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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 calvesremains 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 studyfecal 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 *etal*.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 ºC 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 ºC on blood agar, cell suspensions in Hanks' balanced salts solution (HBSS) were prepared to contain 2.5 X 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 37ºC in an incubator. From the isolates under test (10 µL) were taken at 0 minute and after incubation for180 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*

Virulence factor	Isolates no= 83	
	Positive	
	No	%
Congo red binding assay	83	100
Hemolytic activity	33	39.8
Serum resistance	57	68.7

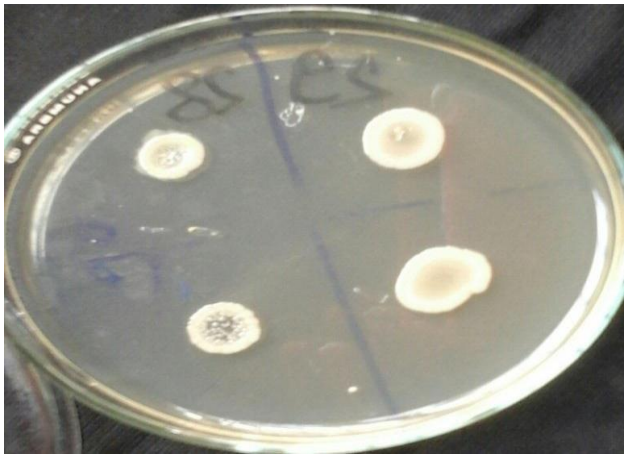

Photo (1): *E. coli* showed serum bactericidal resistance 60% and 100%

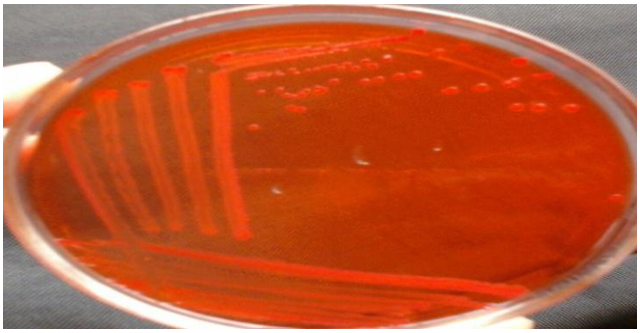

Photo (2): *E. coli* showed positive Congo red activities

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 (H_2S) 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 werehaemolytic 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 seum 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

Aniruddha,U.; Rakesh,S.; Daljeet,.C and Varsha,S. (2009). Study of virulence factors of *Escherichia coli* strains isolated from diarrhoeic calves. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases.30(1): 26-28

Awad, F.I.; Farrag, I.; Shawkat, M.E. and Abeid, M.H. (1979). Studies on enterotoxaemia in young buffalo calves. Egypt J. Vet. Sci., 14 (1): 24-29.

Berkhoff, H. A. and Vinal, A. C. (1986). Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. Avian Dis., 30(1): 117-121

Collee, J. G.; Fraser, A. G.;Marmion, B. P. and Simmons, A. (1996). Practical Medical Microbiology. 14th Ed.

Dastmalchi, S. H., andAyremlou, N. (2012). Characterization of Shiga toxin-producing *Escherichia coli* (STEC) in feces of healthy and diarrheic calves in Urmia region, Iran. *Iranian Journal of Microbiology, 4*(2), 63–69.

De la Fuente, R; Garcia, A; Ruiz-Santa-Quiteria, JA *et al.* (1998). Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. Prev Vet Med; 36:145-152.

El Ayis, A. A.; Elgaddal, A. A. and Almofti,Y. A.(2015). Isolation, identification and enterotoxin detection of *Escherichia Coli* isolated from calf diarrhea and their virulence characteristics. Journal of applied and industrial Sciences, 3(4): 141-149.

El Behiry, A.(2014). Diagnostic aspects and novel approach for treatment of antibiotic-resistant bacteria isolated from diarrheal calves using silver, gold and copper nanoparticles. 5(4):1-9.

EL-Amrousi, S.; Nafie, E.K.; EL- Rohewi, M. and Mottilib, A. (1971). Studies on enteritis in buffalo calves in Assiut. J. Egypt. Vet. Med. Ass., 31: 219-225.

Fecteau, G., Fairbrother, J. M., Higgins, R., Van Metre, D. C., Paré, J., Smith, B. P., … Jang, S. (2001). Virulence factors in Escherichia coli isolated from the blood of bacteremic neonatal calves. *Veterinary Microbiology*, *78*(3): 241–249.

Ferrarezi, MC, Cardoso, TC and Dutra, IS.(2008). Genotyping of Clostridium perfringens isolated from calves with neonatal diarrhea. Anaerobe, 14, 328-331.

Galal, H.M. ;Hakim, A.S. and Dorgham,S. M.(2013). Phenotypic and virulence genes screening of Escherichia coli strains isolated from different sources in delta Egypt. Life Science Journal, 10(2): 352-361.

Garcia, A; Ruiz-Santa-Quiteria, JA;Orden, JA ...de la Fuente R (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp ImmunolMicrobiol Infect Dis.; 23(3):175-183.

Germine, S. S.; Ebied, M. H.; Ibrahim, F. K.; Mettias, K. N and, Daoud, A.M. (2011). Field evaluation of egg yolk antibodies in prevention and treatment of enteric colibacillosis in calves. Benha Vet. Med. J., (1):108-114.

Ghanem, F.M.;El-Sheery,M. N.;Ibrahim, K. M.and El-Sherif, A. M. (2005). Investigation of some virulence factors associated with *E. coli* isolated from diarrheic buffalo calves. Beni-Suef Vet Med J 15 (2): 275-277

Gibbons, J. F., Boland, F., Buckley, J. F., Butler, F., Egan, J., Fanning, S., … Leonard, F. C. (2014). Patterns of antimicrobial resistance in pathogenic *Escherichia coli* isolates from cases of calf enteritis during the spring-calving season. *Veterinary Microbiology*, *170*(1–2), 73–80.

Gjessing, K. M. and Berkhoff, H. A. (1989). Experimental production of air sacculitis and septicaemia by aerosol exposure of 1- day –old chicks using Congo red-positive *Escherichia coli*. Avian Dis., 33(3): 473-478.

Haggag, Y.N. and Khaliel, S.A. (2002). Public health importance of certain bacteria isolated from calves and small ruminants.The 2nd Veterinary Congress, Fac. Vet. Med., Minufyia University, Egypt. 2(1):173-184.

Helal, A.D.; Nagib, H.E.; Abdel-Samer, M.E. and Abdel-Fattah, SH.M.(2014). Using of traditional and quantitative cytochemical methods for identification and enzyme characterization of some *E.coli*serogroups causing enteritis in buffaloes.Assiut Vet. Med. J. 60 (142)121:131 .

Ibrahim, A. M. (1995). Sanitary studies on newly born calves: Ph.D. Thesis (Animal Hygiene), Fac. Vet. Med., Suez Canal Univ.

Izzo, M. M.; Kirkland, P. D.; Mohler, V. L.; Perkins, N. R.; Gunn, A. A.and House, J. K. (2011). Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Australian Veterinary Journal*, *89*(5): 167–173.

Kreig, N. R. and Holt, J. G. (1984). Bergey's Manual of Systematic Bacteriology. Vol.1.Williams and Wilkins. Baltimore.

Magalhães, P.; Pinto, L.; Gonçalves, A.; Araújo, J. E.; Santos, H. M.; Capelo, J. L.; … Igrejas, G. (2016). Could transformation mechanisms of acetylase-harboring

pMdT1 plasmid be evaluated through proteomic tools in Escherichia coli? *Journal of Proteomics*.

Marilda, C.; Ernest, E.; Julio, C.; Amauri, A.; Lvens, G. And Diogenes, S. (1990). Virulence factors of avian *Escherichia coli*. Avian Dis. 34: 531-538.

Osman, K.M.; Mustafa A.M.;Elhariri M. and AbdElhamed, G.S. (2012). Identification of serotypes and virulence markers of *Escherichia coli* isolated from human stool and urine samples in Egypt. Indian J. Med. Microbiol., 30: 308-313.

Osman, K. M.; Mustafa, A. M.; Elhariri, M.and Abdelhamed, G. S. (2013). The distribution of *Escherichia coli*serovars, virulence genes, gene association and combinations and virulence genes encoding serotypes in pathogenic *E. coli* recovered from diarrhoeic calves, sheep and goat. Transboundary and Emerging Diseases, 60(1): 69–78.

Payne, S. M. and Finkelstein, R. A. (1977). Detection and differentiation of iron responsive avirulent mutants on Congo red agar. Infect. Immun. Am. J. Assoc. Microbiol., 18(1): 94-98.

Pluschke, G.; Mayden, J.;Achtman, M., and Levine, R. P. (1983). Role of the capsule and the O antigen in resistance of O18:K1 Escherichia coli to complement-mediated killing. Infection and Immunity, 42(3), 907–913.

Pupo,G. M.;Karaolis,D. K.; Lan,R. and ReevesP.R. (1997). Evolutionary relationships amongpathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and mdh sequence studies.Infect Immun. 65(7): 2685–2692.

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C.; Leonard, F. C. and Maguire, D., (2002). Veterinary Microbiology and Microbial Disease. Published by Blackwell. PP. 113-116.

Refai (1980). *Escherichia coli* infection in calves. Egyptian German Seminar on the mortality of newly born calves. pp 85-86.

Shehata, A.A.E. and Salam, H.S.H (2012). Phenotypic characterization of E. coli isolated from farm animals with diarrhea: use of lectina s a non-specific immune stimulant. Intl. J. Microbiol. Res., 3 (3): 227-237.

Siegfried, L.; Kmetova, M.; Janigova, V.; Sasinka, M. and Takacova, V. (1995). Serum response of *Escherichia coli* strains causing dyspepsia and urinary tract infection: relation to alpha-hemolysin production and O type. Infect. Immun. 63(11):4543-4545.

Vinal, A. C. (1988). The association of Congo red binding and virulence in *Escherichia coli* pathogenic for poultry. Diss. Abstr. Int., 49(6): 2059-2065.

Wray and Morris (1985). Aspects of colibacillosis in farm animals.J Hyg 95(3): 577–593.

Zaki, E. R. (1995). Virulence factors in Escherichia coli and colisepticemia in neonatal calves .Ph.D. Thesis (Microbiology), Fac.Vet.Med.,Cairo, Univ.