national Swiss study that focuses on the spread of 3GC-R Enterobacteriaceae among slaughtered animals.

1. Introduction

Over the past 2 decades, there has been an increasing number of infections worldwide due to third-generation cephalosporin-resistant (3GCs-R) *Escherichia coli* isolates (Coque et al., 2008; Hawser et al., 2011; Rosenthal et al., 2010). The production of Ambler class A extended-spectrum β-lactamases (ESBLs) and class C plasmid-mediated AmpC (pAmpC) enzymes is the most encountered mechanism responsible for this phenomenon. TEM, SHV, and CTX-M types are the 3 main families of ESBLs, whereas 6 families of pAmpCs (CMY, FOX, DHA, MOX, ACC, ACT types) have been described (Jacoby, 2009; Perez et al., 2007). To date, the most frequently detected ESBLs in *E. coli* are of the CTX-M types (Oteo et al., 2010; Peirano and Pitout, 2010), whereas CMY-2 is the most recurrent pAmpC (Jacoby, 2009).

E. coli is the major pathogen responsible for urinary tract and bloodstream infections in humans (Rosenthal et al., 2010). Some pathogenic *E. coli* isolates are also frequently responsible for diarrheal infections, and livestock plays an important role as reservoir (Kaper et al., 2004). Currently, the presence of 3GCs-R *E. coli* (3GCs-R-*Ec*) in clinical settings represents a public-health concern because these infections are challenging the therapeutic armamentarium (Giamarellou and Poulakou, 2009; Pitout, 2010). In fact, ESBL and pAmpC genes are usually carried on mobile plasmids along with other gene-resistance traits (e.g., those for quinolones and aminoglycosides) that render the isolates multidrug resistant (Jacoby, 2009; Perez et al., 2007). As a result, one important task is to monitor the prevalence of these genetic elements among *E. coli* in community, hospital, and environmental settings to implement new strategies that would limit the spread of these elements to life-threatening pathogens.

Healthy animals can be important reservoirs of Gram-negative species that carry genes conferring resistance to β-lactams and other antimicrobial classes. Currently, CTX-M–type ESBLs and CMY-2 pAmpC are increasingly reported in numerous countries, mainly among strains of *E. coli* and *Salmonella* spp. that colonize food-producing animals (e.g., cattle, pigs) and animal companions (Carattoli, 2008; European Food Safety Authority Panel on Biological Hazards (BIOHAZ), 2011; Li et al., 2007). However, in poultry, CMY-2–positive *E. coli* isolates are more rarely described and usually have a lower prevalence than the ESBL producers have (Blanc et al., 2006; Dierikx et al., 2010; European Food Safety Authority Panel on Biological Hazards (BIOHAZ), 2011; Leverstein-van Hall et al., 2011; Li et al., 2007, 2010). Only Smet et al. (2008) reported a high prevalence (i.e., 49%) of CMY-2–positive *E. coli* isolates in Belgian broiler farms.

In Switzerland, national phenotypic surveillances from the past 2 years indicate that the prevalence of 3GCs-R Enterobacteriaceae in humans and in food-producing animals is lower than in other European countries (Büttner et al., 2010, 2011; European Food Safety Authority Panel on Biological Hazards (BIOHAZ), 2011) (http://www.search.ifik.unibe.ch/en/index.html). Nevertheless, data regarding the

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Table 1
Epidemiologic data regarding third-generation cephalosporin-resistant *E. coli* (3GCs-R-*Ec*) isolates from broilers in Switzerland.

Slaughterhouse and 3GCs-R groups	No. of 3GCs-R-*Ec* isolates/overall no. of different holdings (*n*) in the different Swiss Cantons														
	AG (11)	BE (22)	FR (20)	GE (1)	JU (1)	LU (20)	SG (3)	SH (3)	SO (7)	TG (9)	VD (17)	VS (1)	ZG (1)	ZH (2)	Unknown (1)	All holdings (%)
A	2/4	1/7	0/3		1/1	6/13	0/2	0/2	1/2	1/4	1/2	0/1	0/1	0/1		13/43 (30.2)
B	0/1	3/14	2/14			0/3			0/3	0/1	5/14				1/1	11/51 (21.6)
C	1/6					0/4			1/2							2/12 (16.7)
D		1/1	0/1				0/1	0/1		2/4	0/1			0/1		3/10 (30.0)
E			0/2	1/1							0/1					1/4 (25.0)
All slaughterhouses (%)	3/11 (27.3)	5/22 (22.7)	2/20 (10.0)	1/1 (100)	1/1 (100)	6/20 (30.0)	0/3	0/3	2/7 (28.6)	3/9 (33.3)	6/18 (33.3)	0/1	0/1	0/2	1/1 (100)	30/120 (25.0)
CMY-2	2	1	2			4			1		2					12/30 (40.0)
CTX-M-1		2		1	1	1			1		2				1	9/30 (30.0)
CTX-M-15		1														1/30 (3.3)
SHV-12	1	1				1				2						5/30 (16.7)
TEM-52										1	2					3/30 (10.0)

AG = Aargau; BE = Bern; FR = Fribourg; GE = Geneva; JU = Jura; LU = Lucerne; SG = St. Gallen; SH = Schaffhausen; SO = Solothurn; TG = Thurgau; VD = Vaud; VS = Valais; ZG = Zug; ZH = Zurich.

molecular mechanisms responsible for resistance to 3GCs are still lacking. In a recent pilot study at only 1 slaughterhouse, feces from swine and cattle that were sampled in October 2009 were analyzed to determine the percentage of ESBL-producing Enterobacteriaceae. The results demonstrated that 15.2% of the pigs and 17.1% of the cattle were positive, and the CTX-Ms were the only ESBLs found (Geser et al., 2011). However, the possible presence of pAmpCs was not taken into account, and neither the type of bla_{CTX-M} and bla_{TEM} nor the resistance genes against other antibiotics were determined (Geser et al., 2011). Additionally, no data are yet available about the distribution and the molecular characteristics of 3GC-R-*Ec* in broiler production in Switzerland.

In the present study, we used a sampling strategy evenly distributed throughout the months and years and representative for the contemporary Swiss livestock population. We then implemented standard molecular and biochemical tests to detect all possible ESBL and pAmpC producers among the 3GC-R-*Ec* isolates from broilers, cattle, and swine.

2. Materials and methods

2.1. Sample collection

Representative samples were taken according to the guidelines of the Swiss National Monitoring Program on Antimicrobial Resistance in Food Animals (Büttner et al., 2011). The sampling strategy consists of collecting from each slaughterhouse a number of samples that are proportional to the number of animals slaughtered at each establishment per year. The sampling is also evenly distributed across each month of the study period. The samples were randomly collected at the 5 biggest broiler abattoirs (5 to 207 samples per slaughterhouse per year) and at the 9 biggest pig (10 to 102 samples) and 7 biggest cattle (2 to 66 samples) abattoirs where over 80% of livestock in Switzerland are slaughtered. Only 1 sample was taken per animal holding for pigs and cattle, and 1 pool of 5 animals per holding was analyzed for broilers. For this study, broiler samples taken from October 18, 2010, through April 30, 2011, and pig and cattle samples taken from January 1, 2011, through April 30, 2011, were analyzed for the presence of 3GC-R isolates.

2.2. Detection of third-generation cephalosporin-resistant isolates

Five broiler cloacal swabs per holding were vortexed together for 30 s in 1 mL of Tryptone Soy Broth (Becton Dickinson, Franklin Lakes, NJ). This suspension and the fecal swabs from pigs and cattle were transferred into 5 mL of MacConkey broth (Oxoid, Basingstoke, UK) containing ceftazidime (8 mg/L) and incubated at 37 °C for 24 h under agitation. Then, 1 full loop (10 μL) was plated onto selective chromogenic medium for the screening of 3GCs-R Enterobacteriaceae (chromID ESBL; bioMérieux, Marcy l'Etoile, France) and reincubated overnight. From each selective plate, a single colony from those showing a unique color and morphology as described in the manufacturer's product documentation (bioMérieux) was further identified to the species level.

2.3. Species identification and antimicrobial susceptibility tests

Species identification (ID) and antimicrobial susceptibility tests (ASTs) were routinely assessed using the Vitek 2 system on AST-GN38 cards (bioMérieux). The ID was confirmed with matrix-assisted laser desorption/ionization time of flight mass spectrometry (microflex LT, Bruker Daltonik, Bremen, Germany). The MICs were determined by microdilution in Mueller-Hinton broth (BBL, Becton Dickinson) using the Sensititre ESB1F plate (Trek Diagnostics Systems, East Grinstead, England) according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2009).