Screening for *Escherichia coli* O157:H7 in diarrheic patients in Benin City, Nigeria

Esumeh, F.I, *Isibor, J.O., Egbagbe, I.D.S.*

*Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Nigeria*

**ABSTRACT**

*Escherichia coli* O157:H7 is an emerging disease pathogen whose occurrences have been reported in several parts of the world including Nigeria. Factors which predispose humans to infection with the organism abound in Benin City, a cosmopolitan and highly populated city in Nigeria. This fact prompted this prevalence study among 50 hospital patients who reported with symptoms of diarrhea. Stool specimens collected from patients were inoculated onto MacConkey and Sorbitol MacConkey agar, supplemented with Cefuxime-Tellurite medium (Oxoid SR 172), incubated overnight at 37°C. Bacterial colonies were identified using standard bacteriological procedures (Cowan and Steel, 1993; Nataro and Kaper 1998). Sorbitol negative *Esch coli* colonies were confirmed serologically using the O157:H7 Latex agglutination kit (Oxoid DR 620). 23 (46%) of the 50 specimens yielded *Esch coli*. Out of these 11 (48%) were sorbitol positive while 12 (52%) did not ferment sorbitol. As shown from this study, the prevalence rate of 20% was recorded for *Esch coli* O157:H7. The presence of enterohaemorrhagic *Esch coli* O157:H7 in Benin City is no longer in doubt. We advocate that a more intense and well planned public enlightenment be mounted by our sanitary health officials, while cases of gastroenteritis with bloody or non-bloody diarrhea be properly investigated bacteriologically.

**Keywords:** *Escherichia coli* O157:H7, Diarrheic Patients, Benin City, Nigeria.

**INTRODUCTION**

*Escherichia coli* is the pathogen most commonly associated with endemic forms of childhood diarrhea (Huillan et al., 1991). Diarrhea is a leading cause of morbidity and mortality among children in developing countries (Guerrant et al., 1990; WHO 2005). Among the adult populace, diarrhea diseases can lead to drastic loss in man-hours, thus depleting state and national income.

At least six categories of diarrhoeagenic *E. coli* have been described (Prescott et al., 2002). Enterohaemorrhagic *E. coli* can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) through the production of the shiga–like toxins Stx 1 and Stx 2 and other probable virulence factors (Tarr et al., 1990). Outbreaks of *E. coli* O157: H7 infections have been reported in the US (Riley et al., 1983); Britain (Morgan et al., 1988); Democratic Republic
of Congo (Koyange et al., 2004) and Swaziland (Effler et al., 2001). Sources of infection include surface water contaminated with faeces (Effler et al., 2001), undercooked hamburger (Riley et al., 1983), contaminated water and raw milk (Swerdlow et al., 1992) food and drink (Cunin et al., 1999).

In Nigeria, some prevalence studies have been reported in some South Western cities (Ogunsanya et al., 1994; Okeke et al., 2000; Olorunshola et al., 2000). To the best of our knowledge, not much study has been carried out in Edo state of Nigeria. We report here the results of the screening of some diarrheic patients in Benin City, Nigeria.

**MATERIALS AND METHODS**

**Subjects:** 50 patients attending Central Hospital Benin City and the University of Benin Teaching Hospital, Benin City for reasons of gastrointestinal complaints and passing of diarrheic stools were requested to submit their stool specimens in sterile plastic disposable containers. Specimens were transported in Cary-Blair medium and inoculated onto culture media within 24 hours. All ethical considerations were adhered to in accessing the patients.

**Media used:** These included MacConkey Agar (Oxoid CM7) and Sorbitol MacConkey Agar (Oxoid, CM813). For use, appropriate quantities of the dehydrated media were reconstituted and autoclaved following the manufacturers’ instructions. The Sorbitol MacConkey agar was supplemented with Cefuxime – Tellurite medium (Oxoid SR 172).

**Isolation and identification of *E. coli.***

Each stool sample was streaked onto MacConkey medium using a sterile inoculating loop. Plates were incubated overnight at 37°C. Lactose fermenting colonies appearing on the plates were gram-stained, and those showing as gram negative bacilli were tested for indole production. Indole positive colonies were further plated out on Sorbitol MacConkey agar and incubated for 18 – 2hrs at 37°C. All non-Sorbitol fermenting colonies were further subjected to biochemical tests using ducitol, β-glucuronidase, potassium cyanide and cellubiose (Nataro and Kaper, 1998). Colonies suggestive of *E. coli* O157:H7 were tested for agglutination using *E. coli* O157:H7 latex agglutination reagents (Oxoid DR 620).

**RESULTS**

50 stool specimens from diarrheic patients were inoculated on MacConkey and Sorbitol MacConkey agar media and identification of isolates done using standard bacteriological methods (Cowan and Steel, 1993; Nataro and Kaper 1998).

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>No. of suspected <em>E. coli</em></th>
<th>No. of sorbitol Positive <em>E. coli</em></th>
<th>No. of sorbitol Negative <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 2 Serotyping of sorbitol – negative Escherichia coli isolates

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>No. of sorbitol-negative E. coli O157:H7</th>
<th>No. of positive E. coli O157:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Table I shows the number of E. coli isolates recovered, the number of sorbitol fermenting E. coli and non-sorbitol fermenters. 12% of the isolates were non-sorbitol fermenters. The second batch of specimens yielded only sorbitol fermenting E. coli (Table 1). The numbers of strains testing positive in the O157 Latex agglutination test are shown in Table 2. Of the 50 stool specimens examined, 20% yielded E. coli confirmed as O157:H7 serotype (Table 2).

**DISCUSSION**

E. coli O157: H7 has been established as an important etiologic agent of human diarrhea illness. This organism, while being primarily associated with food-borne outbreaks (Chapman et al., 1993; Annon, 1993) has become an important public health concern, being also transmissible through contaminated drinking water (Swerdlow et al., 1994). Benin City is a cosmopolitan city with commercial outfits such as banks, fast food restaurants and commercial motor transport services springing up by the day. Many areas of the city are littered with filth which can conduce to diarrhea diseases.

Routine surveillance for diarrheagenic E. coli has never been carried out in Benin City. The only report, documented to our knowledge, is that of Omoigberale et al., (2002) which reported a zero percent prevalence rate for E. coli O157:H7 in children presenting with diarrhea at the University of Benin Teaching Hospital, Benin City. In this study the presence of E. coli O157:H7 was detected, with a prevalence rate of 20%. Our main focus was to establish the presence of this organism in this area.

In a study done in Lagos by Olorunshola et al., (2000) a detection rate of 6% was recorded for enterohaemorrhagic E. coli O157:H7, while 3.1% prevalence rate was recorded for diarrhea patients in Jos, Nigeria (Ngbede et al., 2006). A prevalence rate of 20% recorded in our study was certainly high. This study suggests that E. coli O157:H7 are an important diarrhea pathogen in subjects in Benin City. The implication of this is that our health inspectors in the local government areas of the state must be vigilant and ensure that campaigns are mounted to educate our citizens on ways of improving on the unsanitary environment. Potential sources of infection as well as food and meat inspection must be followed up. In addition, our physicians should query E. coli O157:H7 infection whenever patients present at their clinics with bloody or non-bloody diarrhea, so as to curb possible outbreaks early enough.

**REFERENCES**