A COMPARISON BETWEEN THE TRADITIONAL AND THE MOLECULAR METHODS IN THE DIAGNOSIS OF DIARRHEAGENIC *ESCHERICHIA COLI*

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ABSTRACT

A total of 126 fecal samples were collected from diarrheic animals and human (43 calves, 24 calf-camel 14 sheep and 45 children). The isolates were identified biochemically, then serotyping of *Escherichia coli* isolates were done to identify somatic (O) serotype and flagellar (H) serotype. The results revealed a wide variety of *Escherichia coli* serogroups implicated as a cause for diarrhea in human and animals. Serotypes recovered from calf-camels were different than the serotypes recovered from children which indicate absence of the zoonotic role of calf-camels to human being. The isolates were then subjected to polymerase chain reaction (PCR) for the detection of species-specific gene (*uidA*), the results were (35/37; 27.8%), (18/20; 14.3%), (8/11; 6.3%) and (7/8; 5.6%) among children, calves, calf-camels and sheep, respectively. These results clear the high sensitivity of the molecular methods for diagnosis comparing to the conventional ones.

INTRODUCTION

Diarrhea remains one of the main causes of morbidity and mortality in today's world and a large proportion is caused by diarrheagenic *Escherichia coli* (***Stuart*, 2001**). Diarrheagenic *Escherichia coli* have been classified into major categories, on the basis of their distinct virulence properties: enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), and enteroinvasive (EIEC) and enteroaggregative (EAEC) (***Nataro* and *Kaper*, 1998).** Assays for identification of diarrheagenic *Escherichia coli* in the developing world are limited to research laboratories. Therefore epidemiological data on diarrheal pathogens from developing countries are scattered (***Shaheen* et al, 2009).**

The molecular methods seem to be the most reliable for identification and studying diarrhea-associated *Escherichia coli* ***(Achtman, 2001),*** but they have not standardized yet in Egypt. Therefore, the present study aimed to provide a molecular identification for diarrhea associated with *Escherichia coli* in Egypt by the detection of the species specific gene (*uidA*) using Polymerase chain reaction (PCR).

MATERIAL & METHODS

Fecal samples:

Each ecal sample was collected separately in a sterile plastic bag and sent to the laboratory within few hours **(*ElSayed*, 1995).**

Bacteriological examination:

A loopful from each sample was inoculated onto MacConkey's agar for primary iso-lation and incubated at 37°C for 24 hours. Lactose fermenting colonies were picked up to be cultured on eosin methylene blue (EMB) and incubated at 37°C for 24 hours for their characteristic greenish metallic sheen. These colony was picked up and streaked on nutrient agar for further identification and also in semi-solid agar for detection of motility and was kept as stock culture.

Morphological examination:

Films from the pure colonies were stained by Gram's stain to examined the morphology, arrangement and staining reaction of the organism **(*Cruickshank* et al., 1975).**

Biochemical identification:

Suspected isolates were confirmed by a series of biochemical tests according to ***Barrow* and *Feltham*, 1993.**

E.coli serotyping:

A - Somatic antigen serotyping :

All the suspected isolates were subjected to slide agglutination test using standard polyvalent and monovalent *Escherichia coli* antisera. Only fresh bacterial cultures from 18- 24 hours colonies onto nutrient agar medium were used.

Determination of O. antigen was done by slide agglutination test according to ***Edwards* and *Ewing*, 1972.**

B- Flagellar antigen stereotyping

Determination of H-antigen was carried out using the test tube method (Augment motility), **(*Davies* and *Wray*, 1997).** The bacteria were cultured in liquid media in Craigie tube. The strains arrived at the surface of the outside Craigie tube were picked up then incubated at 37°C for 18-24 h. Equal 1 %vol. formalin saline was added to the antigen. In a new tube three drops of H-antisera were added and mixed with 0.5ml formalized antigen, then placed in 50°C water bath for 60 min.

Detection of species specific gene (*uidA*) by PCR

All *Escherichia coli* strains tested for the presence of species-specific gene to prove that it is *Escherichia coli uidA* gene ***(Sabrina et al., 2007),*** which amplify 623bp. uidA-F (5'-CCAAAAGCCAGACAGAGT-3') and uidA-R (5'-GCACAGCACA TCAAAGAG -3'). Thirty five amplification cycles at 98 °C for 10 Sec., 62°C for 55 Sec., and 72 °C for 45 Sec. were performed in a thermal cycler (TAKARA, Japan).

RESULTS AND DISCUSSION:

The prevalence of biochemically positive *Escherichia coli* isolates is shown in table (1); isolates 37, 20, 11 and 8 among children, calves, calf-camels and sheep, respectively. The results revealed the importance of biochemical tests for the identification of *Escherichia coli.*

As shown in table (2), a wide variation was found in the serotypes of *Escherichia coli* causing diarrhea among children, these may be due to the variation in the immune status of the patients. the most prevalent serotype was O157:H7 (6/35; 17.4%), which known to cause diarrhea in children ***(Pradel et al., 2000),*** followed by O6:H10 (4/35; 11.4%), which considered to be the most popular in causing diarrhea among the Egyptian children ***(Shaheen et al., 2009).***

The prevalence of different serotypes among calves elucidate that the most prevalent serotypes were O86a :H10 and O157:H7 at a percentage of (3/18; 16.7%) for each, as shown in table (3). Cattle known to be the primary reservoir for O157:H7 ***(Michalowicz and Gawrych, 1996).*** Large number of human illness outbreaks has been traced worldwide to consumption of undercooked ground beef and other beef products contaminated with Shiga toxin-producing *Escherichia coli* (STEC). Although several routes exist for human infection with STEC, beef remains a main source. Thus, beef cattle are considered reservoirs of O157 and nonO157 STEC ***(Hussein, 2007).*** The previous data emphasized the critical need for control measures to assure beef safety.

Data in table (4) reveale *Escherichia coli* serotypes which recovered from diarrheic calf-camels. The most prevalent serotype was O165:H21 (2/8;25%), the serotypes of *E.coli* isolates recovered from calf-camels are completely different from the serotypes recovered from calves, sheep or children. It may be indicating absence of the zoonotic possibility between camel and human which agreed with ***(El-Sayed et al., 2008).***

Escherichia coli serotypes recovered from diarrheic sheep explained in table (5), showed the equal presence of serotype O74:H6 and O157:H7 (2/7; 28.7%) each. The

prevalence of O157:H7 among sheep was very popular (*Oporto* et al., 2008) and ***(Johnsen et al., 2001).*** Serotypes O157:H7 were recovered from diarrheic children, calves and sheep which support the zoonotic hypothesis among them ***(Brett et al., 2003).***

The molecular methods seem to be more reliable than the conventional ones. In the present study *uidA* was used for detection of *Escherichia coli* species, *uidA* identified as *Escherichia coli* Species-specific gene. *uidA* gene encodes the P-glucuronidase (GUD) enzyme that catalyze breakdown of complex carbohydrates ***(Feng and Lampel, 1994).*** As shown in table (6), the biochemically tested *E.coli* strains subjected to PCR for the detection of *uidA*, it was found that (35/37; 27.8%), (18/20; 14.3%), (8/11; 6.3%) and (7/8; 5.6%) among children, calves, calf-camels and sheep isolates, respectively. The results conclude that the detection of species-specific gene (*uidA*) is more specific than the conventional methods for diagnosis of *Escherichia coli* ***(Lluque et al, 2010).***

Table (1): The prevalence of biochemically positive *Escherichia coli* isolates.

Origin	Total No. of suspected isolates	No. of biochemically positive isolates	Percentage*
Children	43	37	29.4%
Calves	36	20	15.9%
Calf-camel	23	11	8.7%
Sheep	12	8	6.3%
Total	114	76	60.3%

*** The percentage was calculated according to the total number of samples (126).**

Table (3): *Escherichia coli* serogroups recovered from diarrheic calves

Serotype	No.	Percentage
O125:HUT	1	5.6%
O25:H40	2	11.1%
OUT:HUT	3	16.7%
O6:HUT	1	5.6%
O1:H21	1	5.6%
O142:HUT	1	5.6%
O148:H2	1	5.6%
O86a:H10	3	16.7%
OUT:HNM	1	5.6%
O157:H7	3	16.7%
O48:HNM	1	5.6%
Total	**18**	**100%**

Table (2): *Escherichia coli* serogroups recovered from diarrheic children

Serotype	No.	Percentage
O27:HUT	1	2.9%
O142:HUT	1	2.9%
O143:HUT	1	2.9%
O15:H45	1	2.9%
O20:HNM	1	2.9%
O20:H9	1	2.9%
O8:H45	1	2.9%
O8:HUT	1	2.9%
O8:H12	1	2.9%
O166:H45	1	2.9%
O74:H21	1	2.9%
O164:HNM	1	2.9%
O157:H7	6	17.1%
O18:H10	1	2.9%
O18:HNM	1	2.9%
O55:H6	1	2.9%
O55:H10	2	5.7%
O1:HUT	1	2.9%
O6:H10	4	11.4%
O6:H16	1	2.9%
O6:HNM	1	2.9%
O125:H6	1	2.9%
OUT:HNM	2	5.7%
OUT:HUT	1	2.9%
OUT:H16	1	2.9%
Total	**35**	**100%**

Table (4): *Escherichia coli* serogroups recovered from diarrheic calf-camels

Serotype	No.	Percentage
O165:H21	2	25%
O126:H28	1	12.5%
O103:H2	1	12.5%
O161:H21	1	12.5%
O143:H16	1	12.5%
O128:H21	1	12.5%
OUT:H2	1	12.5%
Total	**8**	**100%**

Table (5): *Escherichia coli* serogroups recovered from diarrheic sheep

Serotype	No.	Percentage
O74:H6	2	28.6%
OUT:H21	1	14.3%
O157:H7	2	28.6%
O8:H4	1	14.3%
O142:H2	1	14.3%
Total	**7**	**100%**

Table (6): The number and percentage of *Escherichia coli* identified by *uidA*

Species	Total No. of biochemically identified isolates	No. of positive isolates for uidA gene	Percentage*
Children	37	35	27.8%
Calves	20	18	14.3%
Sheep	8	7	5.6%
Calf-camel	11	8	6.3%
Total	**76**	**68**	**54%**

*** The percentage was calculated according to the total number of samples (126)**

Species-specific gene for *Escherichia coli* (*uidA*)

Results of PCR amplification of *uidA* gene shown in photo (1). It gives the specific band size (623 bp) in 100% of the tested strains.

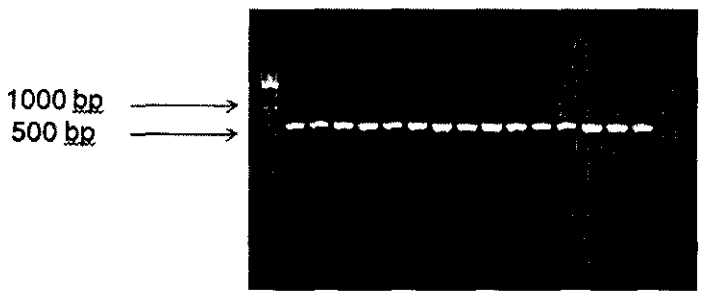

Photo (1): Agarose gel electrophoresis of Amplified *uidA* gene PCR product (623 bp). Lane 1, one-step ladder 500 bp. Lane 2, control positive for *uidA* gene. Lane 3-14, positive strains for *uidA* gene. The last lane represents the negative control.

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الملخص العربى

تم جمع 126 عينه براز من حالات اسهال فى كلا من الحيوان و الانسان(45 من الاطفال و43 من العجول و24 من عجول الجمال و 14 من الماعز) تم تعريف العترات بيوكيميائيا ثم تصنيفها سيرولوجيا لتعريف الانتيجين الجسمى و السوطى و قد تبين تسبب مدى واسع من الانواع السيرولوجيه للايشيريشيا كولاي للاسهال فى الحيوان و الانسان و ايضا اتضح الاختلاف الكامل فى الانواع السيرولوجيه المعزوله من عجول الجمال عن الانواع المعزوله من الاطفال مما يؤكد غياب دور الجمال فى نقل العدوى للانسان. تم تعريف العترات باستخدام تفاعل البلمره المتسلسل للكشف عن الجين الخاص بجنس الإيشيريشياكولاى(uidA) وكانت النتيجة كالتالى(37/35، 27.8%، 20/18، 14.3%، 11/8، 6.3%، 8/7، 5.6%) فى كلا من الأطفال، العجول ، عجول الجمال والماعز على التوالى من الماعز. وقد عكست النتائج مدى حساسية الطرق الجزيئية فى تشخيص الإيشيريشياكولاى عن الطرق التقليدية.