Development of a Simple Latex Agglutination Assay for Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) by Using Polyclonal Antibody against STEC

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ABSTRACT

Rabbit antiserum raised against the whole cell antigen of Shiga toxin-producing Escherichia coli (STEC) strain VT3 (stx₁⁺ stx₂⁺ eae⁺), was repetitively adsorbed with heat-killed cells of different strains of non-STEC and other enteric bacteria. Thus the antiserum obtained was designated VT3 antiserum. VT3 antiserum reacted with intimin type of γ. We assessed the reactivity of VT3 antiserum to whole-cell lysates of 87 strains of E. coli and other enteric bacteria by immunoblotting. The antiserum recognized the 97-kDa protein in whole cell lysate from the strain VT3 and thirty six (83.7%) strains out of the 43 STEC strains were detected positive for STEC antigen. None of the strains of non-STEC and other species examined was positive by immunoblotting. Based on this result we developed a latex agglutination assay for detection of STEC strains. Thirty five (81.4%) of 43 STEC strains were detected positive for STEC antigen by latex agglutination. One (3.3%) of the 30 strains of non-STEC tested and none of the strains of other enteric bacteria included in this study were positive by latex agglutination assay. The corresponding specificity of latex agglutination assay was approximately 98%. Results of this study showed the production of STEC antiserum, and the generation of a simple, cost-effective, sensitive and specific latex agglutination assay for establishing an etiological diagnosis of STEC.

Keywords: Immunoblot, Latex agglutination, Polyclonal antibody, Shiga toxin-producing Escherichia coli, VT3 antiserum.
INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), predominantly of serotype O157:H7 is now one of the most important etiologic agents in hemorrhagic colitis and hemolytic uremic syndrome (HUS) (6, 7, 8, 12, 13, 30). The ability of STEC to cause serious disease in humans is related to the production of one or more Shiga toxins (Stx₁, Stx₂, or their variants), which inhibits protein synthesis of host cells, thus leading to cell death (14, 22). STEC is a serologically diverse group of food-borne, zoonotic pathogens, of which the serotype O157:H7 has been epidemiologically significant worldwide because of its notoriety of being associated with life-threatening disease (13). However, in some geographic areas, non-O157 strains are more commonly isolated from persons with diarrhea or HUS than are O157 STEC strains (25, 28). Hemorrhagic colitis is caused by a number of serotypes of STEC (12). Antibodies to the O157 antigen are used in many assays to detect O157:H7 isolates in clinical and food samples. However, previous studies showed that the anti-O157 sera cross-reacted with *Citrobacter freundii* and other bacterial species (4, 24). Although detection of EPEC and EHEC using monoclonal antibody has been reported earlier (15), developing monoclonal antibody is expensive for many laboratories. Biochemical methods to identify enterohemorrhagic *E. coli* (EHEC) strains, a subgroup of STEC, are based on biochemical markers such as sorbitol fermentation deficiency and β-D-glucuronidase non-productivity of O157 serotype *E. coli* strain (10, 21). Existences of sorbitol fermenting and β-D-glucuronidase positive O157 strains reduce the value of these phenotypes (9). Molecular biology based detection systems for the diagnoses of STEC (3, 5, 16, 18, 23, 26, 29) are expensive for many
laboratories. In this paper we describe the production of antiserum specific for STEC, as well as their use in developing a simple assay system for detection of STEC.

MATERIALS AND METHODS

**Bacterial strains.** The strains used in this experiment were *E. coli* O157:H7 strain EDL933 (STEC, stx$_1^+$ eae$^+$), VT3 (STEC, stx$_1^+$ stx$_2^+$ eae$^+$), ETEC (O125, stx$_1^-$ stx$_2^-$ eae$^-$) and another 43 strains of Shiga toxin (Stx$_1$/Stx$_2$)-producing *E. coli* isolated from different sources in Kolkata, India. Shiga toxin non-producing *E. coli* (stx$_1^-$ stx$_2^-$ eae$^-$) strains DH5$_α$, PC12 (O114), PC26 (O159), PC35 (ND), PC63 (O159) and another 25 non-STEC strains [3 (O128), 3 (O114), 5 (O111), 2 (O26), 3 (O159), 4 (ONT) & 5 (ND)] were also included in the study. Other enteric bacteria (three of *Vibrio cholerae* [one strain for each of O1 (strain NB2), O139 (strain SG24) and non-O1, non-O139 (strain PC2) serotypes], three of *Klebsiella pneumonia*, two of *Pseudomonas aeruginosa*, two of *Flavobacterium multivorum*, one of *Vibrio mimicus*, two of *Enterobacter agglomerans*, and one of *Aeromonas hydrophila* strains) were also used in this study. Virulence gene profiles of the strains used here are given in Results section. Strains were preserved in Luria broth supplemented with 15% glycerol at -70°C and also in nutrient agar stab culture at room temperature.

**Polymerase chain reaction.** Amplification of the target gene was carried out by PCR assay using bacterial cell lysate as the source of template DNA. Strains were grown on Luria agar (HiMedia) for 18 h at 37°C. Single colonies were picked up from the Luria agar and then inoculated into 3 ml Luria broth (LB) (HiMedia) and incubated overnight at 37°C in a shaker. Following overnight incubation, bacterial cells from 100 µl bacterial
culture were washed with normal saline by centrifugation. The cell pellet was
resuspended in 1 ml of double-distilled water and boiled for 10 min. Cell debris was
removed by centrifugation, and the supernatant containing the template DNA was
transferred into a fresh microfuge tube for PCR assay. PCR amplification of the target
dNA was carried out in a thermal cycler (Perkin-Elmer Applied Biosystems, Weiterstadt,
Germany) using 200-µl PCR tubes with a reaction mixture volume of 25 µl. PCR for
detecting both chromosome (\textit{stx}_1, \textit{stx}_2, and \textit{eae})- and plasmid (\textit{hlyA})-encoded virulence
genes was performed as described earlier (12, 19, 20, 32). PCR products were
electrophoresed through 1.5% (w/v) agarose gel to resolve the amplified products which
were visualized under UV light after ethidium bromide staining. The primer sequences
and conditions are given in Table 1.

\textbf{Polyclonal antibody preparation.} Isolated colony of \textit{E. coli} (STEC) strain VT3 from
MacConkey agar (HiMedia) was inoculated into TSB and incubated for 18 h at 37°C
under constant shaking condition. The cells were harvested by centrifugation and washed
thrice with 10mM PBS (pH 7.4). Washed cells were suspended in PBS, and heat killed by
steaming in an autoclave for 10 min. The bacteria were then diluted in PBS to 70%
transmittance at 610 nm (17). This method was used to prepare whole cell antigen. On
day 0, New Zealand white rabbits were immunized subcutaneously with 2 ml emulsion
comprising of 1 ml whole cell antigen and 1 ml Freund’s complete adjuvant (Difco
laboratories, USA). On day 21, each of those rabbits was injected subcutaneously with 2
ml emulsion of 1 ml whole cell antigen and 1 ml Freund’s incomplete adjuvant (Difco).
On day 42 each rabbit was boosted subcutaneously with 1 ml whole cell antigen with out
adjuvant. Rabbits were exsanguinated on day 49. Blood was allowed to clot at room
temperature, and serum was collected and stored at -20°C. VT3 antibody production was
determined by agglutination assay using *E. coli* strain VT3 grown on MacConkey agar or
nutrient agar, as the antigen. Sera obtained by this method were checked for cross-
reactivity with other strains of *E. coli* by slide agglutination method. Agglutination assays
were performed on glass slides by mixing 20 µl of diluted antiserum (in PBS) with a loop
full of bacteria.

**Antiserum adsorption.** Antiserum was adsorbed with heat-killed cells of *E. coli* strain
DH5α, a strain of enterotoxigenic *E. coli* (ETEC) (O125); and non-STEC strains PC12
(O114), PC26 (O159), PC35 (ND) and PC63 (O159) sequentially. The adsorption was
repeated thrice with heat killed cells for each strain. Heat killed bacterial cells were added
to the antiserum at a ratio of 0.1 ml packed cells per ml serum and the mixture was gently
stirred at 25°C for 2 h. After centrifugation, the serum was separated. Adsorbed antiserum
was stored at -20°C for further use. Cross-reactivity of the sera was tested with the cells
of *E. coli* strains PC12, PC26, PC35, PC63, and ETEC; and a strain of STEC (EDL933).

**Reactivity of VT3 antiserum with intimin.** Plasmid pIntg934 encoding intimin type γ
(*E. coli* O157:H7 intimin type) was kindly provided by Dr. J. Sinclair. This plasmid was
transformed by electroporation into *E. coli* strain BL21 (DE3), and cells with the plasmid
were selected for ampicillin resistance. These cells produced full-length intimin molecule
of 934 amino acids of type γ in the outer-membrane of transformed bacteria (27).
Reactivity of transformed and untransformed whole cells with VT3 antiserum was
checked by slide agglutination.

**Immunoblotting.** Whole-cell bacterial lysates were prepared as follow (25). Bacteria
were grown to log phase in TSB, harvested by centrifugation and washed three times in
PBS. Cells were resuspended in 1/10 volume of PBS containing Phenylmethane sulfonyl fluoride and adjusted spectrophotometrically to a concentration of $5 \times 10^9$ cells/ml. SDS-sample buffer (60 mM Tris-HCl buffer, pH 6.8, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% glycerol (v/v) and 0.001% (w/v) bromophenol blue) was added (1:1) immediately, vortex and the solution was heated for 5 min at 100°C. 30 µl of that mixture was loaded per well onto SDS-polyacrylamide gels. Electrophoresis was done on 12% SDS-PAGE gels in a Mini-PROTEAN II Dual Slab Cell (Bio-Rad Laboratories, Richmond, USA). Gels were stained with Coomassie brilliant blue to ensure even loading. Proteins separated by SDS-PAGE were blotted onto nitrocellulose membranes (0.45 µm pore size, BioRad) by use of a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) in 15.6 mM Tris, 129 mM glycine, 20% methanol (pH 8.3) for 5 h at 60 V (31). After washing the nitrocellulose membrane in 10 mM Tris buffered saline (TBS) pH 7.6, the extra binding sites on membrane were blocked by incubating in 3% bovine serum albumin in TBS for 90 min at 37°C followed by washing with 10 mM TBS containing 0.05% Tween 20 (TBS-T). The membranes were then incubated for 1 h with primary antibody (VT3 antiserum) diluted (1:2000) in antibody buffer (1% BSA in TBS-T) at room temperature. After washing with TBS-T, primary antibody exposed membranes were incubated with goat anti-rabbit immunoglobulin G horseradish peroxidase-conjugate (Bangalore Genei, India) in antibody buffer for 1 h at room temperature. After thorough washing with TBS-T, membranes were incubated with substrate (tetramethyl benzidine and 0.02% H$_2$O$_2$ in distilled water) until colour development was sufficient. The developed sheets were washed in distilled water and air dried. Developed sheets
were scanned with a Hewlett-Packard ScanJet 2400 scanner, and the image of the blot was arranged for the figure and labeled with Adobe Photoshop version 7.

**Latex agglutination test.** Latex beads suspended in glycine saline buffer (Bangalore Genei, India) were coated with serially diluted VT3 antiserum in glycine saline buffer. 100 µl of supplied beads were diluted with 200 µl of glycine saline buffer and 300 µl of diluted antiserum was added to it. It was then incubated for 2 h at 37°C and centrifuged at 5000 rpm for 10 min. Supernatant was carefully aspirated out. The pellet was resuspended in 1.5 ml of blocking buffer (10 mM PBS, pH 7.4 containing 3% BSA) and centrifuged. The beads were washed two more times with the blocking buffer. After the final wash, beads were resuspended in 600 µl of blocking buffer and incubated overnight at 4°C. This method was used to coat the bead with antibody. 20 µl of coated beads was mixed with one colony of live cells from either MacConkey or nutrient agar on a glass slide and observed for agglutination reaction within one min.

**RESULTS**

**Polyclonal antibody preparation against the whole cells of STEC strain VT3.** New Zealand albino rabbits were immunized with heat killed cells of a STEC strain VT3. After the third injection antibodies were detected at high dilutions. Sera were collected and kept frozen in aliquots. Average 8 ml of antiserum was obtained per rabbit immunized with heat killed cells of VT3 strain. After adsorption with the heat killed cells of STEC strains, cross reactivity of the adsorbed antiserum was checked with the cells of other enteric bacteria (*K. pneumoniae*, *P. aeruginosa*, *V. cholerae*, *A. hydrophila*, *K. oxytoca*, *V. fluvialis*, *V. vulnificus*, *Ent. amnigenus*, *Ent. agglomerans*, *F. odoratum*, *F.
multivorum, and S. marcescens). It was found that the antiserum agglutinated the cells of a strain of K. pneumoniae (PC47), a strain of F. multivorum (PC69), and a strain of Ent. agglomerans (PC56). The antiserum was then further adsorbed with those cross reactive strains following the method as described in Materials and Methods. Thus the antiserum specific for VT3 was obtained and designated VT3 antiserum. From average 8 ml of crude sera, 6 ml VT3 antiserum was obtained. Slide agglutination experiments were first performed with serial dilutions of the antiserum with the live homologous E. coli strain VT3 cells to select an appropriate working dilution. The reciprocal of the working dilution of the VT3 antiserum was 20. This antiserum did not agglutinate the cells of non-STEC strains [strains DH5α, PC12 (O114), PC26 (O159), PC35 (ND), PC63 (O159) and ETEC (O125)]; K. pneumoniae; E. agglomerans; and F. multivorum using which the antiserum was adsorbed. It did cross-react with a STEC strain EDL933 (Table 2).

PCR. PCR analysis was performed to confirm the presence of stx1 [encodes Shiga toxin variant 1 (Stx1)], stx2 [encodes Shiga toxin variant 2 (Stx2)], hlyA (encodes hemolysin) and eae (encodes intimin) gene sequence/s. STEC strains were either positive for stx1 or stx2 or both of stx1 and stx2 gene sequences. Genotypes of the strains of non-STEC and other bacterial species used in this study were stx1⁺ stx2⁻ eae⁻. Virulence gene profiles of E. coli strains used in the present study are shown in Table 3.

Reactivity of VT3 antiserum with intimin. VT3 antiserum agglutinated the whole cells of E. coli strain BL21 (DE3) with plasmid pIntg934 encoding intimin type γ (E. coli O157:H7 intimin), and did not agglutinate the untransformed cells. As the cells produced full-length intimin molecules in the outer-membrane of transformed bacteria (27), the results indicated that VT3 antiserum reacted with intimin.
**Immunoblotting.** We assessed the reactivity of VT3 antiserum to whole-cell lysates of the strains of *E. coli* and other enteric bacteria (listed in Materials and Methods) by immunoblotting. It was found that VT3 antiserum recognized the 97-kDa protein in whole cell lysates from the strains VT3 and EDL933; and did not cross-react with the ETEC (O125) strain [Fig. 1]. Immunoblot analysis with the whole cell antigens prepared from the 87 strains using VT3 antiserum was performed. Thirty six (83.7%) strains out of the 43 STEC strains were detected positive for STEC antigen and seven (three cattle isolates and four environmental isolate) were negative (Table 4). None of the strains of non-STEC and other enteric bacteria examined here was positive by immunoblot analysis.

**Development of a simple latex agglutination method to detect STEC.** Based on the results of immunoblotting assays, a simple latex agglutination assay was developed using VT3 antiserum for detection of STEC in the present study. Determination of working dilution of VT3 antiserum to coat the latex beads is shown in Table 5. Results obtained here indicated that latex agglutination test showed positive test with antiserum at higher dilution (between 1:1000 and 1:2000) as compared with slide agglutination test (1:20). It was also calculated that one ml of VT3 antiserum was sufficient to perform 10,000 tests. By latex agglutination assay developed in this study, it was found that thirty five (81.4%) of 43 STEC strains were detected positive for STEC antigen and eight (four cattle isolates and four environmental isolate) were negative (Table 4 & 6). The corresponding sensitivity of latex agglutination assay was 81.4%, whereas it was 83.7% for immunoblot assay. One (3.3%) of the 30 strains of non-STEC and none of the strains of other enteric
bacteria included in this study were positive by latex agglutination assay (Table 6). The corresponding specificity of latex agglutination assay was approximately 98%.

**DISCUSSION**

The purpose of the study described here was the production of polyclonal antibody specific for STEC, and their use for development of a sensitive and specific immunodiagnostic assay for detection of Shiga toxin-producing *Escherichia coli* (STEC) that would avoid expensive reagents and equipment. VT3 antiserum was specific, as could be seen by the reactivity in immunoblotting assay with the different strains of STEC and non-reactivity with non-STEC strains used in the present study. A band at around 97-kDa was detected by the reaction of the whole cell antigens of the STEC strains with VT3 antiserum. Several authors have documented that among the surface antigen on EHEC, intimin (encoded by *eae* gene) is the most immunogenic and its size is around 97-kDa (4, 16, 25). In the present study, serologically it was confirmed that VT3 antiserum reacted with intimin type γ and it could be indicated here that the 97-kDa protein recognized by VT3 antiserum was intimin. Previous studies reported the existence of at least three immunologically distinct groups of intimins, i.e., those similar to intimins from RDEC-1, EPEC E2348/69 (O127:H6), and EHEC (O157:H7); and this cross-reactivity did not appear to be serogroup specific (2, 11). The primer sequences for *eae* gene (encodes intimin) used in our study for PCR, was developed based on *eae* gene sequences of EHEC (O157:H7) (31). There might be some diversity in the *eae* gene sequences between that of O157 and non-O157 STEC. That might be the reason for which *eae* gene sequences was not detected by PCR for most of the STEC strains
included in the present study. However, despite the diversity in the polypeptide domain, two stretches of six and seven amino acids (WLQYGQ and WAAGANKY) are identical in all intimins for EPEC strains (1). It was also reported earlier that a group of EPEC strains did not produce the PCR product with either Int-α or Int-β primers, but was recognized poorly by both anti-intimin α and anti-intimin β sera (1). In the present study it was found that the strains of STEC were recognized by the VT3 antiserum and majority of them did not produce the PCR product with the primer for EHEC specific *eae* sequence used in the study. Immunoblotting assay confirmed the extent of serological cross-reactivity among the STEC strains of different serotypes isolated from different sources including human, cattle and environment. By latex agglutination test, STEC at a concentration of $5 \times 10^6$ to $5 \times 10^7$ CFU/ml could be easily detected using latex beads coated with antiserum diluted 1:2000. Some of the STEC strains evaluated were detected negative by latex agglutination tests. The strains that were negative by latex agglutination assay were isolated from either cattle or environment. This could be suggested that those strains were unable to express sufficient antigen. Only 2% of non-STEC and other enteric bacteria used (listed in Materials and Methods) were detected positive using latex agglutination assay, supporting the specificity of the assay. Although the specificity of the immunoblotting assay was higher than that of latex agglutination method to detect STEC, the latex agglutination assay would avoid costly equipment and required minimal laboratory facilities. Furthermore, the production of polyclonal antiserum specific for STEC would be much less expensive than that of monoclonal antibody. Overall, the results of our study had shown the production of highly specific polyclonal antiserum, and the generation of a simple, cost-effective, sensitive and specific latex agglutination
assay for establishing an etiological diagnosis of STEC. This method may also be employed for epidemiological surveillance.

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polymorphism assay for the epidemiological analysis of Shiga toxin-producing

nucleolin with equivalent affinity but lower avidity than to the translocated

detection and subtyping methods, pp. 331-356, In J.B. Kaper and A.D. O'Brien
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American Society for Microbiology, Washington, D.C.

Development of a rapid PCR method using the insertion sequence IS1203 for


Legend to the figure:

Fig. 1. Nitrocellulose immunoblot using VT3 antisera with whole cell preparations of *E. coli* strains VT3 (O157, STEC) lane 1, and EDL933 (O157, STEC) lane 2. Marker size in kilodaltons (kDa) [phospholipase B (103-kDa), BSA (77-kDa), ovalbumin (50-kDa), carbonic anhydrase (34.3-kDa), and soybean trypsin inhibitor (28.8-kDa)].
<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence of primers</th>
<th>Target</th>
<th>Length of PCR product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVT1</td>
<td>5’-CAACACTGGATGATCTCAG-3’</td>
<td>stx family&lt;sup&gt;a&lt;/sup&gt;</td>
<td>349</td>
<td>19</td>
</tr>
<tr>
<td>EVT2</td>
<td>5’-CCCCCTCAACTGCTAATA-3’</td>
<td>stx family&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>EVS1</td>
<td>5’-ATCAGTCGTCACCTCAGGGT-3’</td>
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<td>110</td>
<td>19</td>
</tr>
<tr>
<td>EVC2</td>
<td>5’-CTGCTGTCACAGTGACAAA-3’</td>
<td></td>
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<td>EAE1</td>
<td>5’-AAACAGGTTGGAGACTGTTGCC-3’</td>
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<tr>
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<tr>
<td>hlyA1</td>
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<td>hlyA4</td>
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<td>hly&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>PCR consists of 30 cycles, each of which consisted of three steps in the following order: denaturation at 94°C for 60s, annealing at 55°C for 60s, and extension at 72°C for 60s. After 30 cycles, final extension step of 10 min at 72°C was performed.

<sup>b</sup>30 cycles, each of which consisted of three steps: denaturation at 94°C for 60 s, annealing at 55°C for 90 s, and extension at 72°C for 90 s.

<sup>c</sup>30 cycles, each of which consisted of three steps: denaturation at 94°C for 30 s, annealing at 57°C for 60 s, and extension at 72°C for 90 s.
### Table 2. Slide agglutination reactions of anti-STEC antiserum against different strains of *E. coli*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>VT3</th>
<th>EDL933</th>
<th>ETEC</th>
<th>DH5α</th>
<th>PC12</th>
<th>PC26</th>
<th>PC35</th>
<th>PC63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reactivity with unadsorbed sera&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<td>Cross-reactivity with adsorbed sera&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>eae&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

<sup>a</sup>+++; Strong agglutination; ++, moderate agglutination; +, agglutination; −, No agglutination.
**TABLE 3.** Virulence gene profiles obtained by PCR analysis of *E. coli* strains used in the study

<table>
<thead>
<tr>
<th>Virulence gene(s)</th>
<th>Human</th>
<th>Cattle</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;</td>
<td>3</td>
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<td>1</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>stx</em>&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5</td>
<td>3</td>
<td>1</td>
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<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>hlyA</em>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>hlyA</em>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
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</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>stx</em>&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>hlyA</em>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>eae</em>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt; <em>hlyA</em>&lt;sup&gt;+&lt;/sup&gt; <em>eae</em>&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td><em>stx</em>&lt;sub&gt;1&lt;/sub&gt; <em>stx</em>&lt;sub&gt;2&lt;/sub&gt; <em>hlyA</em>&lt;sup&gt;-&lt;/sup&gt; <em>eae</em>&lt;sup&gt;-&lt;/sup&gt;</td>
<td>10</td>
<td>2</td>
<td>18</td>
</tr>
</tbody>
</table>

*stx*<sub>1</sub> [encodes Shiga toxin variant 1 (*Stx*<sub>1</sub>)], *stx*<sub>2</sub> [encodes Shiga toxin variant 2 (*Stx*<sub>2</sub>)], *hlyA* (encodes hemolysin) and *eae* (encodes intimin) gene sequences were detected by PCR (Materials and methods).

<sup>a</sup>Non-STEC strains.
**TABLE 4.** Results obtained with immunoblotting and latex agglutination on the STEC strains

<table>
<thead>
<tr>
<th>Source</th>
<th>Serotype</th>
<th>No. of positive (no. of strains tested) by following assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Immunoblotting</td>
</tr>
<tr>
<td>Human</td>
<td>O29/O29:H1/</td>
<td>12 (12)</td>
</tr>
<tr>
<td></td>
<td>O28ac:H1</td>
<td>4 (4)</td>
</tr>
<tr>
<td>cattle</td>
<td>O124</td>
<td>8 (10)</td>
</tr>
<tr>
<td>cattle</td>
<td>ONT</td>
<td>1 (1)</td>
</tr>
<tr>
<td>cattle</td>
<td>O114</td>
<td>2 (2)</td>
</tr>
<tr>
<td>cattle</td>
<td>O28ac</td>
<td>1 (1)</td>
</tr>
<tr>
<td>cattle</td>
<td>O111</td>
<td>1 (2)</td>
</tr>
<tr>
<td>cattle</td>
<td>O55</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Environment</td>
<td>O111</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Environment</td>
<td>O136</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Environment</td>
<td>O125</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Environment</td>
<td>ONT</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

ONT, O-antigen non type able.
**TABLE 5.** Results obtained in Latex agglutination test

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>Beads coated with VT3 antiserum at the dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4×</td>
</tr>
<tr>
<td>EDL933</td>
<td>+++</td>
</tr>
<tr>
<td>VT3</td>
<td>+++</td>
</tr>
<tr>
<td>PC12</td>
<td>-</td>
</tr>
</tbody>
</table>

+++‚ Strong agglutination; ++, moderate agglutination; +, Weak agglutination; −, No agglutination.
### TABLE 6. Analysis of 87 bacterial strains (clinical and environmental) for STEC antigen by latex agglutination and immunoblotting assays

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>No. of strains tested</th>
<th>No (%) positive by the following assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Immunoblotting</td>
</tr>
<tr>
<td>STEC</td>
<td>43</td>
<td>36 (83.7)</td>
</tr>
<tr>
<td>Non STEC</td>
<td>30</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other enteric bacteria(^{a})</td>
<td>14</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\(^{a}\)Listed in Materials and Methods.
<table>
<thead>
<tr>
<th>kD</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.8</td>
<td>8</td>
</tr>
</tbody>
</table>