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Molecular Identification of Extended-Spectrum-β-Lactamase Genes from *Enterobacteriaceae* Isolated from Healthy Human Carriers in Switzerland

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In this study, fecal samples from 586 healthy humans were investigated to determine the occurrence of extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* in Swiss people. A total of 5.8% of the human fecal samples yielded ESBL producers, and all of the 34 isolated strains were *Escherichia coli*. PCR analysis revealed that 14 strains produced CTX-M-15, 10 produced CTX-M-1, 7 strains produced CTX-M-14, and 2 strains produced CTX-M-2 ESBLs. One strain produced SHV-12 ESBL. Of the 34 isolates, 15 produced additional TEM-1 broad-spectrum β-lactamases. By serotyping, a high degree of diversity among the strains was found.

Antimicrobial resistance in bacteria has emerged as a problem in both human and veterinary medicine. One of the currently most important resistance mechanisms in *Enterobacteriaceae*, which reduces the efficacy even of modern expanded-spectrum cephalosporins (except cephamycins and carbapenems) and monobactams, is based on plasmid-mediated production of extended-spectrum β-lactamases (ESBL). Until now, more than 600 ESBL variants are known. Among them, the over 100 CTX-M enzymes so far reported may be grouped into five main subgroups (3). As a matter of growing concern, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole (5, 15). In the past few years, there has been an increase in the detection of ESBL-producing strains in the general community (26). Three studies have been published about ESBL prevalence in healthy humans, establishing prevalences between 6 and 7% (25, 26, 36). More recently, several alarming studies have reported the dissemination of ESBL-producing *Enterobacteriaceae* to (i) healthy food-producing animals in several countries in Europe and Asia (7, 8, 16, 27, 35) and (ii) food products like meat, fish, and raw milk (17, 19, 20). Moreover, ESBL producers are also reported increasingly among infection-associated enterobacterial isolates in France (9, 23), Italy (4), the Czech Republic (21), and Austria (10). These studies were the reason to look for ESBL producers also in Switzerland, a country—located between the mentioned ones—with a tight policy of antibiotic prescription and a low level of multidrug resistance in bacteria (12). The aim of the present study was to screen for the occurrence of ESBL-producing *Enterobacteriaceae* in healthy human carriers in Switzerland and to further characterize isolated strains and ESBLs.

In an ongoing study of routine stool samples from staff members of meat-processing companies, fecal samples were collected from September to November 2010 from 586 healthy humans. The native samples were transported in sterile tubes without transport medium and were processed within 24 h of collection. All samples were collected in urban areas, and each person was tested only once. The population consisted of adults without diarrhea aged between 20 and 60 years, a quarter being female.

One loopful of each sample was enriched for 24 h at 37°C in 10 ml of EE broth (BD, Franklin Lakes, NJ), streaked onto Brilliance ESBL agar (Oxoid, Hampshire, United Kingdom), and incubated at 37°C for 24 h under aerobic conditions. Colonies of different morphology were selected and subcultured onto triple sugar iron (TSI) agar (BD, Franklin Lakes, NJ) at 37°C for 24 h. Nonfermenters were discarded, and oxidase-negative colonies were subjected to identification by API ID 32 E (bioMérieux, Marcy l'Etoile, France). Serotyping was performed according to standard methods (32).

All isolated strains were subjected to susceptibility testing against 15 antimicrobial agents (Table 1) by the disk diffusion method according to CLSI protocols and evaluated according to CLSI criteria (6). Presumptive ESBL producers were confirmed on Mueller-Hinton agar plates using Etest-ESBL strips containing cefotaxime, cefepime, or ceftazidime, each alone and in combination with clavulanic acid (bioMérieux, Marcy l'Etoile, France).

Bacterial strains confirmed as producing ESBLs were further analyzed by PCR and by sequencing the whole open reading frames (ORF) of *bla* genes. DNA was extracted by a standard heat lysis protocol. Thereafter, five specific published primer sets (custom synthesized by Microsynth, Balgach, Switzerland) and PCR protocols (13, 30, 33, 38) were used to search for β-lactamase-encoding genes belonging to bla_{TEM}, bla_{SHV}, and three bla_{CTX-M} subfamilies. In order to ensure coverage of all entire *bla* ORFs, the primer sets were supplemented with the following newly designed primers from up- and downstream bla_{CTX-M} flanking regions: 5′ AAACACACGTGGAATTTAGGG3′, 5′CCGTCGGTGACGATT TTAGCC3′, 5′CCGATGACTATGCGCACTGGG3′, 5′TTTTGC CGTACCTGCGTACCC3′, 5′CCGTGGGTTACGATTTTCGCC 3′, 5′TTGGTCCAGAAAAAAGAGCGG3′, 5′TGATGTAACACG

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TABLE 1 Identification and further characterization of the 34 ESBL producers isolated from fecal samples of 586 healthy human carriers in Switzerland[a]

Sample no.	Serotype[d]	β-Lactamase(s) identified	β-Lactam antibiotic resistance									Further resistances
			AM	AMC	CF	CXM	CPD	CTX	CAZ	FEP	FOX	
1519	O2:H48	CTX-M-1 and TEM-1[c]	r	s	r	r	r	r	s[b]	s[b]	s	
1038	O8:H21	CTX-M-1	r	s	r	r	r	r	s[b]	i[b]	s	SXT, TE, CIP
1582	O11:H12	CTX-M-1	r	s	r	r	r	r	s[b]	s[b]	s	TE
2238	O23:H16	CTX-M-1 and TEM-1	r	i	r	r	r	r	s[b]	s[b]	s	SXT, TE
2332	O24:H26	CTX-M-1 and TEM-1	r	i	r	r	r	r	s[b]	s[b]	s	SXT, TE
2018	O32:H19	CTX-M-1	r	s	r	r	r	r	s[b]	s[b]	s	SXT, TE
2291	O53:H18	CTX-M-1 and TEM-1	r	i	r	r	r	r	i[b]	s[b]	s	SXT, TE
2290	O68:H21	CTX-M-1	r	s	r	r	r	i[b]	s[b]	s[b]	s	SXT, TE
1559	O107:H27	CTX-M-1 and TEM-1	r	s	r	r	r	r	s[b]	s[b]	s	SXT, TE
2333	Or:H26	CTX-M-1 and TEM-1	r	i	r	r	r	i[b]	s[b]	s[b]	s	SXT, TE
1348	O20:H33	CTX-M-2 and TEM-1	r	i	r	r	r	r	s[b]	s[b]	s	SXT, TE
2294	Ont:H7	CTX-M-2 and TEM-1	r	r	r	r	r	r	s[b]	i[b]	s	K, SXT, TE
1877	O2:H48	CTX-M-14	r	s	r	r	r	i[b]	s[b]	s[b]	s	
2241	O15:H18	CTX-M-14	r	i	r	r	r	r	s[b]	s[b]	s	TE
2242	O15:H18	CTX-M-14	r	i	r	r	r	r	s[b]	s[b]	s	TE
1999	O33:H4	CTX-M-14	r	r	r	r	r	r	s[b]	r	s	TE, CIP
1018	O73/77:H18	CTX-M-14	r	s	r	r	r	r	s[b]	s[b]	s	GM
1545	O153:H30	CTX-M-14 and TEM-1	r	i	r	r	r	r	s[b]	i[b]	s	K, SXT, TE
1495	Or:H51	CTX-M-14	r	i	r	r	r	r	s[b]	s[b]	s	SXT, TE
2310	O1:H6	CTX-M-15	r	r	r	r	r	r	i[b]	i[b]	s	SXT, TE, CIP
1887	O15:H1	CTX-M-15	r	i	r	r	r	r	s[b]	s[b]	s	GM, SXT, TE
2225	O86:H4	CTX-M-15	r	s	r	r	r	r	i[b]	i[b]	s	SXT, CIP
150	O88:H8	CTX-M-15 and TEM-1	r	s	r	r	r	r	i[b]	i[b]	s	
1866	O102:H6	CTX-M-15	r	r	r	r	r	r	i[b]	r	s	K, SXT, TE, CIP
503	O102:H6	CTX-M-15 and TEM-1	r	s	r	r	r	r	r	i[b]	s	K, SXT
506	O123:H12	CTX-M-15	r	s	r	r	r	r	s[b]	i[b]	s	SXT, TE
1330	O153:H6	CTX-M-15	r	r	r	r	r	r	i[b]	i[b]	s	K, GM, CIP
171	O153:H6	CTX-M-15 and TEM-1	r	i	r	r	r	r	r	r	s	GM, SXT, TE, CIP
1024	O153:H6	CTX-M-15 and TEM-1	r	i	r	r	r	r	i[b]	i[b]	s	SXT, TE, CIP
2017	O184:H2	CTX-M-15	r	s	r	r	r	r	s[b]	s[b]	s	SXT, TE
2200	Or:H5	CTX-M-15	r	s	r	r	r	r	r	i	s	SXT, TE, CIP
1507	Ont:H21	CTX-M-15 and TEM-1	r	r	r	r	r	i[b]	s[b]	s[b]	s	K, SXT, TE, CIP
1027	Ont:H30	CTX-M-15 and TEM-1[c]	r	r	r	r	r	r	s[b]	s[b]	s	SXT, TE, CIP
490	O138:H48	SHV-12	r	s	r	r	r	i[b]	i[b]	s[b]	s	TE

[a] Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CXM, cefuroxime; CPD, cefpodoxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; K, kanamycin; GM, gentamicin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; CIP, ciprofloxacin; PB, polymyxin; s, sensitive; i, intermediate; r, resistant.
[b] It is known that many ESBL producers may appear susceptible or intermediate to oxyimino cephalosporins *in vitro* if CLSI criteria are applied strictly but do not respond to the respective therapies. Consequently, for clinical reporting these results have to be corrected to "resistant."
[c] Query coverage, 100%; maximal identity, 99% (http://www.ncbi.nlm.nih.gov/blast/).
[d] All isolates listed were identified as *E. coli*.

GATTGACCG3′ 5′AAACCAGTTACAGCCCTTCGG3′, and 5′TGGAGCCACGGTTGATGAGGG3′ (this study). The resulting amplicons were purified using the PCR purification kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. Custom sequencing was performed by Microsynth (Balgach, Switzerland), and the nucleotide and protein sequences were analyzed with Codon Code Aligner v. 3.7.1.1. For database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was used.

ESBL-producing strains were isolated from 34 healthy human carriers (5.8%). This is an average carriage rate compared to an estimated <3%, 5.5%, an estimated >10%, and 13.2% in Sweden, Spain, India, and Saudi Arabia, respectively (34). All 34 ESBL producers showed a synergy effect with at least one of the three Etest-ESBL strips, and they yielded factors >8 when ratios of MIC (cephalosporin)/MIC (cephalosporin + clavulanic acid) were calculated. Identification of all 34 isolates yielded *Escherichia coli* (Table 1). The β-lactamase genes of all ESBL-producing isolates were further characterized by PCR and sequencing (Table 1). One *bla* gene coded for a SHV ESBL (SHV-12), and 33 genes coded for CTX-M ESBLs. Of the $bla_{\text{CTX-M}}$ genes, 24 (70.6%) coded for CTX-M group 1 ESBLs (CTX-M-1, 10 strains; CTX-M-15, 14 strains), 7 (20.5%) for CTX-M group 9 (all CTX-M-14), and 2 (6%) for CTX-M group 2 (both CTX-M-2). All of the CTX-M group 2-positive isolates, 50% of the CTX-M group 1-positive *E. coli* isolates, and only 14.3% of the members of CTX-M group 9 harbored additional $bla_{\text{TEM-1}}$.

Besides β-lactam resistance, susceptibility to other classes of antibiotics was also tested; 26 strains were found resistant to tetracycline (76.5%), 23 strains were resistant to trimethoprim-sulfamethoxazole (67.6%), 10 strains were resistant to ciprofloxacin (29.4%), and 9 were resistant to aminoglycosides (26.4%). Four strains (11.8%) showed resistance to β-lactam antibiotics only (Table 1).

To identify relationships between the ESBL-producing strains, serotyping was carried out. All 34 strains were serotyped, resulting in as many as 29 different serotypes (Table 1). Only three serotypes, O2:48, O15:H18, and O102:H6, were found twice, and one, O153:H6, was found three times. This is in contrast to results for northwestern Spain, for example, where a strong predominance of O25b:H4 expressing CTX-M-15 was observed (28).

Currently, no studies describing the prevalence and characteristics of ESBL-producing *Enterobacteriaceae* in healthy human carriers are available in Switzerland, but there are two studies from Switzerland presenting data about infection-associated human ESBL producers (22, 31). The prevalence (0.7%) in hospitalized patients determined in 2007 was lower than in our study, but the CTX-M type distribution was the same (22). Despite the strict policy of antibiotic prescription in Switzerland (12), the determined prevalence rate of ESBL producers is almost identical to rates in other countries (25, 26, 34, 36). The increasing predominance of CTX-M group 1 enzymes, as has recently been described in strains from healthy food-producing animals in Denmark, France, the Netherlands, Portugal, and Switzerland (1, 13, 14, 16, 24) and in humans in the Netherlands, Norway, and Sweden (11, 24, 37), is also confirmed by our study. However, SHV-12—first described in Switzerland in 1997 (31)—has persisted in this country to the present day. The type mostly found in Switzerland, CTX-M-15, is distributed worldwide (4, 34). In contrast, CTX-M-9 together with CTX-M-14 (18, 28, 29), CTX-M-1 (4) and CTX-M-2 are the predominant types in Spain, Italy, and South America/Japan/Israel (2, 3, 4, 18, 29).

The fact that ESBL-producing strains are often also resistant to other classes of antimicrobial agents has been described in the past, and it is known that plasmids with bla_{CTX-M} genes often carry genes conferring resistance to, e.g., quinolones, aminoglycosides, and co-trimoxazole, etc. (14, 15). This location of different resistance genes on single plasmid replicons could explain the successful dissemination of bla_{CTX-M} genes by coselection (5, 15).

By serotyping, a high diversity within our ESBL producers was found (Table 1). The relatively high rates of intestinal carriage of ESBL producers in the general healthy human public and the high diversity among these strains, which is an indicator for high transmissibility of resistance factors, are worrisome. Further studies are necessary to assess future trends.

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