Occurrence and characterisation of Big Seven Shiga toxin-producing *Escherichia coli* (STEC) serotypes from healthy cattle in South Africa

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Shiga toxin-producing *Escherichia coli* (STEC) also termed Verotoxin producing *Escherichia coli* (VTEC) are foodborne zoonotic pathogens.

STEC disease in humans is characterised by mild to severe diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (kidney failure).

Cattle are considered the main reservoir of STEC and ingestion of foods of cattle origin is a risk for humans.
STEC Introduction


- In 1968, Kibel and Bernard in South Africa hypothesized that HUS was caused by a mutant *E. coli* lysogenized by bacteriophage.

- In 1992, STEC O157 outbreak was reported in Swaziland and South Africa (40,912 persons) - Contaminated water
“Big seven” STEC

- STEC serogroups O26, O45, O103, O111, O121, O145 and O157 are termed “Big seven” - associated with severe human disease including HUS.

- Most human STEC disease outbreaks have been attributed to STEC O157:H7 - **63,000** cases annually (US).

- Non-O157 STEC are responsible for **113,000 cases** of STEC diseases annually in the US (O26, O111, O103, O121 & O145).

STEC Transmission
Ruminants are central in transmitting STEC to humans (feces)

Minced meat = hamburger disease

Unpasteurized apple cider

manure

Water-Usutu River-Swaziland outbreak

Minced meat

Water

Spinach

Unpasteurized apple cider

Cattle: remain healthy, but humans become sick
STEC Chromosomal Virulence Genes

Bacteriophage-encoded toxins:

- Shiga toxins (Verotoxins):
  - Stx1 and variants (Stx1c, Stx1d)
  - Stx2 and variants (Stx2a - Stx2g)

Locus of Enterocyte Effacement (LEE)

- eaeA gene - encodes intimin
- Type III Secretion System (T3SS)

Type III Secretion System

- Complex protein secretion system employed by many Gram-negative pathogenic bacteria
- Transport bacterial effector proteins across three membrane barriers into eukaryotic host cytoplasm
- The effector proteins delivered by TTSS are capable of modulating and interfering with the host cellular processes,
  - plague,
  - typhoid fever,
  - bacterial dysentery
- Composed of more than 20 structural proteins, effector proteins, and chaperones.
Plasmid encoded Virulence Genes

- **ehxA** (enterohemolysin)- lyse erythrocytes
- **espP** (extracellular serine protease protein)-
  (autotransporter) enhances haemorrhage.
- **katP**- catalase peroxidase- neutralizing host cytotoxic oxidants.
- **etpD**- responsible for exoprotein secretion.
- **saa** (STEC autoagglutinating adhesion)
Non-LEE encoded Virulence Factor

- **PAI OI-122** - commonly found in STEC strains associated with severe STEC human disease

- Other PAI include OI-57, OI-71, OI-36, OI-43/48

- Nle genes are used as markers in molecular risk assessment (MRA) of STEC to predict the potential virulence of STEC strains - “seropathotyping”. .
OBJECTIVES

To identify and characterize Big seven STEC serotypes, virulence genes and antimicrobial resistance profiles.

Specific objectives:

- To identify “Big seven” STEC serogroups and serotypes in healthy cattle.

- To characterize STEC isolates for genes encoding virulence genes and markers and antimicrobials resistance profiles.
Collection of Shiga toxin-producing *E. coli* isolates (n=961)

Re-confirmation of *E. coli* & STEC by PCR

STEC (140 isolates)
- Major virulence factors and markers by PCR
- Stx variants, Plasmid encoded, PAI

Molecular Serotyping (PCR for O & H-typing)

Antimicrobial Resistance Profiling by Disk diffusion
Results: Serotyping

- The overall occurrence of “Big seven” STEC was 16.5% (92/559) and 43% for other serotypes.

![Bar chart showing serogroup/Cattle positive results]

- Serogroup O26: 10.2%
- Serogroup O45: 2.9%
- Serogroup O145: 2.5%
- Serogroup O157: 1.4%
- Serogroup O121: 1.1%
- Serogroup O103: 0.4%
## Results on Serotyping

<table>
<thead>
<tr>
<th>SERO GROUP</th>
<th>TOTAL ISOLATE</th>
<th>ID SEROTYPES</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26</td>
<td>156</td>
<td><strong>H2</strong> (42), H4 (2), H7 (4), <strong>H8</strong> (31), <strong>H11</strong> (10), H16 (2), <strong>H19</strong> (18), <strong>H21</strong> (17), H28 (2), H38 (7), H45 (1), NT (20)</td>
</tr>
<tr>
<td>O45</td>
<td>38</td>
<td><strong>H2</strong> (1), H8 (3), H11 (8), H16 (3), H19 (3), H21 (2), H28 (1), H38 (5), NT (12)</td>
</tr>
<tr>
<td>O145</td>
<td>23</td>
<td><strong>H2</strong> (1), H7 (1), <strong>H8</strong> (1), H11 (1), H19 (13), <strong>H28</strong> (3), NT(3)</td>
</tr>
<tr>
<td>O121</td>
<td>10</td>
<td><strong>H8</strong> (8), H21 (1), NT(1)</td>
</tr>
<tr>
<td>O157</td>
<td>12</td>
<td>H2 (1), <strong>H7</strong> (9), H19 (1), H28 (1)</td>
</tr>
<tr>
<td>O103 OTHER</td>
<td>2</td>
<td><strong>H2</strong> (1), <strong>H21</strong> (1)</td>
</tr>
<tr>
<td>NON-O157</td>
<td>175</td>
<td>42 O:H serotypes</td>
</tr>
</tbody>
</table>
Result - Major Virulence Genes

**Chromosomal Virulence Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>68.3%</td>
</tr>
<tr>
<td>stx1c</td>
<td>20.7%</td>
</tr>
<tr>
<td>stx1d</td>
<td>15.2%</td>
</tr>
<tr>
<td>stx2</td>
<td>96.3%</td>
</tr>
<tr>
<td>stx2a</td>
<td>91.8%</td>
</tr>
<tr>
<td>stx2c</td>
<td>97.0%</td>
</tr>
<tr>
<td>stx2d</td>
<td>56.0%</td>
</tr>
<tr>
<td>stx1 &amp; stx2</td>
<td>66.5%</td>
</tr>
<tr>
<td>eaeA</td>
<td>7.1%</td>
</tr>
</tbody>
</table>
katP and etpD genes were prevalent in eaeA-positive strains (p<0.000)
Non LEE effector \((nle)\) genes were prevalent in \(eaeA\) positive strains \((p < 0.000)\)
## Result- Antimicrobials

### Antimicrobial Test

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Susceptible (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>AMP</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>GEN</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>NAL</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CHL</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CEF</td>
<td>99.3</td>
<td>0.7</td>
</tr>
<tr>
<td>KAN</td>
<td>99.3</td>
<td>0.7</td>
</tr>
<tr>
<td>STX</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CT</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>TET</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>AMC</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CAZ</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CRO</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>AMK</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CFP</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

### Percentage

- **CIP**: 100%
- **AMP**: 100%
- **GEN**: 100%
- **NAL**: 100%
- **CHL**: 100%
- **CEF**: 99.3%
- **KAN**: 99.3%
- **STX**: 100%
- **CT**: 100%
- **TET**: 100%
- **AMC**: 100%
- **CAZ**: 100%
- **CRO**: 100%
- **AMK**: 100%
- **CFP**: 100%
Conclusions

- Cattle in S. Africa are an important reservoir of STEC O26, O45, O103, O121, O145 and O157 and many other non-O157:H7 serotypes.

- 75 serotypes were identified, but only 13 serotypes have been previously implicated in human disease worldwide.

- The majority of STEC isolates carried mainly \textit{stx1} and \textit{stx2} but lacked \textit{eaeA}, a key STEC adhesin.
Conclusions

- STEC carried *ehxA, subA, saa* but lacked plasmid-encoded genes: *katP* and *etpD*.
- *Nle* genes were mainly associated with STEC isolates carrying intimin (*O157:H7*, *O145:H28*, *O26:H2* and *O103:H2*).
- **HUMAN STEC SPORADIC AND MILD DISEASE MAINLY??**
Acknowledgements:

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References:


- Coombes, B.K., Wickham, M.E., Mascarenhas, M., Gruenheid, S., Finlay, B.B. & Karmali, M.A. 2008, "Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing Escherichia coli strains