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# Antigenic evaluation of extracted fimbrial protein obtained from pathogenic *Escherichia coli* isolated from diarrheic camel neonates

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***Abstract***---Enteropathogenic *Escherichia coli* (EPEC) were considered prime reason of diarrhea among neonatal livestock animals in developing countries and could be of public health importance via contaminated milk and meat. Continual attempts were conducted to combat this illness using various antigenic determinants. This study was performed on four *E. coli* serotypes O26, O45, O103 and O111, which were previously recovered from diarrheic camel calves in Giza, Egypt. Extraction of fimbrial proteins was carried out through dialysis then evaluation of their immunogenic activity was preceded. SDSPAGE electrophoresis was performed on crude extracted fimbrial proteins and revealed single band for each isolate ranged from 22 to 33kDa. Immunobloting was implemented on the extracted crude fimbrial proteins against *E.coli* O26 antisera formerly prepared in rabbit.These findings suggested that the fimbrial proteins are of immunogenicity importance and can serve as a protective passive vaccine antigen in prevention of diarrhea caused by EPEC and ETEC infection in camel calves.

***Keywords***---camel calves, entropathogenic *Escherichia coli*enterotoxigenic, *Escherichia coli*, fimbrial protein, immunoblot, SDS PAGE-electrophoresis.

## Introduction

Entropathogenic particularly enterotoxigenic *Escherichia coli* (ETEC) organism is a significant reason of bacterial diarrhea in developing countries not only in human but only in various neonatal animals as camel calves (Shahein et al., 2021). It is believed that ETEC diarrhea and consequent dehydration are due to the production of fimbrial colonization agents (CFs) that eased small intestinal adherence and elaboration of enterotoxins (either heat stable or heat labile) that encourages the loss of electrolytes and water; results in the complexity of the case that may be ended by death (Bin et al., 2018). Fimbriae are proteineous thread appendices which found to be jutted from the bacterial cell surface and noticed to have ability of erythrocytes agglutination (Duguid et al., 1955).

*E. coli* fimbriae were described mainly on enterotoxigenic *E. coli* (ETEC) from animals as well as humans thereafter, described on enteropathogenic (EPEC), necrotoxigenic (NTEC), septicaemic (SePEC), and uropathogenic (UPEC) *E. coli* (Mainil, 2013). Fimbriae are denoted by the letter F followed by numbers, and encoded by genes which can be plasmid-located, or chromosome-located (Rasko et al., 2008). F1 (type A) is a mannose-sensitive haemagglutination factor, found to be produced by nearly all strains of *E. coli* and probably engaged in the early strides of the colonization and encoded by fimH gene (Nataro, 2005). Because of the considerable morbidity and mortality rates associated with pathogenic especially zoonotic ETEC diarrhea, development of accurate diagnosis then protective vaccine which can combat these pathogens in-between livestock animals as camels has been followed up to diminish either economic or public health importance (Bourgeois et al., 2016). In spite of the common LT and LT-like vaccines have been demonstrated to give protection but it is found to be hindered

by finite coverage and sturdiness. Today, the direction to whole-cell live and inactivated vaccines comprising both LT and fimbriae -based constituents are clinically settled (Zhang et al., 2018). Our study targeted the extraction of fimbrial (F1) protein from local enteropathogenic *Escherichia coli* previously isolated from diarrheic camel calves, then assessment of its immunogenic ability as a step of prospect vaccine development.

## Material and Methods

### Samples

Fourvarious *E. coli* serotypes; O26, O45, O103 and O111 that obtained from diarrheic newly born camel calves (Shahein et al., 2021) and carried fimH gene which responsible for fimbriae type A formation.

### Extraction of Crude Fimbrial Protein from *E.Coli* Strains

The four serotyped *E.coli* isolates were grown onto veal heart infusion broth at 37°C for 24 hours. The developed colonies were harvested, washed three times using 0.01M Tris hydrochloride, and then centrifuged at 6000 xg for15minutes. Each pellet was resuspended using the same previous buffer, then autoclaved at 121°C for 20 minutes. The bacterial fimbriae were obtained from cells by agitation in a homogenizer set at 70% speed for 5 minutes at 4°C. Cells were then centrifuged at 3000 xg for30 minutes, and then each supernatant which contained the fimbriae was separated. The supernatants were dialyzed overnight at 4°C in 6000 to 8000 molecular weight restriction dialysis tubing against 2 L of 0.05M Tris buffer (pH 9.5) with 0.01M sodium azide. Fimbriae were precipitated by adding ammonium sulfate to 50% saturation. The precipitated proteins were gathered and centrifuged, then the pellets were resuspended in 0.01M Tris hydrochloride and dialyzed in several changes of phosphate buffer saline (PBS pH 7.4) for 2 days at 4°C. Finally, each solution was aspirated, and protein concentration was determined and stored at -70°C till used (Buckles et al., 2004).

### Purification of Fimbrial Protein from *E.Coli*Strains using SDS-PAGE Electrophoresis:

The prepared fimbrial proteins were purified by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) on 12% NuPageBis-Tris gradient gels (BIO-RAD). Fimbrialpreparation was diluted at 4-fold with sample buffer (0.25M TrisHcl, 0.5% SDS, 2.5% glycerol and 1µg/ml bromophenol blue), and drying occurred in 80°C for 2 hours (Laemmli, 1970).Quantitation of different fractions of each sample was performed using BIO-RAD (GS- 700 Imaging Densitmeter) by molecular analysis software.

### Preparation of *Escherichia coli* Antisera in Rabbits Ethical Approval

Handling and inoculation of with animals followed the ethical guidelines of the Ethical Committee of the Cairo University, Egypt. The antisera were prepared in female New Zealand white rabbits aged 2 month old by subcutaneous inoculation of acetone killed *E.coli* O 26 isolate emulsified in equal volumes of Freund's

complete adjuvant at days; 1, 5, 10, 15, 20 by a dose of 0.5ml, 1ml, 2ml, 2ml, 2ml respectively. Rabbits were bled from the ear vein at days 25, 30 and bled out on day 35 and the antibody fractions were precipitated by bringing it to 50% saturation with ammonium sulfate (Brade et al., 1986).

**Immunoblotting**

Electrophoretic transfers of fimbrial proteins from SDS-PAGE gels to nitrocellulose sheets; using a Mini Trans-Blot electrophoretic cell (Bio-Rad) for 60 min at 100 V. The membrane was blocked with Starting Block supplemented with 0.05% Tween 20 (Pierce). Incubations with rabbit polyclonal antisera against acetone killed *E.coli* O 26 was carried out for 1 h at room temperature (Lymberopoulos et al., 2006).

**Results**

The crude fimbrial proteins were extracted from the four *E.coli* isolates using overnight dialysis and addition of ammonium sulfate to 50% saturation. Fimbrial proteins were purified using 12% SDS-PAGE electrophoresis and declared one fraction band for each *E.coli* isolate at molecular weight of 22, 25, 33 and 33kDa for *E.coli* O26, O45, O103 and O111 respectively figure (1).

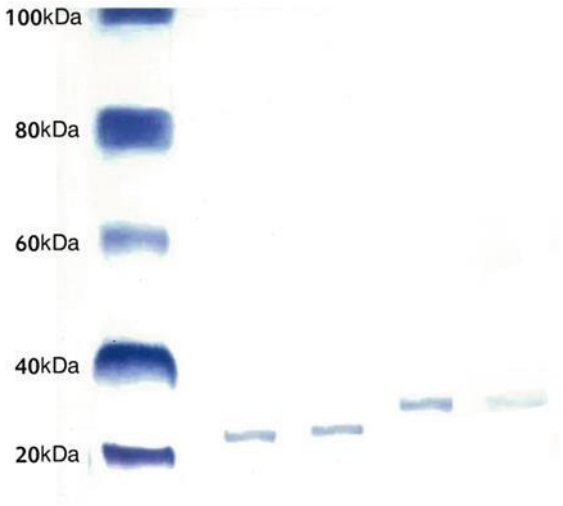


Figure 1. SDS-PAGE analysis of purified fimbriae isolated from *Escherichia coli* isolates. The fimbriae were electrophoresed on SDS-polyacrylamide gel and stained with Coomassie brilliant blue. Lane 1: marker (20 kDa protein ladder), Lanes: 2,3,4,5 revealed fimbrial proteins' fractions of *E.coli* O26, O45, O103 and O111 respectively

The immunogenicity of the extracted fimbrial protein was assessed against rabbit polyclonal antisera for acetone killed *E.coli* O 26 using western blotting. Figure (2) showed formation of immunoblot at 22 kDa.

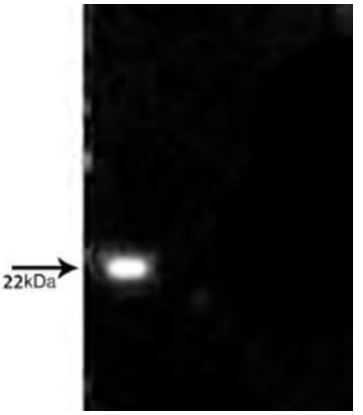


Figure 2. Western blot analysis of *Escherichia coli* fimbriae. The SDS-PAGE was electrophoretically transferred to a nitrocellulose membrane and incubated with polyclonal antisera for acetone killed *E.coli* O 26 (Mw 22-kDa)

**Discussion**

In spite of *Escherichia coli* generally considered one of microbiota of animal's gut, it is known that the microorganism has another face that in many conditions become pathogenic and causes several illnesses (Rossi et al., 2021). In developing countries as Egypt, enterotoxigenic *E. coli* (ETEC) is believed to be reason of diarrheal disease; enteric colibacillosis encountered in neonatal ruminants not only in cattle's (Galal et al., 2013) and buffalo's (Hakim et al., 2017) but also in camel's calves (Shahein et al., 2021). ETEC are noninvasive bacteria that its pathogenicity relies on their ability to adhere and colonize the intestinal epithelium via the important proteinaceous fimbriae. After that, the pathogen produces its virulence factors; either heat-labile or heat-stable enterotoxin or both resulting in diarrhea (Bai et al., 2020).

Type 1 fimbriae are appeared to be the most significant mannose-sensitive fimbriae, which are encoded by a gene bunch in the bacterial chromosome. FimHadhesin constitutes a dimer component at the tip of the fimbriae and probably represents the prime agent in the binding of the pathogen to epithelial cells (Sheikh et al., 2017). The purification of the fimbrial protein of previous mentioned *E. coli* serotypes isolates were performed in this study by SDS-PAGE analysis. It was declared fromfigure (1) single bands of apparent molecular weight of 22, 25, 33 and 33 kDa represented *E.coli* O26, O45, O103 and O111 respectively.Our result was harmonized with the former investigations which concluded that various fimbrial proteins were exhibited as one separate band with their molecular weights ranged from 15 to 40kDa (Sheikh et al., 2017; Blackburn et al., 2021).

For Western blotting, membranes were incubated with rabbit polyclonal antisera against acetone killed *E.coli* O 26 and the presence of the blot of 22 kDa support the immunogenicity of fimbrial protein. This result coincided with the data obtained from several investigations kDa (Luiz et al., 2015; Sincock et al., 2016; AsadiKaram et al., 2019; ) which support that fimbrial proteins are good immunogenic and become a prospective target for vaccine production.It was

demonstrated that fimbriae type 1 are highly conserved; expressed in majority of diverse ETEC clinical isolates so they are a common target for prospective vaccine development(Sahl et al., 2015; Sheikh et al., 2017; Song et al., 2020).

## Conclusion

In our study we succeeded in extraction of crude fimbrial protein from four serotyped *E.coli* isolates that obtained from diarrheic camel calves. The immunogenicity of these fimbrial proteins was achieved against polyclonal antisera prepared in rabbit. Further investigations will be recommended to realize more purification and fractionation of these immunogenic proteins to achieveproper future vaccines to combat colibacillosis in neonates.

## Conflict of interest

The authors declared that no conflict of interest.

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