Isolation, Identification and Enterotoxin Detection of *Escherichia Coli* Isolated from Calf Diarrhea and their Virulence Characteristics

Abubaker A. El Ayis¹, Ali A. Elgaddal², Yassir A. Almofti³

¹Department of Medicine, College of Veterinary Medicine, University of Bahri (Sudan)
²Department of Bacteriology, Veterinary Research Institute (Suba/ Sudan)
³Department of Biochemistry and Molecular Biology, College of Veterinary Medicine, University of Bahri (Sudan).

*Corresponding author E. mail: yamofti99@gmail.com*

(Received: June 17, 2015; Accepted: September 30, 2015)

Abstract—A total of 260 *E. coli* isolates out of 342 bacterial isolates were obtained from faecal samples collected from 300 diarrhoeic calves from different localities in Khartoum State. The isolates were confirmed using API 20E and VITEK2 identification systems. Ninety isolates were confirmed by API 20E strips and twenty isolates scored high probability percentages by using VITEK2. Then 150 *E. coli* isolates were subjected to sensitivity test to ten antimicrobial agents used for treatment of calf diarrhea in Sudan. They exhibited high susceptibility to Gentamycin, Ampicillin, Ciprofloxacin, Sulphamethoxazole-trimethoprim, Cephalothin, Kanamycin, Chloramphenicol and Tetracycline but they demonstrated less sensitivity to Erythromycin and high resistance to Procaine Penicillin. Fifty *E. coli* isolates were examined for enterotoxins production using suckling mouse test for heat stable (STa) production and reversed passive latex agglutination test for heat labile (LT) production. Forty five isolates were positive for STa and ten isolates were positive for LT production. Also 110 of *E. coli* isolates were tested for specific fimbrial antigens possession. The agglutination reactions of fimbrial adhesions showed different percentages for K99(F5), K88(F4) and 987p(F6) which was detected in two isolates. Taken together the study showed that *E. coli* is the predominant bacterial spp isolated from diarrhoeic calves and the incidence rate of calf diarrhea in Khartoum State is 60%.

Index terms — Calf diarrhea, *E. coli*, Enterotoxins, Khartoum State, Virulence

I. INTRODUCTION

Diarrhea in newborn farm animals, particularly calves under 30 days of age, is one of the most common disease complexes that the large-animal clinician encounters in practice. It is a significant cause of economic loss in cattle herds and continues to assume major importance as livestock production becomes more intensified [1]. *Escherichia coli* can be isolated and classified as a cause of calf diarrhea using traditional methods, i.e. identifying its biochemical or antigenic characteristics. The pathogenic mechanisms may be studied in cell cultures and animal method assays, as well as more up to date molecular biology methods for study and diagnosis. The latter have proven that genes are involved in pathogenesis [2]. More over multiple studies used API 20E for isolation and identification of Enterobacteriaceae. API 20E strips were compared to many other identification systems. For instance in the evaluation of API 20E in one study approximately 93.0% of the 129 Enterobacteriaceae and five Aeromonas strains were correctly identified to species level [3, 4]. Another study showed an overall accuracy rate of 96.4% in testing 366 enteric strains [4, 5]. Therefore API 20E because of its large acceptability by the clinical microbiology laboratory market, became somewhat of a “gold standard” among commercial systems.

The virulence factors of pathogenic strains of *E. coli* include capsules, endotoxin, structures responsible for colonization, endotoxins and other secreted substances [6]. Two types of enterotoxins, heat labile (LT) and heat stable (ST) have been identified and each type of enterotoxin has two subgroups. Many strains of Enterotoxigenic *E. coli* (ETEC) from pigs produce LT1 which induces hyper secretion of fluids into the intestine through stimulation of adenylate cyclase activity [6]. Most ETEC isolates which produce LT1 also possess K88 adhesins. *E. coli* strains, containing virulence factors such as a heat-stable enterotoxin (STa) and fimbria such as F5 (K99) and F41, are mainly associated with diarrhea in newborn calves [1, 7, 8]. The F17 fimbrial family was also detected on bovine pathogenic *E. coli* (PEC) isolates implicated in diarrhea or septicemia [8] in the infected animals. A second heat-labile toxin, LT2, has been demonstrated in some EC strains isolated from cattle [6]. One of the heat-stable enterotoxin subgroups, STa has been identified in strains of ETEC isolated from porcine, bovine, ovine and human specimens. This toxin induces increased guanylate cyclase activity in enterocytes and the resultant increase in intracellular guanosine monophosphate stimulates fluid and electrolyte secretion into the small intestine lumen and inhibits fluid absorption from the intestine [6]. The precise cytotoxic effect of the other heat-stable enterotoxin, STb, is not known [6]. The pathological effects of infection with pathogenic *E. coli*, other than those attributed to endotoxin, derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Unlike enterotoxins which affect only the functional activity of enterocytes, verotoxic necrotizing factors can produce demonstrable cell damage at their sites of action. Enterotoxigenic *E. coli* (ETEC) is the most common enteropathogen that causes diarrhea in newborn farm animals [9].
In Sudan multiple studies reported the involvement of *E. coli* as a cause of diarrhea, haemorrhagic colitis and dysentery in calves [10-12]. Moreover a considerable progress has been made in the treatment of the effects of diarrhea such as dehydration and acidosis [9] but less so in the control of these diseases complexes. The problem of diarrhea is complex and attaining hazardous proportions, despite few fragmented studies have been carried out in Sudan to investigate calf diarrhea problem [10-15]. However, no studies were carried out to investigate the prevalence and virulence characteristics of *E. coli* as a cause of calf diarrhea.

The objective of this research work was to investigate the characteristics of *E. coli* isolated from diarrhoeic dairy calves using rapid and automated systems and to study the enterotoxins production of the isolated *E. coli* by bioassay and Immunoassay.

**II. MATERIALS AND METHODS**

**Area of the study**

From 500 visited farms, total of 300 faecal samples from diarrhoeic calves were collected from the three localities in Khartoum State in Sudan. In Bahri and East Nile localities samples were collected from Parlor 2 and 3, Shigla farms, Selate North and South farms, Ed Babeiker farms, Mygoma Farms, Shambat farms, Helat kuku farms, University of Khartoum farm and University of Sudan for Science and Technology farm. In Omdurman locality, samples were collected from El Ruduan farms, Gabal Toureia Farms and El Fetaihab farms. In Khartoum and Gabel Awleia localities samples were collected from El Saig farms, Soba farms and El Azhari farms. All these farms were visited twice during different seasons of the years 2013 and 2014 (dry, wet and cold seasons).

**Faecal sample collection**

Faecal samples were collected directly from the rectums of less than one month old calves. These calves showed clinical signs of diarrhea, but had not been treated with antibiotics before. The characteristics and the colors of the faecal samples were observed during the collection. Samples were placed in ice box containing ice and transported immediately to the laboratory (Veterinary Research Institute- Soba). Samples were stored at -20°C. Next day samples were thawed and subjected to bacteriological analysis.

**Isolation, identification and characterization of the *E. coli* isolates**

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of *E. coli*, a loop full faecal sample was streaked onto blood agar, McConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37°C for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Colonies which showed Gram positive cocci, Gram positive bacilli and Gram-negative bacilli were sub cultured on nutrient agar. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4°C.

**Biological and biochemical identification**

The purified isolates were identified as previously described [16, 17]. The identification include: Gram’s reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, oxidation fermentation test, motility test, glucose breakdown test, fermentation of carbohydrates, methyl red reduction (MR), Voges-Proskauer reaction, indole production, urease activity, citrate utilization, gelatin hydrolysis test, Eijkman's test, arginine decabyslet test, potassium cyanide test, nitrate reduction test).

**Identification of *E. coli* isolates using API 20E and VITEK2 identification systems**

A standardized identification system for Enterobacteriaceae and other Gram-negative rods was used [18]. For identification of *E. coli*, API 20E and VITEK2 (BIOMERIEUX, France) identification systems were used. Pure *E. coli* isolates were sub cultured on blood agar and incubated at 36°C ± 2 for 18–24 hours. The identification test of *E. coli* isolates was conducted according to the manufacturer BIOMERIEUX protocol. Homogeneous bacterial suspension was obtained by using API 20E medium. Both tubes and cubules of API 20E were filled with the inoculated API 20E media. Anaerobiosis was ensured in the ADH, LDC, ODC, URE and H₂S tests by filling the cubules with sterile mineral oil to form a convex meniscus. The incubation boxes were closed and incubated at 36°C ± 2 for 18–24 hours. Identification was obtained according to the numerical profile of API 20E.

The VITEK2 (BIOMERIEUX, France) test was performed as previously described [19]. Pure colonies were suspended in 3.0 ml sterile saline with turbidity of 0.50-0.63 MacFarland. The tested samples and the GN (Gram negative) identification cards were placed into a cassette. The cassette was placed into a vacuum chamber station inside the VITEK2 analyzer machine in which GN cards were inoculated with bacterial suspensions. The inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed. The cassette was manually inserted in the VITEK2 reader-incubator modules at 35.5°C and every card automatically subjected to a kinetic fluorescence measurement every 15 minutes. The results were interpreted by the ID-GNB database after the incubation period.

**Antibiotic susceptibility test**

Antibiotic susceptibility test was performed according to the Standard Disc Diffusion method as previously described [20]. Briefly, *E. coli* isolates were sub cultured onto blood agar and incubated at 37°C for 18-24 hrs.
Homogenous bacterial suspensions (0.5 MacFarland) were prepared. 2 ml of diluted culture were spread evenly over the surface of the media. Oxoid antimicrobial susceptibility test discs (Basingtoke, Hampshire, England) of antimicrobial drugs were applied to the surface of the medium and pressed gently using sterile forceps. Plates were incubated at 37°C for 24-48 hours. Zones of inhibition were measured in (mm) to determine whether the organism was sensitive or resistant to the tested antibiotics [21].

**Suckling mouse test (SMT)**
The biological method of SMT was performed as previously described [22] and standardized by Giannella 1976 [23]. The principal of the test based on the injection of extracted STA preparation of *E. coli* into the stomach of 2-4 days old suckling mouse for accumulation of the fluid in the intestine. The inoculated mice per os were kept at room temperature for 4 hours and then decapitated or killed. The abdomen was incised; the small intestines were examined for distension and then removed by forceps. The intestines were then weighed using a sensitive balance. The ratio of gut weight to the body weight was calculated. Ratios of less than 0.070 were considered negative, those in range of 0.070-0.090 were considered doubtful positive and those over 0.090 were positive [23].

**Detection of the fimbrial adhesions of E. coli isolates**
For detection of the fimbrial adhesion of *E. coli* isolates, the commercially available kits, monoclonal antibodies based immunoassay (Mab) was used. Monoclonal antibodies were coated onto K88, 987p, K99, and F41 latex particles at concentration of 10% (W/V) by standard method. Fimbrix is a rapid agglutination test used for identification of the fimbrial adhesions of *E. coli* grows on an appropriate medium. The reaction was checked with the positive control antigen. Valid tests were those only showed agglutulation with R1 and not with R2. Positive results showed agglutination with R1 within 3 minutes and lacked agglutination with R2. Negative results showed no agglutination with R1 or R2 after three minutes.

**Detection of heat-labile (LT) enterotoxin**
Reversed passive latex agglutination test was performed as previously described [24]. Polystyrene latex particles were sensitized with purified antiserum taken from rabbits immunized with purified *Vibrio cholerae* enterotoxin. These latex particles agglutinated in the presence of *Vibrio cholerae* enterotoxin (CT) or *E. coli* heat-labile enterotoxin (LT). If either toxin is present, agglutination occurs. Preparation and extraction of *E. coli* LT enterotoxin was performed as previously described [23]. The plate was arranged so that each row consisted of 8 wells. Each sample needed the use of 2 such rows. 25µl of diluents were added in each well of the two rows except for the first well in each row. 25µl of test sample was added to the first and second well on each row. 25µl was picked up and a double dilution was performed along each of the 2 rows. The 7th well was left to contain diluents only. 25µl of sensitized latex was added to each well of the first row and 25 µl of latex control was added to each well of the second row. The plate was rotated gently to mix the contents by hand, covered and put in moist place to avoid evaporation. The plates were left at room temperature for 24hours. The agglutination pattern was judged by comparison with the illustrated results classified as (+), (++) and (+++) are considered positive. Results in the row of wells containing latex control were negative.

**Statistical analysis**
Statistical analysis was done through Microsoft office Excel 2007.

**III. RESULTS AND DISCUSSION**

**Characteristics of the collected samples and the isolated microorganisms**
As shown in figure-1, a total of 300 faecal samples were collected from different localities of Khartoum State. The characteristics of the samples were classified as watery yellowish diarrhea, watery-mucoid diarrhea and bloody-watery diarrhea. According to the cultural characteristics, bacterial morphology and biochemical reactions results, and API 20E and VITEK2 results, a total of 342 bacterial isolates were isolated (Figure-2). The majority of the isolated and identified bacterial species were *Escherichia coli* (260 isolates)

![Fig. 1: Number and percentage of samples collected from different localities in Khartoum State](image-url)
Identification of E. coli using API20E and VITEK2 systems
Different identification percentages ranged from 89.8% to 99.8% were scored by ninety E. coli isolates after using API 20E identification system. Moreover according to the interpretation of the results after using VITEK2 identification system, twenty E. coli isolates scored high probability percentages ranged from 96.00% to 99.00%.

Antimicrobial susceptibility test
Figure-3, showed the result of the sensitivity test for 150 isolates out of 260 E. coli isolates. The isolates exhibited high sensitivity to Gentamycin, Ampicillin, Ciprofloxacin, Sulphamethoxazole-trimethoprim, Cephalothin, Kanamycin, Chloramphenicol and Tetracycline. However the isolates were less sensitive to Erythromycin and highly resistant to Procaine Penicillin.

Suckling mouse test (SMT)
Table -1 showed the result of SMT. Among the 50 tested isolates, 43 isolates (90%) measured more than 0.09, while 5 isolates (10%) measured 0.07 to 0.09. on the other hand only 2 isolates (4%) measured less than 0.07.

Detection of STa enterotoxin
Out of the fifty isolates used in the SMT, forty five isolates (90.0%) gave positive results for production of STa toxins (table 2). The percentages of positive tests were higher in Bahri and East Nile locality compared to Omdurman and Khartoum and Gabel Awleia localities.

Detection of heat-labile (LT) enterotoxin
The fifty E. coli isolates used in SMT test were also subjected to LT enterotoxins production ability. As shown in table (2) ten isolates (20%) gave positive results, i.e., agglutination was noticed as a net onto the bottom of the wells. Also the percentages of positive tests were higher in Bahri and East Nile locality compared to Omdurman and Khartoum and Gabel Awleia locality. Most importantly, 5 isolates (10%) were positive for both STa and LT production.
Fig. 4: Agglutination reaction of fimbrial adhesions and antigens

Table 1: Suckling mouse test (SMT): The table showed the weight of the intestines, the carcass weight and the ratios.

<table>
<thead>
<tr>
<th>E. coli Isolates</th>
<th>Intestine weight (g)</th>
<th>Carcass weight (g)</th>
<th>Ratio</th>
<th>E. coli Isolate</th>
<th>Intestine weight (g)</th>
<th>Carcass weight (g)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.096</td>
<td>1.000</td>
<td>0.09</td>
<td>26</td>
<td>0.124</td>
<td>1.000</td>
<td>0.124</td>
</tr>
<tr>
<td>2</td>
<td>0.216</td>
<td>1.304</td>
<td>0.165</td>
<td>27</td>
<td>0.224</td>
<td>1.274</td>
<td>0.176</td>
</tr>
<tr>
<td>3</td>
<td>0.101</td>
<td>1.224</td>
<td>0.083</td>
<td>28</td>
<td>0.146</td>
<td>1.144</td>
<td>0.128</td>
</tr>
<tr>
<td>4</td>
<td>0.082</td>
<td>1.620</td>
<td>0.078</td>
<td>29</td>
<td>0.188</td>
<td>1.144</td>
<td>0.164</td>
</tr>
<tr>
<td>5</td>
<td>0.238</td>
<td>1.476</td>
<td>0.161</td>
<td>30</td>
<td>0.242</td>
<td>1.416</td>
<td>0.170</td>
</tr>
<tr>
<td>6</td>
<td>0.180</td>
<td>1.240</td>
<td>0.145</td>
<td>31</td>
<td>0.224</td>
<td>1.200</td>
<td>0.187</td>
</tr>
<tr>
<td>7</td>
<td>0.332</td>
<td>1.692</td>
<td>0.196</td>
<td>32</td>
<td>0.332</td>
<td>1.242</td>
<td>0.267</td>
</tr>
<tr>
<td>8</td>
<td>0.212</td>
<td>1.468</td>
<td>0.212</td>
<td>33</td>
<td>0.146</td>
<td>1.000</td>
<td>0.146</td>
</tr>
<tr>
<td>9</td>
<td>0.260</td>
<td>1.376</td>
<td>0.189</td>
<td>34</td>
<td>0.202</td>
<td>1.058</td>
<td>0.191</td>
</tr>
<tr>
<td>10</td>
<td>0.322</td>
<td>1.322</td>
<td>0.244</td>
<td>35</td>
<td>0.990</td>
<td>1.086</td>
<td>0.091</td>
</tr>
<tr>
<td>11</td>
<td>0.264</td>
<td>1.616</td>
<td>0.163</td>
<td>36</td>
<td>0.124</td>
<td>1.132</td>
<td>0.109</td>
</tr>
<tr>
<td>12</td>
<td>0.240</td>
<td>1.084</td>
<td>0.221</td>
<td>37</td>
<td>0.174</td>
<td>1.174</td>
<td>0.154</td>
</tr>
<tr>
<td>13</td>
<td>0.275</td>
<td>1.668</td>
<td>0.165</td>
<td>38</td>
<td>0.258</td>
<td>1.138</td>
<td>0.227</td>
</tr>
<tr>
<td>14</td>
<td>0.356</td>
<td>1.532</td>
<td>0.232</td>
<td>39</td>
<td>0.231</td>
<td>1.146</td>
<td>0.201</td>
</tr>
<tr>
<td>15</td>
<td>0.212</td>
<td>1.432</td>
<td>0.170</td>
<td>40</td>
<td>0.291</td>
<td>1.168</td>
<td>0.249</td>
</tr>
<tr>
<td>16</td>
<td>0.265</td>
<td>2.080</td>
<td>0.127</td>
<td>41</td>
<td>0.243</td>
<td>1.296</td>
<td>0.187</td>
</tr>
<tr>
<td>17</td>
<td>0.292</td>
<td>1.486</td>
<td>0.197</td>
<td>42</td>
<td>0.027</td>
<td>1.184</td>
<td>0.022</td>
</tr>
<tr>
<td>18</td>
<td>0.660</td>
<td>1.312</td>
<td>0.503</td>
<td>43</td>
<td>0.091</td>
<td>1.192</td>
<td>0.076</td>
</tr>
<tr>
<td>19</td>
<td>0.146</td>
<td>1.230</td>
<td>0.119</td>
<td>44</td>
<td>0.175</td>
<td>1.300</td>
<td>0.135</td>
</tr>
<tr>
<td>20</td>
<td>0.218</td>
<td>1.276</td>
<td>0.171</td>
<td>45</td>
<td>0.188</td>
<td>1.378</td>
<td>0.136</td>
</tr>
<tr>
<td>21</td>
<td>0.149</td>
<td>0.998</td>
<td>0.150</td>
<td>46</td>
<td>0.099</td>
<td>1.225</td>
<td>0.080</td>
</tr>
<tr>
<td>22</td>
<td>0.184</td>
<td>1.044</td>
<td>0.176</td>
<td>47</td>
<td>0.100</td>
<td>1.301</td>
<td>0.077</td>
</tr>
<tr>
<td>23</td>
<td>0.175</td>
<td>1.214</td>
<td>0.144</td>
<td>48</td>
<td>0.120</td>
<td>1.299</td>
<td>0.092</td>
</tr>
<tr>
<td>24</td>
<td>0.146</td>
<td>1.016</td>
<td>0.144</td>
<td>49</td>
<td>0.083</td>
<td>1.300</td>
<td>0.064</td>
</tr>
<tr>
<td>25</td>
<td>0.202</td>
<td>1.152</td>
<td>0.175</td>
<td>50</td>
<td>0.162</td>
<td>1.205</td>
<td>0.134</td>
</tr>
</tbody>
</table>
Table 2. Detection of fimbrial antigens possessed by E. coli isolates and detection of STa and LT enterotoxins produced by E. coli isolates using suckling mouse test (SMT). Five isolates demonstrated both STa and LT enterotoxin production.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Fimbrial Antigens</th>
<th>STa and LT enterotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of tested isolates</td>
<td>K88 (F4)</td>
</tr>
<tr>
<td>Bahri &amp; East Nile</td>
<td>50</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>Omdurman</td>
<td>40</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Khartoum &amp; Gabal Awleia</td>
<td>20</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>17 (15.5%)</td>
</tr>
</tbody>
</table>

Fimbrial antigens

E. coli isolates (110 isolates) were selected randomly and tested for specific fimbrial antigens possession. As shown in figure (4) and table (2), the agglutination reactions of fimbrial adhesins were detected in the tested isolates with different percentages. K99 (F5) (17.3%), K88 (F4) (15.5%) and 987p (F6) was detected in two of Omdurman area isolates (1.8%).

Newborn calf diarrhea is considered as the top leading cause of morbidity and mortality in dairy calves and beef calves [25, 26]. Some reports demonstrated that diarrhea is one of the most common diseases reported in calves up to three months old and the first weeks of calf's age are the most hazardous and the risk decreases with old ages [9, 27, 28]. In this report 500 dairy farms in different localities of Khartoum State were investigated for the problem of calf diarrhea. The incidence rate of calf diarrhea reported in this study was 60%, which is lower than that previously reported in Khartoum State which was 75% [9].

The incidence rate was greater in Bahri and East Nile locality (64.5%) than Omdurman locality (60.6%) and Khartoum and Gabel Awleia locality (52.9%) (un published data). This may be attributed to high population density in Bahri and East Nile locality and also the stall surfaces in the animal farms were different.

This study reported that E. coli are the predominant bacterial spp. of calf diarrhea among Gram-negative aerobes. Moreover multiple reports revealed the involvement of E. coli as the main agent in calf diarrhea [9-12, 16, 29-42]. This report revealed that beside E. coli other bacterial spp are implicated in calf diarrhea. Moreover it was found that the Gram-negative bacteria were the most prevalent organisms (94.2%) than Gram-positive bacteria (5.8%) as previously reported [43]. Some reports as well as in this report showed the implication of Klebsiella pneumonia sub spp. Ozaenae and Proteus mirabilis in calf diarrhea [6, 13, 40]. Moreover Proteus mirabilis was thought to be involved in less cases of calf diarrhea [6, 44]. Beside the Gram negative bacteria, Gram positive bacteria also are isolated in this study which was Enterococcus spp. This result is similar to another report finding [12].

For the identification of the E.coli isolates, 90 E. coli culture isolates were used on API 20E strips. They showed different identification percentages ranging from 99.8% to 89.8%. Slight variations in the biochemical behavior of the microorganism may be attributed to variations in the genetic constituents of different strains resulting in different phenotypic characteristics. These genetic variations may be of chromosomal or plasmid origin [4, 5]. In both circumstances, transfer of genetic material between strains of the same species does occur through different mechanisms. These findings generally fulfilled the API 20E requirements for identification as E. coli [45, 46]. Twenty E. coli isolates were selected randomly and inserted into VITEK2 automated identification system. They scored high probability percentages and there were slight variations in the results of their biochemical tests. Although the results of biochemical reactions revealed many differences between E. coli strains, they fulfilled the requirements for identification as E. coli by VITEK2 system [47, 48]. The present findings showed that VITEK2 system as an automated system provided very good and trustable accuracy and reproducible results as shown in repeated samples of same source. Economic studies estimate a good future for automated systems in microbiology lab [49-54]. It is noteworthy that this is the first report in Sudan using VITEK 2 automated identification system for identification of E. coli.

Isolation of E. coli doesn't necessarily means the presence of the disease unless, virulence factors are identified i.e. toxins and/or fimbrial antigen [6, 55-57]. In this study 110 E. coli isolates from different localities of Khartoum State selected randomly and tested for specific fimbrial antigens possession.
Two *E. coli* isolates (5.0%) in Omdurman locality, as a new finding, was found to be positive for F6. The reasonable explanation for this may be the availability of contact between pigs which rose by some people and dairy herd. These findings are discussed and supported by multiple reports that demonstrated the most significant fimbrial antigens of pathogenic *E. coli* strains to domestic animals are F4, F5, F6 and F41 fimbrial antigens [6, 31, 56, 58, 59]. In Sudan F4, F5, F6 and F41 fimbrial antigens of *E. coli* strains were isolated from diarrhoeic camel calves in Butana, Upper Atbara River and Northwest Kordofan areas [12, 60]. Also *E. coli* F4, F5 and F41 adhesion antigens were detected in diarrhoeic calves in Khartoum North, Butri, Masoudia, Um Haraz, River Nile State and Kenana areas [10]. These findings collectively proved the significance of *E. coli* fimbrial antigens as pathogenic factor.

In this study 50 randomly selected *E. coli* isolates were tested by the sucking mouse test (SMT) for production of heat-stable (STa) enterotoxin. The result of SMT in this report is in accordance with Ellaithi (2004) [10] who found that 85.7% of isolated *E. coli* produced STa enterotoxin. The reliability of the sucking mouse was also confirmed by Dean et al. (1972) [22]. Nevertheless this disagrees with Gyles (1971) [61] who stated that the infant mouse test is unsatisfactory as a method for detection of STa enterotoxins. In addition to that, in this report ten isolates (20%) were positive for LT production. The same findings were reported by Salih et al. (1999) [60] who detected LT in 8 (27.6%) of 29 *E. coli* isolated from camel calves and also by Elgaddal (2009) [12] who detected LT in 16.6% *E. coli* isolated from camel calves. It is noteworthy that this is the first report in Sudan of detection of LT in *E. coli* isolated from diarrhoeic dairy calves by using RPLA kit. Also five *E. coli* isolates reported to have both STa and LT enterotoxins, the possession of the two enterotoxins may increases the bacterium virulence [6].

In this study 150 isolates out of 260 *E. coli* isolated were subjected to sensitivity test. The isolates demonstrated slight variation in their sensitivity to antimicrobials tested. It is reported that *E. coli* showed variation in their susceptibility to various chemotherapeutic agents in use for treatment of calf diarrhea [6]. However Gentamycin has improved stool consistency in calves with experimentally induced *E. coli* diarrhea [9]. Other studies reported that all *E. coli* isolates were sensitive to Choramphenicol and Erythromycin and 97% of the isolates showed different patterns of sensitivity to the other antibiotics used in those studies [10, 11]. In this report the resistance of some *E. coli* isolates to the tested antimicrobials may be attributed to the presence of CTX-M-14-like enzyme that has been detected in the *E. coli* recovered from the faeces of diarrhoeic dairy calves [9]. The enzyme is an extended-spectrum beta-lactamase (ESBL), which confers resistance to a wide range of beta-lactam compounds (Penicillin and Cephalosporin) [9]. Organisms possessing ESBL are considered to be resistant to second-, Third-, and fourth-generation Cephalosporines. In addition to this enzyme, the isolates produced a TEM-35 (IRT-4) beta-lactamase that conferred resistance to the Amoxicillin/ Calvulanate combination [9].

VI. CONCLUSION

This study clearly revealed that *E. coli* is the predominant bacterial spp. (76.0%), isolated from diarrhoeic calves and the incidence rate of calf diarrhea in Khartoum State is 60%. Moreover VITEK2 auto analyzer machine is used for identification of *E. coli* isolates, and the isolates scored high probability percentages ranged from 96% to 99%. The significance of *E. coli* in calf diarrhea is becoming well known and further studies should be carried out to investigate the predisposing factors related to the incidence of neonatal calf diarrhea and to identify different causes of calf diarrhea. Moreover the serotyping of *E. coli* isolates obtained from different areas should be given more attention

V. REFERENCES


