Detection of Shiga toxin- and extended-spectrum β-lactamase-producing
Escherichia coli O145:NM and Ont:NM from calves with diarrhoea

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Sir,

Shiga toxin-producing Escherichia coli (STEC) are recognized as important human pathogens. Ruminants, in particular bovines, are the primary reservoir of STEC and transmission principally occurs via the food chain. So far, extended-spectrum β-lactamases (ESBLs) have been rarely observed among STEC strains. In humans, such findings are currently limited to five isolates belonging to serogroups O26:NM, O26:H11, O64 and O157:H7, expressing β-lactamases TEM-52, CTX-M-2, CTX-M-3, CTX-M-18 or CTX-M-1, and to the O104:H4-CTX-M-15 strain associated with an outbreak of haemolytic uraemic syndrome (HUS) in Germany.2,4,5 Even rarer is the finding of an extended-spectrum β-lactamase-producing STEC strain from animal sources, where only one O157-CTX-M-2 chicken isolate and one O111:H8-CTX-M-15 bovine isolate have been described so far.1,6

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 (HIT23778), further typed as stx1a 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 O145.6 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 enterohaemorrhaglic 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 P0157, i.e. the putative serine protease precursor-encoding gene espR and EHEC haemolysin-encoding gene hlyA.1 Genes located on the genetic element O island 122 (efA1 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.3 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 HIT23778 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 (HIT23778).
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</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB932491</td>
<td>Lower Saxony, 05/2010</td>
<td>O111:NM</td>
<td>301/165</td>
<td>blaoCTX-M-1, bluTEM-1</td>
<td>FIB, FII (Tc), XI, Y</td>
<td>BLA, GEN, TET, TOB, ENR, MBX, SXT</td>
<td>S83L, D87N/S80I</td>
<td>aphA, arr-1, ereB, mphA, mphB, mxr, rrs, dfr13, dfrA17, dfrF, strB, sul2</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), BO/FIB</td>
<td>BLA, SXT</td>
<td>wild-type/wild-type</td>
<td>aac3IVa, aac6, oaadA1, arr-1, ereB, mphB, rmtA, rrs, ant21a, dfrA17, dfrF, strAB, sul1/2</td>
</tr>
</tbody>
</table>

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

1 An ESBL: extended-spectrum β-lactamase.

2 The most virulent serotypes are O111 and O157.

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Beyond their resistance to β-lactams, both isolates showed further resistance to trimethoprim/sulfamethoxazole, while the O111: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 Sts have already been described as STEC 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 capable of causing devastating disease in humans. Uninvasive disease due to STEC are suspected, humans are usually not treated with antibiotics, as different agents might promote the induction of the Stx prohage and subsequent release of Stx toxin from lysed bacterial cells or the movement of toxin-encoding prophages to E. coli commensals. Indeed, low selective pressure may be a reasonable explanation for the infrequent finding of STEC ESBL in human samples. However, it remains unknown why STEC still represent a minor subpopulation of strains that have acquired ESBL genes in cattle, although these animals constitute the primary reservoir of STEC and are at the same time frequent 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.

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The carbapenemase threat in the animal world: the wrong culprit

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Sir,

We read with great interest two recent publications dealing with the same topic, i.e. the public health risk related to the occurrence of acquired carbapenemase-producing Gram-negative species in the animal world and the environment.1,2 Woodford et al.1 highlight the series of reports of carbapenemases found either in bacteria isolated from non-human sources or in Salmonella enterica subs. enterica, a zoonotic species. This raises concern about the real spread of these threatening organisms in the food chain and other non-human sources of infection. Abraham et al.2 focus on the possible role of companion animals as a source of carbapenemase-producing strains in humans, building their hypotheses on two studies reporting NDM-1-producing Escherichia coli isolates from dogs in the USA, and OXA-48-producing E. coli and Klebsiella pneumoniae isolates, also from dogs, in Germany. Both reports encourage public health authorities to implement measures to better evaluate the spread of carbapenemase-producing strains in animals, and reinforce efforts leading to a reduction of antibiotic consumption in veterinary practice.

We fully agree that the implementation of surveillance studies aimed to better evaluate and trace multidrug resistance in general, and carbapenem resistance in particular, is crucial in the fight against antibiotic resistance. We also agree that any effort towards a reduction of antibiotic consumption is valuable and must be sustained. However, we believe that the spread of carbapenemase-producing isolates among animals is not the main explanation for their occurrence in humans. Carbapenemases are not registered for use in veterinary medicine, even though they may be used in specific circumstances in companion animals or horses when dealing with multidrug-resistant Enterobacteriaceae. This usage, at least in developed countries, remains rare.3 The occurrence of carbapenemase-producing isolates in companion animals, as for extended-spectrum β-lactamase (ESBL) producers, most probably results from contamination from the animal keeper, who is statistically more exposed to broad-spectrum antibiotics, and in particular to broad-spectrum β-lactams, than the animal itself. In this regard, an increasing and irresponsible use of carbapenemases in companion animals might contribute to the selection and dissemination of carbapenem-resistant strains, and all efforts to avoid carbapenem use in veterinary practice should be pursued.3

The real threat related to carbapenemase resistance in humans comes from two main facts. The first corresponds to the increased consumption of carbapenems worldwide, as a consequence of an increased rate of resistance to broad-spectrum cephalosporins among human isolates. Therefore, carbapenems, although being last-resort antibiotics, are now considered to be first-line therapeutic options in certain geographical areas where multidrug resistance is endemic. The second main explanation comes from the overall increase in human population movements worldwide, including migration and tourism.4

In humans, carbapenemase-producing Enterobacteriaceae may be either hospital acquired (mostly K. pneumoniae), or community acquired (mostly E. coli), either as colonizers or infectious agents. Carbapenemase-producing E. coli are mainly the source of community-acquired infections or colonization.5 Of note, E. coli, by contrast with K. pneumoniae, may be identified in the food chain. Numerous studies have been conducted over the past decade to evaluate the possible link between the occurrence of ESBL producers among food-producing animals on one hand, and in humans on the other. Despite the fact that the rate of colonization...