Original Research Article

Some virulence factors of *Escherichia coli* isolated from diarrheic calves

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**ABSTRACT**

*Escherichia coli* are the most common cause of diarrhea in calves. Diarrhea in calves remains one of the most important problems faced by livestock, causing great economic losses. Some strains of *E. coli* characterized by the presence of specific virulence factors including haemolysin production, resistance to bactericidal effects of serum and Congo red binding activity. In this study, fecal samples were collected from 115 diarrheic calves aged from 3 days to one year and from different localities in Egypt along the period from February to August 2015. The prevalence of *E. coli* in diarrheic calves was 72.2%. 39.8% of isolated *E. coli* were haemolytic to sheep blood agar, 68.7% were serum resistant, 100% showed Congo red binding activity.

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Introduction

*Escherichia coli* (*E. coli*) are a normal inhabitant of the gastrointestinal tract of worm blooded animals (Wray and Morris, 1985; Magalhães et al., 2016). There are commensal strains and a variety of pathogenic strains, including enteropathogenic (*EPEC*), enterohemorrhagic (*EHEC*), enterotoxigenic (*ETEC*), enteroinvasive (*EIEC*), and urinary tract infection (*UTI*) strains (Pupo et al., 1997). Neonatal calf diarrhea (NCD) is a common condition of young calves and can be caused by pathogenic strains of *E. coli* (Gibbons et al., 2014). NCD still a major cause of economic losses directly through mortality and the need for treatment, and indirectly from poor growth after clinical disease (De la Fuente et al., 1998).

The organisms of *E. coli* are divided into pathogenic and nonpathogenic based on their ability to cause diseases. Differentiation of pathogenic strains from normal flora is based on identification of virulence characteristics (Pupo et al., 1997). Among these factors, Congo red binding activity, haemolytic activity and toxin production are the most commonly studied ones. Several studies confirmed that, binding of Congo red dye has been associated with pathogenicity of the *E. coli* (Berkhoff and Vinal, 1986; Vinal, 1988; Gjessing and Berkhoff, 1989). Also Some strains of *E. coli* characterized by resistance to bactericidal effects of serum (Fecteau et al., 2001).

Fecal bacteria culturing is a common laboratory method for isolation and identification of bacterial pathogens in feces (Izzo et al., 2011). Colony morphology, physical characteristics, microscopic features, and biochemical tests are used to characterize and identify the isolated bacteria (Ferrarezi et al., 2008).

**Aim of the work:**

The present work aimed to identify *E. coli* causing diarrhea in calves and detect some virulence factors of the isolated *E. coli* strains.

This aim is fulfilled through the following objectives:

1. Isolation of *E. coli* from feces of diarrheic calves.
2. Biochemical identification of the isolates suspected as *E. coli* strains.
3. Detection of some virulence factors of the isolated *E. coli* strains.

**Materials and Methods**

2.1. Samples:

A total of 115 rectal swabs were collected from diarrheic calves of age range from 3 days to one year and from different localities in Egypt along the period from February to August 2015. Calves exhibited signs of systemic disease; poor appetite, dehydration, decreased mentation, reduced suckling reflex and had pasty or watery feces.

2.2. Isolation and Biochemical Identification:

Isolation and identification of *E. coli* were done according to Collee et al. (1996).

All the recovered isolates of *E. coli* were identified biochemically according to schemes described by Kreig and Holt (1984), Collee et al. (1996) and Quinn et al (2002).

2.3. Phenotypic detection of virulence traits of *E. coli* isolates:
2.3.1. Congo red binding assay: in accordance to Berkhoff and Vinal (1986)

E. coli isolates were tested for their growth status on Congo red medium after incubation for 24 hours at 35°C, then left at room temperature for additional 2 days (not to exceed 4 days). Congo red positive (CR+) E. coli was indicated by the development of red colonies while Congo red negative (CR-) did not bind the dye and appeared as white to yellow colonies. The degree of redness of the colonies varied from one isolate to another.

2.3.2. Hemolytic activity: in accordance to Marilda et al. (1990)

Biochemically identified isolates as E. coli were tested for its hemolytic activity by its cultivation onto sheep blood agar media (7%) and incubated at 37 °C for 24 h.

2.3.3. Serum resistance test: in accordance to Siegfried et al. (1995)

From overnight cultures of the biochemically identified isolates as E. coli grown at 37 °C on blood agar, cell suspensions in Hanks’ balanced salts solution (HBSS) were prepared to contain $2.5 \times 10^8$ CFU/mL.

a. Test tubes were used for incubation of bacterial suspensions (0.05 mL) with serum (0.05 mL). Control wells contained 0.05 ml of HBSS instead of serum.

b. The tubes were mixed by shaking for a minute and incubated at 37°C in an incubator. From the isolates under test (10 µL) were taken at 0 minute and after incubation for 180 min at 37°C and spread on MHA.

c. The plates were further incubated for 18 h at 37°C. Susceptibility of bacteria to serum bactericidal activity expressed as the percentage of bacteria surviving after 180 min and overnight incubation in relation to the original growth of bacteria determined at 0 min in the controls.

d. Strains termed serum sensitive if the viable count dropped to 1% of the initial value and resistant if >90% of organisms survived after 180 min and overnight incubation and results in between were considered intermediate.

Results

3.1. Isolation and Biochemical Identification:

Out of 115 collected fecal samples from diarrheic calves, 83 E. coli were recovered with a prevalence of 72.2%.

3.2. Phenotypic detection of virulence traits of E. coli isolates:

As shown in table (1).
Table (1) showed phenotypic virulence character of isolated *E. coli*

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congo red binding assay</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Hemolytic activity</td>
<td>33</td>
<td>39.8</td>
</tr>
<tr>
<td>Serum resistance</td>
<td>57</td>
<td>68.7</td>
</tr>
</tbody>
</table>

Photo (1): *E. coli* showed serum bactericidal resistance 60% and 100%
Photo (2): *E. coli* showed positive Congo red activities

**Discussion**

Neonatal calf diarrhea remains one of the most important causes of calf mortality and a major disease problem facing livestock that leads to great economic losses not only from calf mortality and treatment costs, but also from losses in future growth and production (Germine et al., 2011). Calves are at greatest risk of developing diarrhea within the first month of life and the incidence of diarrhea decreases with age (Garcia et al., 2000).

In the present study, trials were made to examine 115 fecal samples of diarrheic calves exhibiting signs of systemic disease; poor appetite, dehydration, decreased mentation and reduced suckle reflex, and had pasty or watery feces, which were suspected to be infected with *E. coli*.

A total of 83 bacterial isolates were recovered which were identified morphologically and biochemically as *E. coli* isolates with the prevalence was (72.2%)(83/115)

The prevalence of *E. coli* in the current study; in comparison to the other studies in Egypt, was nearly similar to that of Helal et al. (2014); 72%, nearly coincided to the findings of Awad et al. (1979); 80%, while higher than those of El-Amrousi et al. (1971); 36%, Refai (1980); 62% and and lower than that obtained by Ibrahim (1995); 100% and Haggag and Khaliel (2002); 82%.

As regarding to biochemical and other characteristics of *E. coli* isolates recovered from diarrheic calves. (Table), all isolates of *E.coli* were positive for indole production, methyle red (MR) and motility tests and grown onto MacConkey's agar media giving pink colonies and fermented glucose, lactose, mannose, arabinose, maltose and mannitol with acid and gas production while fermentation of sucrose was variable.

They were all negative for oxidase, citrate utilization, hydrogen sulphide (H2S) on TSI, VogesPrauskeur (VP) and urea hydrolysis tests. These results go hand to hand to that obtained by Dastmalchi and Ayremlou (2012), El Behiry (2014) and El Ayis et al. (2015).

Binding of congo red dye by *E. coli* is associated with the pathogenicity of the organism and can be used as a phenotypic marker to distinguish invasive and non-invasive isolates of *E. coli* (Berkhoff and Vinal, 1986 and Osman et al., 2012).

Agar medium containing Congo red dye differentiates virulent and avirulent colonies of *E. coli* as they absorb the dye and produce red colonies (Payne and Finkelstein, 1977)
All isolates of E. coli were Congo red positive (CR+) 100%. The obtained results are in agreement with findings of Shehata and Salam (2012), Osman et al. (2013) in delta Egypt and Galal et al. (2013) who reported that all E. coli isolates were Congo red positive and higher than Helal et al. (2014) who reported that 65.88% of E. coli isolates isolated from calves in Cairo and Giza governorates were Congo red positive and Ghanem et al. (2005); 90.30% E. coli were positive.

Out of 83 isolates, 33 (39.8%) were haemolytic to sheep blood agar which lower than Helal et al. (2014) who reported that 72.22% of E. coli isolates isolated from diarrheic calves in Cairo and Giza governorates were haemolytic to sheep blood agar and higher than Aniruddha et al. (2009) 12.9% E. coli isolates were hemolytic on trypticase soya agar (TSA) containing 5% sheep blood.

57 (68.7%) isolates out of 83 E. coli isolates were serum resistant which was similar to Zaki (1995) 68.6% E. coli strains were serum resistant. That was lower than that obtained by Ghanem et al. (2005) who reported that results of serum resistance of E. coli isolates were (93.50%) survived in calf serum and (74.19%) were able to grow in calf serum.

That was due to the roles played by the O antigen and the K antigen in complement resistance and serum resistance. Pluschke et al. (1983).

Conclusion

Neonatal calf diarrhea is one of the most important problems faced by livestock, causing great economic losses. The prevalence of E. coli in diarrheic calves was 72.2%.

References


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