Antimicrobial Resistance Testing of Verocytotoxin-Producing
Escherichia coli and First Description of TEM-52 Extended-Spectrum
β-lactamase in Serogroup O26

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We have investigated the antimicrobial resistance of verocytotoxin-producing Escherichia coli (VTEC) strains
isolated from humans, animals, food, and the environment in Belgium. Resistance was more frequent in
non-O157 strains from humans than in O157 strains from humans or other sources, and among non-O157
VTEC strains, intimin-positive strains were more resistant than intimin-negative strains. We also report the
first VTEC strain producing an IncI1 extended-spectrum β-lactamase encoded by plasmid-borne blaTEM-52;
this β-lactamase was previously associated with Salmonella enterica and E. coli isolates from different origins.

Verocytotoxin-producing Escherichia coli (VTEC) is associated
with gastrointestinal illness, and especially strains belonging
to serogroups O157, O26, O103, O111, and O145 may
cause hemolytic-uremic syndrome (HUS) (4). The cardinal
virulence trait involved in HUS development is the production
of one or more verocytotoxins types encoded by genes located
on temperate lambdoid bacteriophages (14). Although most postdiarrheal HUS cases are linked to VTEC infection, anti-
biotic therapy has not demonstrated beneficial effects after
HUS development (11, 14, 17). The underlying mechanism is
unknown, but bacterial lysis could increase the amount of
verocytotoxin released into systemic circulation and/or induce
verocytotoxin-containing bacteriophages.

Antimicrobials are widely used for disease prevention and
growth promotion in cattle and other farm animals identified
as important VTEC reservoirs [8; European Medicines
Agency, Joint opinion on antimicrobial resistance (AMR) fo-
duced on zoonotic infections, 2009 (http://www.ema.europa.eu
/pdfs/vet/sagam/44725909en.pdf)]. Consequently, resistance
may be promoted in VTEC commensally present in the intesti-
nal tracts of these animals. Recent reports indicate that antimi-
crobial resistance of VTEC is rising (15). Mora et al. re-
ported that bovine VTEC O157:H7 strains were significantly
more resistant to streptomycin, tetracycline, and sulfisoxazole
than those from humans, whereas non-O157 VTEC strains
isolated from humans and beef were more resistant than bo-
vine non-O157 strains (8). Most non-O157 strains showing
multidrug resistance belonged to HUS-associated serotypes.
Although the overall frequency of β-lactamases in E. coli iso-
lated from humans and farm animals is increasing (13, 16;
European Antimicrobial Resistance Surveillance System,
Susceptibility results for E. coli in Belgium, 2010 [http://www
.rivm.nl/ears/database/j], only a few extended-spectrum β-
lactamase (ESBL)-producing VTEC strains have been reported (3, 6, 10).

We evaluated the antimicrobial resistance of VTEC strains
isolated in Belgium from different origins in relation to estab-
lished virulence factors and report the first TEM-52-producing
VTEC isolate.

(Part of this research has been presented at the Pathogenic
Escherichia coli Network Conference Control and Manage-
ment of Pathogenic Escherichia coli, Dublin, Ireland, 17 and 18
September 2009.)

A total of 302 unduplicated, consecutive VTEC strains iso-
lated in Belgium from humans, as well as from animals (n =
48), food (n = 21), and the environment (n = 1), referred to our laboratory between 2004 and 2009 by several Belgian lab-
oratories were investigated. Among strains from humans, 153
belonged to serogroup O157 and 149 to non-O157 serogroups,
whereas all strains of nonhuman origin (n = 70) belonged to
serogroup O157. These strains were isolated from cattle (n =
47), a dog (n = 1), ground beef (n = 20), cheese (n = 1), and
dust (n = 1). Four established virulence genes, the verocyto-
toxin 1 and 2 genes (vtx1 and vtx2), the intimin gene (eaeA),
and the enterohemolysin gene (ehxA) were searched for by
PCR (9). The flagellar type (flfC) was determined by PCR-
restriction fragment length polymorphism (RFLP) (7). In vitro
susceptibility tests were performed by the disk diffusion
method for the antimicrobials listed in Table 1 using the
EUCAST and CLSI potency Neo-Sensitabs tablets (Rosco,
Taastrup, Denmark), with interpretation of zones according to
CLSI, as described by the manufacturer (Rosco Diagnostica
A/S; Neo-Sensitabs user’s guide, document 3.1.0, 2010 [http:
//www/rosco.dk/j]). In addition, cefotaxime plus clavulanate
and ceftazidime plus clavulanate were systematically tested, and an
ESBL was considered to be present if the inhibition zone
increased by ≥5 mm in comparison with that for the antibiotic
alone.

Results are shown in Table 1. One hundred two (102/302;
33.8%) human isolates showed resistance to at least one antibiotic. Combined resistance to streptomycin, sulfonamide, and tetracycline occurred in 15 (9.8%) of 153 O157 VTEC isolates; 22 of 26 (84.6%) streptomycin-resistant strains were also resistant to sulfonamide. In non-O157 strains, ampicillin-streptomycin-sulfonamide-tetracycline was the most frequently observed multidrug resistance profile (29/149; 19.5%). Most (58/59) sulfonamide-resistant non-O157 strains were also resistant to sulfonamide. In non-O157 strains, ampicillin-streptomycin-sulfonamide-tetracycline occurred in 15 (9.8%) of 153 O157 VTEC isolates.

No significant difference in levels of resistance was observed among O157 VTEC strains from humans and other sources, or when only isolates from cattle were considered.

ESBL production was detected in one isolate from an afebrile 70-year-old man with nonbloody diarrhea and abdominal cramps. Fecal cultures were positive for VTEC O26:H- (fliC type H11) and Campylobacter jejuni. The O26:H- strain showed resistance to ampicillin, susceptibility to B-lactam combinations, and intermediate susceptibility to all tested cephalosporins except cepime. It was confirmed as an ESBL producer using Etest ESBL cefotaxime/cefotaxime plus clavulanic acid (CT/CTL) and ceftazidime/ceftazidime plus clavulanic acid (TZ/TZL) strips (AB Biodisk, Solna, Sweden). PCR sequencing of the whole 850-bp coding sequence using primers TEMFL-F (5'-AGT ATT CAA CAT TTY CGT G-3') and TEMFL-R (5'-TTA CCA CAT TTY CGT G-3') revealed a sequence identical to that of blaTEM-52 (2). This gene was borne on an IncI1 replicon type-containing plasmid as determined by PCR-based replicon typing (1). The presence of TEM-52 and its association with plasmids belonging to the IncI1 incompatibility group have been previously demonstrated for several Salmonella enterica serovars isolated from poultry and humans and recently in E. coli strains from healthy humans and broilers (2, 12, 13, 16).

Antimicrobial resistance among food-borne bacteria has been rising worldwide since the early 1990s, albeit to a lesser extent in VTEC (15). Our data show that both O157 and non-O157 strains are frequently resistant to ampicillin, streptomycin, sulfonamide, and tetracycline. Compared to O157 isolates, non-O157 VTEC strains were significantly more resistant to 8 of the 21 antimicrobials tested. This is in contra-

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**TABLE 1. Antimicrobial resistance and correlation with the presence or absence of the intimin gene (eaeA) in VTEC isolated from humans and other sources in Belgium**

<table>
<thead>
<tr>
<th>Antimicrobial(s)</th>
<th>All (n = 302)</th>
<th>O157 (n = 153)</th>
<th>Humans</th>
<th>Non-O157</th>
<th>Animals and food (all O157; n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Ampicillin*&amp; &amp;</td>
<td>43 (14.2)</td>
<td>8 (5.2)</td>
<td>35 (23.5)</td>
<td>23 (26.1)</td>
<td>12 (19.7)</td>
</tr>
<tr>
<td>Piperacillin-tazobactam*</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Amoxicillin + clavulanic acid*&amp; &amp;</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cefazolin* &amp;</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cefuroxime*</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cefotaxime*</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Chloramphenicol*</td>
<td>11 (3.6)</td>
<td>1 (0.7)</td>
<td>9 (6.0)</td>
<td>5 (5.7)</td>
<td>4 (6.6)</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cefepime*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Aztreonam*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Meropenem*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Nalidixic acid*</td>
<td>16 (5.3)</td>
<td>16 (10.7)</td>
<td>16 (10.7)</td>
<td>12 (13.6)</td>
<td>4 (6.6)</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Streptomycin&amp;</td>
<td>84 (27.8)</td>
<td>58 (38.9)</td>
<td>40 (45.5)</td>
<td>18 (29.5)</td>
<td>12 (17.1)</td>
</tr>
<tr>
<td>Gentamicin* &amp;</td>
<td>2 (0.7)</td>
<td>2 (1.3)</td>
<td>2 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Kanamycin&amp;</td>
<td>25 (8.3)</td>
<td>20 (13.4)</td>
<td>17 (19.3)</td>
<td>3 (4.9)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Amikacin*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>59 (19.5)</td>
<td>44 (29.5)</td>
<td>31 (35.2)</td>
<td>13 (21.3)</td>
<td>4 (5.7)</td>
</tr>
<tr>
<td>Chloramphenicol&amp;</td>
<td>11 (3.6)</td>
<td>9 (6.0)</td>
<td>5 (5.7)</td>
<td>7 (6.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sulfonamide*</td>
<td>81 (26.8)</td>
<td>59 (39.6)</td>
<td>40 (45.5)</td>
<td>19 (31.1)</td>
<td>11 (15.7)</td>
</tr>
<tr>
<td>Trimethoprim* &amp;</td>
<td>28 (9.3)</td>
<td>24 (16.1)</td>
<td>16 (18.2)</td>
<td>8 (13.1)</td>
<td>3 (4.3)</td>
</tr>
</tbody>
</table>

* a, antimicrobials used in human medicine; &, antimicrobials used in veterinary medicine.

b By PCR.
diction to earlier findings by Mora et al., who showed similar resistance levels among O157 and non-O157 VTEC strains (8). Moreover, we provide further evidence for an enhanced resistance to streptomycin, kanamycin, and tetracycline among non-O157 strains carrying intimin, an adhesin associated with more-severe disease (5, 8). Antibiotics do not beneficially influence clinical outcome and may even increase HUS risk. Resistance could still worsen the outcome by selecting VTEC in the guts of treated patients. To our knowledge, only three ESBL-producing VTEC isolates have been described in the literature, two belonging to serogroup O26 (CTX-M-3 and CTX-M-18) and one to O157 (CTX-M-2) (3, 6, 10). With the isolation of a TEM-52 VTEC O26:H- strain, three of the four reported ESBL-positive VTEC strains belong to O26, suggesting a higher propensity of this O serogroup to acquire ESBL genes.

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We declare that we have no conflict of interest.

REFERENCES