In the present study, two E. coli strain collections either known for a positive ESBL phenotype or for possession of Shiga toxin genes were screened for a combination of these two determinants. In detail, 62 ESBL-positive E. coli isolates recovered through routine diagnostics (Vet Med Labor GmbH, Germany) from the faecal contents of cattle/calves with diarrhoea between 2010 and 2012 in Germany, determined as ESBL producers by MIC testing (Vitek® 2; AST-GN38 or AST-N062) and the combination disc test (CLSI document M31-A3), were investigated for the possession of Shiga toxin genes in this study. A DNA array (Identibac EC, Alere, Germany) identified the stx2 gene in one strain (VB932491), which was further determined as stx2a by sequence analysis (Table 1). Another 87 E. coli, predominantly isolated from calves with diarrhoea in the past 30 years in the diagnostic laboratory of the Institute of Hygiene and Infectious Diseases of Animals, Germany, and known to harbour either an stx1 or an stx2 gene, as determined by PCR analyses,9 were screened by overnight incubation on Mueller–Hinton agar containing 4 mg/L cefotaxime. Here, one stx1-positive isolate (IHIT23778), further typed as stx1α by sequence analysis, showed growth, was further examined by MIC testing and could be finally confirmed as an ESBL producer by the combination disc test.

The ESBL-producing STEC strains were determined as Ont:NM and O145:NM, respectively. O145 represents a serotype that is counted among the ‘big six’ of non-O157 STEC O antigens, namely O26, O45, O103, O111, O121 and O145.7 Multilocus sequence typing (MLST) assigned this strain to sequence type (ST) 32, which is currently represented by another seven E. coli isolates from Germany and Norway, six of which were defined as human STEC or enterohaemorrhagic E. coli (EHEC) strains associated with HUS in the years 1996 to 2009, all expressing O145 (http://mlst.ucc.ie/mlst/dbs/Ecoli). The Ont:NM strain was assigned to ST301. Another two bovine ST301 STEC strains (O80:NM and O4:NM), isolated in 1992 and 1994, respectively, are available in the MLST database. However, none of these strains showed an ESBL phenotype, as confirmed in the present study.

Both ESBL-producing STEC strains possessed genes formerly detected in plasmid pO157, i.e. the putative serine protease precursor-encoding gene espP and EHEC haemolysin-encoding gene hlyA.1 Genes located on the genetic element O island 122 (efAI and nleABC) initially identified in EHEC strain EDL933 and associated with STEC capable of causing HUS and foodborne outbreaks were further detected.10 In addition, both strains harboured adherence-conferring protein gene iha, which is located on a chromosomal island recently acquired by O157:H7 STEC strains.11 Genes aggR, irp2 and pAA, which are related to the 2011 German O104:H4-CTX-M-15 Shiga toxin-producing enter-aggregative haemorrhagic E. coli outbreak strain, were absent.5 The ESBL-producing STEC strains possessed different types of eae genes (variants ε and γ). They also differed partly in the genes linked with the type III secretion system and with effector proteins located on the locus of enterocyte effacement (LEE) pathogenicity island (Table 1). Here, O145:NM strain IHIT23778 harboured tcpC, which encodes a Tir cytoskeleton coupling protein, and cif, which encodes type III secretion effector cycle inhibiting factor, in addition to esp genes, nleABC and tir. Finally, it differs from the Ont:NM strain in the possession of the catalase-peroxidase-encoding gene katP. These three genes are tightly associated with strains causing complicated EHEC infections underlining the serious threat of the acquisition of an ESBL plasmid by such a strain. PCR and sequence analysis revealed blaCTX-M-1 in our STEC strains. The transferability of ESBL plasmids was explored by filter mating using E. coli K-12 strain J53 as the recipient strain and tryptic soy agar (TSA) plates containing 100 mg/L sodium azide and 4 mg/L cefotaxime. According to PCR-based replicon typing, the donor strains contained four and three plasmids of different sizes and replicon types, respectively. Self-transferable plasmids carrying the blaCTX-M-1 genes were either of replicon type IncFII (VB932491) or IncII (IHIT23778).
Beyond their resistance to β-lactams, both isolates showed further resistance to trimethoprim/sulfamethoxazole, while the Ont:NM strain revealed additional resistance to gentamicin, tetracycline, tobramycin, enrofloxacin and marbofloxacin (Table 1). S83L and D87N substitutions in GyrA and an S80I substitution in ParC were most likely the mechanism of fluoroquinolone resistance in VB932491, as DNA array analysis proved the absence of plasmid-mediated quinolone resistance genes.

This is the first study revealing the presence of CTX-M-1 in a bovine STEC isolate, which has recently been associated with a human O64 STEC strain from India and which principally represents a common ESBL type among bovine isolates. Isolate IHIT23778 expressed one of the STEC-typical serotypes, i.e. O145, and both ESBL–STE C STs have already been described as STE C and/or EHEC strains in humans, underlining a putative zoonotic risk. The recent O104:H4-CTX-M-15-ST678 E. coli outbreak has once more shown that STEC with a presumed primary bovine source are carriers of ESBLs, suggesting a possible transfer of ESBL plasmids. A better understanding of the emergence, dissemination and characteristics of ESBL–producing STEC strains from bovines should therefore be a particular concern of global research.

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Transparency declarations
None to declare.

References
2 Buvens G, Bogerts P, Gluczynski Y et al. Antimicrobial resistance testing of verocytotoxin-producing Escherichia coli and first description

Table 1. Characteristics of ESBL-producing STEC isolated from the faeces of calves with diarrhoea from Germany

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Region, date of isolation</th>
<th>Serotype</th>
<th>ST/ST complex</th>
<th>bla genes</th>
<th>Plasmid replicon types</th>
<th>Phenotypic resistance</th>
<th>Mutations in GyrA/ParC</th>
<th>Genes detected by DNA array related to antimicrobial resistancea</th>
<th>Genes detected by DNA array related to virulenceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB932491</td>
<td>Lower Saxony, 05/2010</td>
<td>Ont:NM</td>
<td>301/165</td>
<td>blaoCTX-M-1, blaoTEM-1</td>
<td>FIB, FII (Tc), X1, Y</td>
<td>BLA, GEN, TET, TOB, ENR, MBX, SXT</td>
<td>S83L, D87N/S80I</td>
<td>aphA, arr-1, ereB, mphA, mphB, mxr, mrtA, rrs, dfr13, dfrA17, dfrA7, dfrV, strB, sul2</td>
<td>stx2a, cdtB, hlyA</td>
</tr>
<tr>
<td>IHIT23778</td>
<td>Baden-Wuerttemberg, 07/2013</td>
<td>O145:NM</td>
<td>32/32</td>
<td>blaoCTX-M-1</td>
<td>I1 (Tc), B/O, FIB</td>
<td>BLA, SXT</td>
<td>wild-type/wild-type</td>
<td>aac3Iva, aac6, oadA1, arr-1, ereB, mphB, mrtA, rrs, ant2Ia, dfrA17, dfrV, strAB, sul1/2</td>
<td>eae (γ), efα1, iha, iroN, cma, strA/B, mphB, astA, espB, espF, espP, nleABC, tir, tc cp, katP</td>
</tr>
</tbody>
</table>

BLA, β-lactams; ENR, enrofloxacin; GEN, gentamicin; MBX, marbofloxacin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TOB, tobramycin; D, dox purpose; I, isolucine; L, leucine; N, asparagine; S, serine; Tc, transconjugant.

aarr-1 (rifampicin); ereB (erythromycin); mph, mxr (macrolides); aac3Iva, aac6, oadA1, ant2Ia, aphA, mrtA (aminoglycosides); rrs, strA/B (streptomycin); sul1/2 (sulphonamides); dfrA7, dfr13, dfrA17, dfrV (trimethoprim).
bastA, enteroreaggregative heat-stable toxin; stx, Shiga toxin; cdt, cytolethal distending toxin; hlyA, EHEC haemolysin; toxB, toxin B; eae, intimin; efα1, EHEC factor for adherence; iha, iron-regulated homologue adhesion; iroN, salmonelhin siderophore receptor; cma, colicin B activity gene; cma, colicin M phosphatase; mch, microcin; is, increased serum survival protein; esp, EHEC secreted protein; cif, type III secretion effector cycle inhibiting factor; nle, non-LEE-encoded factor; tir, translocated intimin receptor; tc cp, Tir cytoskeleton coupling protein; katP, catalase-peroxidase.

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