

## CMY-2-Producing *Escherichia coli* in the Nose of Pigs

The spread of extended-spectrum cephalosporin-resistant (ESC-R) *Escherichia coli* isolates represents a public health concern. Extended-spectrum  $\beta$ -lactamases (ESBLs) or plasmid-mediated AmpC  $\beta$ -lactamases (pAmpCs) are the most frequently encountered mechanisms responsible for this phenomenon (10, 15). ESBL- and pAmpC-producing *E. coli* isolates are now constantly reported in humans, as well as among companion and food-producing animals (3, 10, 15).

Pigs are recognized as an important source of multidrug-resistant organisms, such as methicillin-resistant *S. aureus* (MRSA) and ESC-R *E. coli*, which have strong impacts in the human medical setting (9, 11). In Switzerland, 3.3% of slaughtered pigs are intestinal carriers of ESC-R *E. coli* and 5.9% are colonized in the nose with MRSA (5, 13). To date, nothing is known about the role of the nose of pigs as a possible reservoir for ESC-R Gram-negative bacteria.

In this study, 24 nasal swabs from swine raised in 24 different herds from 7 Swiss cantons were collected at 9 slaughterhouses from December 2010 to April 2011. Specimens were plated onto selective chromID ESBL (bioMérieux) directly and after a preliminary overnight incubation in MacConkey broth (Oxoid) containing ceftazidime (4  $\mu$ g/ml). One ESC-R colony (if any) was randomly selected for the following analyses: species identification using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) (Bruker Daltonik), MIC determination using the Sensititre microdilution plates ESB1F and EUMVS2 (Trek Diagnostics) and the Vitek 2 card AST-GN38 (bioMérieux), microarrays for detection of *bla* (CT-101; Check-Points) and other resistance genes (AMR-ve version 0.5m; Alere) (1, 2), analytical isoelectric focusing (aIEF) (6), PCR DNA sequencing for *bla*<sub>ESBLs</sub>, *bla*<sub>pAmpCs</sub>, *qnrA*, -*B*, and -*S*, and *qepA* genes (7, 14), PCRs for phylogenetic group (4), PCRs for plasmid replicon typing (PBRT kit; Diatheva), pulsed-field gel electrophoresis (PFGE) (5), and multilocus sequence typing (MLST) ([www.pasteur.fr/recherche/genopole/PF8/mlst/](http://www.pasteur.fr/recherche/genopole/PF8/mlst/)). Plasmids were extracted using the NucleoBond PC100 kit (Macherey-Nagel) and electrotransformed into *E. coli* SURE cells (Stratagene, Agilent technologies); transformants were selected on LB agar plates containing ampicillin (20  $\mu$ g/ml).

As shown in Table 1, the noses of 3 pigs (an indicative preva-

lence of 12.5% [95% confidence interval {CI}, 2.6 to 32.4%]) harbored *E. coli* isolates producing CMY-2, the most frequently detected pAmpC in *Enterobacteriaceae* causing infections in humans (10). The isolates were nonclonally related as they displayed different PFGE profiles (data not shown) and belonged to different sequence types (ST2, ST532, and ST539) but carried plasmids with a common I1 replicon type (Table 1). The association of *bla*<sub>CMY</sub> with IncI1 plasmids was confirmed by PCRs using DNA of transformants obtained from all of the three original ESC-R *E. coli* isolates.

This is the first description of ESC-R *Enterobacteriaceae* in the nose of pigs. Of note, the ESC-R *E. coli* isolates found in this study produced CMY-2, whereas the ones isolated from pig feces during our recent Swiss national surveillance on food-producing animals produced only CTX-M-type ESBLs. Conversely, a high prevalence (i.e., 40% [95% CI 22.6% to 59.4%]) of CMY-2-producing *E. coli* was observed in cloacal swabs of broiler chickens, where several isolates also belonged to ST539, like one of the pig isolates from the present study (5).

The presence of pAmpC-producing *E. coli* isolates in the nose of slaughtered pigs represents an additional critical source of contamination of materials, carcasses, and meat at slaughterhouses, amplifying the risk of diffusion of pAmpCs in the human food chain. Moreover, as in the case of MRSA (11), we speculate that occupational contact with pigs carrying ESC-R *E. coli* might have a future clinical impact on our health care systems (8, 12). Larger epidemiological studies should be implemented to comprehend this overall phenomenon and to help in taking appropriate measures to limit the spread of multidrug-resistant bacteria from food-producing animals to the community.

Published ahead of print 5 June 2012

Address correspondence to Vincent Perreten, vincent.perreten@vetsuisse.unibe.ch.

\* Present address: Institute for Infectious Diseases, University of Bern, Bern, Switzerland.

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doi:10.1128/AAC.00389-12

## ACKNOWLEDGMENTS

We thank Alexandra Rossano, Alexandra Collaud, Daniel Kunz, Werner Lehmann, Isabelle Bertschy, Isabelle Brodard, and Andreas Thomann for their assistance.

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## Andrea Endimiani\*

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

## Markus Hilty

Institute for Infectious Diseases, University of Bern, Bern, Switzerland

## Vincent Perreten

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

TABLE 1 Phenotypic and molecular characteristics of the extended-spectrum cephalosporin-resistant *E. coli* isolates detected in the nose of Swiss pigs

Isolate (abattoir code, canton) <sup>a</sup>	ST (PFGE pattern)	Phylogroup	MIC ( $\mu$ g/ml) in antimicrobial susceptibility test <sup>b</sup>																		CT-101 and Alere microarray analyses <sup>c</sup>	PCR/DNA sequencing for genes	Plasmid replicon typing					
			AMP	FOX	TZP	CAZ	CTX	FEP	IPM	MEM	CIP	NAL	KAN	GEN	TOB	AMK	SMX	TMP	TET	CHL				FN	NIT	PoB	aIEF	
MSA935 (K, Bern)	ST539 (A)	D	$\geq 32$	$\geq 128$	$\geq 128$	128	$\geq 64$	2	$\leq 0.25$	$\leq 0.5$	0.06	$\leq 4$	$\leq 4$	$\leq 2$	$\leq 1$	4	$> 1024$	$\leq 0.5$	2	4	4	$\leq 16$	1	9.0	CT-101 and Alere microarray analyses <sup>c</sup>	CMY-2	II, Y	
MSA967 (N, St. Gallen)	ST12 (B)	A	$\geq 32$	$\geq 128$	32	128	32	$\leq 0.5$	$\leq 0.25$	$\leq 0.5$	8	$> 64$	$> 128$	$\leq 2$	$\leq 1$	$\leq 2$	$> 1024$	$\leq 0.5$	$> 64$	8	8	8	$\leq 16$	0.5	9.0	CT-101 and Alere microarray analyses <sup>c</sup>	CMY-II/aadA4, CMY-2 <sup>d</sup>	II
MSA969 (N, St. Gallen)	ST532 (C)	A	$\geq 32$	$\geq 128$	16	128	32	$\leq 0.5$	$\leq 0.25$	$\leq 0.5$	0.25	$> 64$	$\leq 4$	$\leq 2$	$\leq 1$	$\leq 2$	$> 1024$	$> 32$	$> 64$	8	8	64	0.5	5.4, 9.0	CT-101 and Alere microarray analyses <sup>c</sup>	CMY-II, TEM	II	

<sup>a</sup> Nasal swabs were collected at 9 slaughterhouses from 24 pigs raised in 24 different herds from 7 Swiss cantons (Aargau, Lucerne, Obwald, St. Gallen, Thurgau, Vaud, and Zurich).

<sup>b</sup> AMP, ampicillin; FOX, cefoxitin; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; NAL, nalidixic acid; KAN, kanamycin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; SMX, sulfamethoxazole; TMP, trimethoprim; TET, tetracycline; CHL, chloramphenicol; FN, florfenicol; NIT, nitrofurantoin; PoB, polymyxin B. MICs for TOB, AMK, NIT, and PoB were obtained using the Vitek II system (bioMérieux), whereas all others were obtained using the Sensititre plates ESBIF and EUNV52 (Trek Diagnostics).

<sup>c</sup> The CT-101 microarray detects bla<sub>SHV</sub>, bla<sub>TEM</sub> (distinguishing bla<sub>TEM</sub> from bla<sub>non-TEM</sub>), bla<sub>CTX-M</sub>, bla<sub>KPC</sub>, bla<sub>NDM</sub>, bla<sub>DHA</sub>, bla<sub>NDX</sub>, bla<sub>OXA</sub>, bla<sub>ACTV</sub>, bla<sub>OXA</sub>, and bla<sub>ACC</sub>. The AMR-ve 0.5 m microarray detects bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>OXA</sub>; genes conferring resistance to quinolones (qnrA, -B, and -S), aminoglycosides (aadA1, -aadA2, and aadA4-like, aac(3)-Ia, aac(3)-Ib, ant(2'')-Ia, strA, and strB), tetracyclines (tet(A), tet(B), tet(C), tet(D), and tet(G)), sulfonamides (sul1, sul2, and sul3), trimethoprim (dhfrA1, dhfrA5, dhfrA7, dhfrA12, dhfrA14, dhfrA17, and dhfrA19), and phenicols (catA1, catII, catB3, floR), and class I and II integron-associated integrase (intI/III).

<sup>d</sup> The presence of bla<sub>TEM</sub> in MSA967 was not confirmed by PCR.