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High Prevalence of Extended-Spectrum-Cephalosporin-Resistant *Enterobacteriaceae* in Poultry Meat in Switzerland: Emergence of CMY-2- and VEB-6-Possessing *Proteus mirabilis*

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The spread of extended-spectrum-cephalosporin-resistant (ESC-R) *Escherichia coli* in poultry meat is a serious concern (1–3). However, data regarding this problem in Switzerland are lacking. Moreover, the role played in this matter by other *Enterobacteriaceae* remains undetermined.

We explored the prevalence of extended-spectrum-β-lactamase (ESBL)- and plasmid-mediated-AmpC (pAmpC)-possessing *Enterobacteriaceae* as contaminants of raw poultry meat retailed in Bern, Switzerland. Twenty samples were purchased on various days during November and December 2012 in three grocery stores (Table 1). Ten grams of meat was incubated overnight in LB broth, and then 50 μl was plated on plates containing selective chromID ESBL agar, MacConkey agar plus ceftazidime (2 μg/ml), and Drigalski agar plus cefotaxime (1.5 μg/ml) (bioMérieux). Colonies were identified by MALDI-TOF MS (matrix-assisted laser desorption ionization–time of flight mass spectrometry; Bruker). ESC-R *Enterobacteriaceae* were characterized using phenotypic and molecular methods as previously done (4–7) (see Table S1 in the supplemental material).

Fourteen samples (70%) contained ESC-R *E. coli* ($n = 11$), ESC-R *P. mirabilis* ($n = 2$), or both resistant species ($n = 1$) (Table 1). ESC-R *E. coli* strains found in meat originating from Switzerland possessed $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$, whereas those detected in meat imported from other countries harbored $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$, or $bla_{\text{CMY-2}}$. Several ESC-R *E. coli* isolates belonged to the same sequence type (ST), but pandemic clones (e.g., ST131) often responsible for human infections were not found (8, 9). The recorded high prevalence of ESC-R *E. coli* (60%) was consistent with prevalences reported in other countries (1–3). However, we note that in Switzerland, CTX-M-1- and CMY-2-producing *E. coli* strains are common colonizers of food animals and may also cause human infections (4, 6, 10).

More intriguing was the detection of ESC-R *P. mirabilis* that grew in MacConkey-ceftazidime and Drigalski-cefotaxime plates. In particular, one meat sample imported from Austria harbored $bla_{\text{CMY-2}}$, whereas two from an unspecified European country carried $bla_{\text{VEB-6}}$. These samples came from the same store and same packing plant but were wrapped on different dates.

CMY-2-positive *P. mirabilis* isolates are commonly found in humans (11), but only unique VEB-6-possessing *P. mirabilis* isolates had been previously reported as responsible for infection in France, Oman, and Australia (7, 12, 13). In our isolates, $bla_{\text{VEB-6}}$ was situated in an ~17-kb class I integron which also carried *aacA4*, *aadB*, *dfrA1*, *sul1*, *tet*(A), and *qnrA1* resistance genes and had a nucleotide sequence identical to that found in the human VEB-6-positive *P. mirabilis* isolate (VB1248) reported in France (12). Both VEB-6-positive *P. mirabilis* isolates also possessed a previously reported class II integron of ~4.6 kb containing *dfrA1*, *sat2*, and *aadA1* resistance genes (GenBank no. DQ268533) and a single ~50-kb IncP plasmid. Electroporation experiments failed to transfer $bla_{\text{VEB-6}}$, suggesting the chromosomal location of the integron. By repetitive extragenic palindromic PCR (rep-PCR), the two VEB-6-positive *P. mirabilis* isolates were identical to each other and showed 94% genomic identity with VB1248. Based on these results, we investigated the possible presence of VEB-6-positive *P. mirabilis* among 484 human isolates identified in Bern during 2011-2012, and we identified only four CMY-2-positive *P. mirabilis* isolates which were not clonally related to those found in poultry meat (see Fig. S1 in the supplemental material).

This is the first report of bla_{VEB}-possessing *P. mirabilis* in a non-human clinical setting and the first description of ESBL- and pAmpC-positive *P. mirabilis* strains in the food chain. Our findings emphasize that not only ESC-R *E. coli* but also other species are responsible for the spread of multidrug-resistant mobile genetic elements in the raw meat. CMY-2- and VEB-6-positive *P. mirabilis* strains might represent an additional group of life-threatening pathogens that can be transmitted through the food chain to humans (8).

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TABLE 1 Phenotypic and molecular characteristics of the bla_{ESBL}- and bla_{pAmpC}-possessing *E. coli* and *P. mirabilis* isolates found in poultry meat retailed in Bern during the study period[a]

Meat (store)	Species	bla_{ESBL}, bla_{pAmpC}	Plasmid Inc group(s)	Phylogenetic group	ST	Antimicrobial agents tested (MICs, μg/ml)[b]	Country[c] Production	Country[c] Packaging (packing plant)
Turkey slices (A)	*E. coli*	CMY-2	F, A/C	D	ST117	CTX (4), CAZ (4), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER (I)
Turkey slices (A)	—	—	—	—	—	—	GER	GER (I)
Chicken slices (A)	*E. coli*	SHV-12, TEM-1	F, X1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (≥32), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	GER	GER (Un)
Chicken slices (A)	*E. coli*	TEM-52	F, I1, X1	A	ST23	CTX (4), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER (Un)
Chicken ministeaks (A)	*E. coli*	CTX-M-1	F, I1	D	ST2485	CTX (≥64), CAZ (2), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken ministeaks (A)	—	—	—	—	—	—	CH	CH (II)
Ground chicken (A)	*E. coli*	CTX-M-1, TEM-1	F, FII, I1	A	ST1141	CTX (16), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken legs (A)	—	—	—	—	—	—	CH	CH (II)
Ground chicken (A)	*E. coli*	CTX-M-1	F, I1	B1	ST602	CTX (8), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken legs (A)	—	—	—	—	—	—	CH	CH (II)
Chicken wings (B)	*E. coli*	CTX-M-1	F	B1	ST1304	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Chicken wings (B)	—	—	—	—	—	—	CH	CH (III)
Chicken legs (B)	*E. coli*	CMY-2	F, B/O, A/C, K	D	ST38	CTX (8), CAZ (8), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Chicken wings (B)	*E. coli*	CTX-M-1	I1, Y	D	ST2485	CTX (16), CAZ (≤0.5), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Ground chicken breasts (B)	*E. coli*	SHV-12	F, FII, I1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (16), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	AUT	CH (III)
	P. mirabilis	CMY-2	I1	NA	NA	CTX (2), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (2), AMK (8), CIP (≤0.125), SXT (≤0.25), COL (≥8), TGC (2)		
Turkey slices (C)	*P. mirabilis*	VEB-6, TEM-1[d]	P	NA	NA	CTX (16), CAZ (≥32), FEP (8), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≥16), AMK (16), CIP (≥4), SXT (≥8), COL (≥8), TGC (2)	EU	CH (IV)
Turkey slices (C)	*P. mirabilis*	VEB-6, TEM-1[d]	P	NA	NA	CTX (16), CAZ (≥32), FEP (16), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (8), AMK (16), CIP (≥4), SXT (≥8), COL (≥8), TGC (2)	EU	CH (IV)
Ground chicken (C)	*E. coli*	CTX-M-1	F, I1, P, U	A	ST23	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (V)
Chicken ministeaks (C)	—	—	—	—	—	—	CH	CH (V)
Chicken slices (C)	*E. coli*	CTX-M-1	F, I1	A	ST23	CTX (32), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (V)

[a] —, isolates possessing bla_{ESBLs} or bla_{aAmpCs} were not detected. NA, not applicable.

[b] Antibiotics and interpretation of MICs according to EUCAST criteria (14) were as follows: CTX, cefotaxime (susceptible [S], ≤ 1 μg/ml); CAZ (S, ≤ 1 μg/ml); FEP, cefepime (S,≤ 1 μg/ml); ATM, aztreonam (S, ≤ 1 μg/ml); TZP, piperacillin-tazobactam (S, ≤ 8 μg/ml); MEM, meropenem (S, ≤ 2 μg/ml); ERT, ertapenem (S, ≤ 0.5 μg/ml); GEN, gentamicin (S, ≤ 2 μg/ml); AMK, amikacin (S, ≤ 8 μg/ml); CIP, ciprofloxacin (S, ≤ 0.5 μg/ml); SXT (S, ≤ 2 μg/ml); COL, colistin (S, ≤ 2 μg/ml); TGC, tigecycline (S, ≤ 1 μg/ml). MICs were determined by using GNX2F plates (Trek Diagnostics).

[c] GER, Germany; CH, Switzerland; AUT, Austria; EU, European Union (the country was not specified on the package sticker). I, II, III, IV, V, and Un (unknown) indicate the different meat-packing plants.

[d] Both isolates possessed the following non-*bla* resistance genes according to the AMR-ve 0.5-m microarray (Alere): bla_{VEB}, bla_{TEM}, *aadA*, *aadB*, *catA*, *ant2Ia*, *aac6-Ib*, *aphA*, *qnr*, *sul1*, *sul2*, *tetA*, *strB*, *intI*, and *intII*. bla_{VEB-6} was carried by a chromosomally located class I integron. For each isolate, analytical isoelectric focusing showed two β-lactamase bands at pI 5.4 (TEM-1) and 7.2 (VEB-6).

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REFERENCES

1. **Kola A, Kohler C, Pfeifer Y, Schwab F, Kuhn K, Schulz K, Balau V, Breitbach K, Bast A, Witte W, Gastmeier P, Steinmetz I.** 2012. High prevalence of extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat, Germany. J. Antimicrob. Chemother. **67:**2631–2634.
2. **Egea P, Lopez-Cerero L, Torres E, del Gomez-Sanchez MC, Serrano L, Navarro Sanchez-Ortiz MD, Rodriguez-Bano J, Pascual A.** 2012. Increased raw poultry meat colonization by extended spectrum β-lactamase-producing *Escherichia coli* in the south of Spain. Int. J. Food Microbiol. **159:**69–73.
3. **Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ.** 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. **17:**873–880.
4. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J. Antimicrob. Chemother. **68:**2249–2254.
5. **Hilty M, Betsch BY, Bögli-Stuber K, Heiniger N, Stadler M, Kuffer M, Kronenberg A, Rohrer C, Aebi S, Endimiani A, Droz S, Mühlemann K.** 2012. Transmission dynamics of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the tertiary care hospital and the household setting. Clin. Infect. Dis. **55:**967–975.
6. **Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V.** 2012. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. Diagn. Microbiol. Infect. Dis. **73:**31–38.
7. **Zong Z, Partridge SR, Iredell JR.** 2009. A $bla_{\text{VEB-1}}$ variant, $bla_{\text{VEB-6}}$, associated with repeated elements in a complex genetic structure. Antimicrob. Agents Chemother. **53:**1693–1697.
8. **Seiffert SN, Hilty M, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: an emerging problem for human health? Drug Resist. Updat. **16:**22–45.
9. **Woodford N, Turton JF, Livermore DM.** 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. **35:**736–755.
10. **Endimiani A, Hilty M, Perreten V.** 2012. CMY-2-producing *Escherichia coli* in the nose of pigs. Antimicrob. Agents Chemother. **56:**4556–4557.
11. **D'Andrea MM, Literacka E, Zioga A, Giani T, Baraniak A, Fiett J, Sadowy E, Tassios PT, Rossolini GM, Gniadkowski M, Miriagou V.** 2011. Evolution and spread of a multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type cephalosporinases in Europe. Antimicrob. Agents Chemother. **55:**2735–2742.
12. **Siebor E, Neuwirth C.** 2011. The new variant of *Salmonella* genomic island 1 (SGI1-V) from a *Proteus mirabilis* French clinical isolate harbours $bla_{\text{VEB-6}}$ and qnrA1 in the multiple antibiotic resistance region. J. Antimicrob. Chemother. **66:**2513–2520.
13. **Potron A, Poirel L, Elhag K, Al Yaqoubi F, Nordmann P.** 2009. VEB-6 extended-spectrum β-lactamase-producing *Proteus mirabilis* from Sultanate of Oman. Int. J. Antimicrob. Agents **34:**493–494.
14. **EUCAST.** 2013. European Committee on Antimicrobial Susceptibility Testing clinical breakpoints. Version 3.0.