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ORIGINAL ARTICLE

Screening for *Escherichia albertii* in Children with Gastroenteritis in Pediatric Hospital at Assiut University

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ABSTRACT

Background: *Escherichia albertii (E. albertii) was isolated from feces of the people suffering from gastroenteritis. It is problematic to differentiate between it and other species of Enterobacteriaceae.* ***Objective:*** *The work aimed to determine the prevalence of E. albertii in stool samples from the children with gastroenteritis and its relation to some clinical and demographic factors.* ***Methodology:*** *In this study, 296 fecal samples were cultivated on MacConkey, xylose lysine deoxycholate and Hektoen enteric agar plates. Vitek®2 was used to analyze the isolates. By PCR lysP, mdh and eae genes were detected for E. albertii.* ***Results:*** *Out of 296 samples, 35 isolates (11.8%) were recognized as E. albertii. Younger children were more vulnerable. The gender was not a risk factor. Diarrhea was watery in all included cases. Vomiting, abdominal pain and fever were not presented in all included cases.* ***Conclusion:*** *E. albertii can cause gastroenteritis. For precise diagnosis, the use of PCR can be beneficial.*

INTRODUCTION

Albert[1, 2] and colleagues, Huys et al.[3], and Hyma et al.[4] initially He described the enteric bacteria; *Escherichia albertii*, which is linked to infectious gastroenteritis. that causes watery diarrhea, abdominal distention, vomiting, and fever in human[5]. Gastroenteritis is a leading cause of mortality and morbidity, particularly in areas where sanitation is lacking[6]. *Escherichia* is a genus of enteric bacteria that includes five species: *Escherichia coli* (the most frequent one), *Escherichia hermannii*, *Escherichia vulneris*, *Escherichia fergusonii and Escherichia blattae* (less frequent species)[7].

Later, the sixth one; *Escherichia albertii* was related to diarrhea in Bangladeshi children[3]. *Escherichia albertii* is a pathogen that spreads through food or water contamination, posing a public health risk. It has been classified as an attaching and effacing pathogen[3]. *Escherichia albertii* are Gram-negative, nonspore forming, non-motile, non-lactose fermenting pathogens but d-glucose fermenters with both acid and gas production and incapable of fermenting xylose or dulcitol[8].

There are few studies on human *E. albertii* strains' phylogeny, phenotypic features, and virulence factors[9]. *E. albertii's* phenotypic characteristics are enough varied to make traditional phenotypic identification methods unreliable[7]. The collective action of intimin; an *eae* gene encoded outer membrane protein, and type III secretion system effectors produced by *E. albertii* causes damages on intestinal epithelial cell surfaces[10]. *E. albertii* makes up a representable percentage of the strains previously classified as *eae*-positive *Escherichia coli*; enteropathogenic *E. coli* (EPEC), or enterohemorrhagic *E. col* (EHEC)[8]. The cytolethal distending toxin gene *(cdt)* can also be expressed by *E. albertii*[11] and seldom Shiga toxin 2 genes (stx_{2a}, stx_{2f})[12].

The exact clinical significance and prevalence of *E. albertii* are unknown, in part due to the failure to detect isolates using commercially available biochemical identification techniques like Vitek and API biochemical test strips. Isolates may be falsely identified as *Hafnia alvei*, *Shigella* spp., and *E. coli*, or since *E. albertii* is not mentioned in databases of API and Vitek.[13].

To discriminate *E. albertii*, molecular genetic methods like PCR were used[8]. *E. albertii* has been recognized via 16S rRNA gene sequence analysis [14], however, it is not discriminatory enough[15].

The PCR is used to detect two genes: *mdh* (encoding malate dehydrogenase) and *lysP* (encoding lysine Permease) as specific genes in *E. albertii*[16].

There are currently no published data on *E.albertii* in humans from Assiut or even Egypt to the authors ' knowledge. The worldwide data on involvement in

generating diseases in humans and animals, and how to detect *E.albertii* is still sparse. The work aimed to determine *E. albertii* prevalence in stool samples from children with gastroenteritis and how it related to clinical and demographic features.

METHODOLOGY

Ethical statement

The Ethical Committee of the Faculty of Medicine at Assiut University, Egypt, approved the research in accordance with the World Medical Association's code of ethics (Declaration of Helsinki) with IRB number:17300440 dated 26/7/2020. A complete history was obtained, including demographic and clinical informations such as name, age, sex, type of feeding (breast or artificial) and history of certain clinical data such as occurrence of vomiting, diarrhea, abdominal pain, distension, and fever.

Study design

This cross-sectional study was directed to determine *E. albertii* prevalence in stool samples from children with gastroenteritis and its relation to clinical and demographic features.

Studied population

Between August 2020 and December 2020, Children with suspected gastroenteritis and presented with diarrhea in combination with abdominal cramps, abdominal pain, or fever attending Pediatric Hospital at Assiut University, Assiut, Egypt were included.

Sample size

Collection of 296 stool samples from children with suspected gastroenteritis was done.

Macroscopic observations

Mucus or blood presence in these stool samples was physically checked. The classification of stool was done as formed if the consistency was ordinary, semi-formed if it appeared semi-solid, and watery if it was accompanied by a large amount of water.

Isolation and presumptive recognition of *E.albertii*

The samples were cultured on MacConkey agar plates aseptically and incubated at 37°C overnight[5]. Non- mucoid and non-lactose fermenting, colonies were chosen for plating on xylose lysine deoxycholate agar (XLD) and Hektoen enteric agar (HEA) (HEA) (Hardy Diagnostics, Santa Maria, CA) and at 37°C. These plates were incubated overnight. On XLD, suspected isolates of *E. albertii* appeared as pink with a somewhat yellow or cream-colored core colonies while on HEA, they appeared as green colonies). In 10% glycerol[17] with Luria-Bertani (LB) liquid medium (Hardy Diagnostics, Santa Maria, CA), selected isolates were frozen at -20°C[18].

Phenotypic characterization of *E. albertii* isolates using Vitek®2

The Vitek®2 Compact System (bioMérieux, France) was used for presumptive identification of the *E. albertii* isolates. For biochemical analysis, Vitek test card (GN ID card) (Gram negative for identification) was used, and testing was done consistent with the manufacturer's instructions. After a 15-hour incubation period, the findings were interpreted using AES 8.01 software[17].

Molecular characterization of *E. albertii*

DNA extraction

Scraping 5 colonies from the agar plate and dissolving them in 100 µL of molecular grade water in an Eppendorf tube were done to isolate genomic DNA from overnight cultures. For ten minutes, tubes were incubated at 100°C in a heating block. To each tube, 900 µL of molecular grade water was added, then vortexing and centrifugation were done to homogenize the solutions. The supernatants were put into sterile Eppendorf tubes and stored at - 20 °C until PCR analysis[19].

Polymerase chain reaction (PCR) for expected E. albertii genes

The isolates were tested for presence of three genes: two housekeeping genes (*lysP* and *mdh*) which are *E. albertii* specific genes (amplicon size 252 bp and 115 bp, respectively) [19] and one virulence gene (*eaeA*), intimin, the attaching and effacing gene[4].

The PCR was carried out using thermocycler instrument (Thermo Fisher Scientific, England). Total reaction volume was 20 µL. Table (1) lists the components needed for each reaction.

Table 1: PCR reaction components

Reaction components	(Volume) µl
Water	12.6
Buffer (10X) PCR	2
Mg Cl_2 (50 mM)	1.5
dNTPs (10 mM)	0.4
Primers (From each one)	1.2
Taq DNA Polymerase (5 U/µl)	0.3
Template DNA (10 ng/µl)	2
Sum	20

Primer selection

The primers for the three genes (*eae*, *mdh*, and *lysP*) were acquired from Invitrogen Company, as stated in table (2) (United Kingdom). The chosen primer pairs were diluted (1:10) to the defined standard by adding certain amounts of sterile distilled water.

Table 2: Primer sequences used in this study

Gene	Primer name	Sequence ('3-'5)	Amplicon size (bp)	Reference
mdh	mdh_50F mdh_164R	5′-CTG GAAGGC GCA GAT GTG GTA CTG ATT-3′ 5′-CTT GCT GAA CCA GAT TCT TCA CAA TAC CG-3′	115	4
lysp	lysP_107F lysP_358R	5′-GGG CGC TGC TTT CAT ATA TTC TT-3′ 5′-TCC AGA TCC AAC CGG GAG TAT CAG GA-3′	252	4
eae	eae1 F eae1 R	5′-ATA TCC GTT TTA ATG GCT ATC T-3′ 5′-AAT CTT CTG CGT ACT GTG TTC A-3′	425	22

Thermal cycling of PCR reaction

The thermal cycle began with a 5-minute initial denaturation at 95°C, followed by 35 cycles. Denaturation at 95°C for 30 s, primer annealing at various temperatures for each primer (Annealing temperatures in this work for *eae*, *mdh*, and *lysP* primers were 67, 65, and 64°C, respectively) for 30 s, and extension at 72°C for 60 s were all conducted over these 35 cycles. Lastly, final extension was done at 72℃ for 5 min after the cycles were completed[4, 21].

Electrophoresis of PCR products on agarose gel

In 2% agarose gel. DNA electrophoresis was done.

Statistical analysis:

The analysis statistically was carried out using the Statistical Package for Social Sciences, version 16 (SPSS Inc., Chicago, USA). The Chi-square test and the Student's t-test were employed to compare categorical and continuous variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Escherichia albertii **identification by culture, VITEK® 2 and PCR:**

The culture findings revealed that 35 out of 296 (11.8 %) of the samples had *E. albertii* properties. However, analysis of these isolates biochemically was performed using Vitek®2 Compact system. There were no significant phenotypic differences between *E. albertii* and *E. coli*. This system was not programmed to detect *E. albertii*. The PCR findings showed that *mdh*, *lysP* and *eaeA* genes were detected in these 35 samples as shown in fig. (1), fig. (2) and fig. (3) respectively.

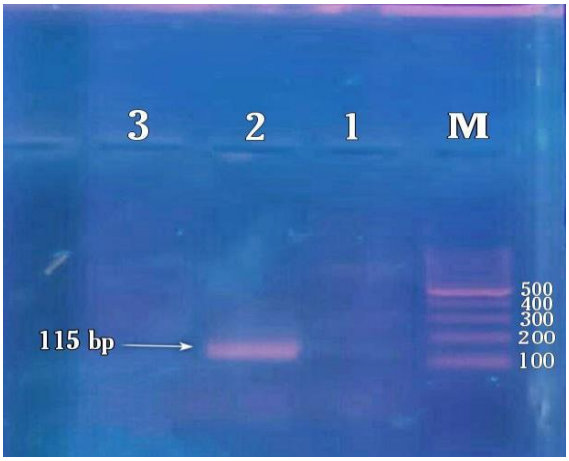

Fig 1: Amplification results of *mdh* gene in *E. albertii* isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control (water); Lanes 2 = positive sample (115 bp); and Lane 3 = negative sample.

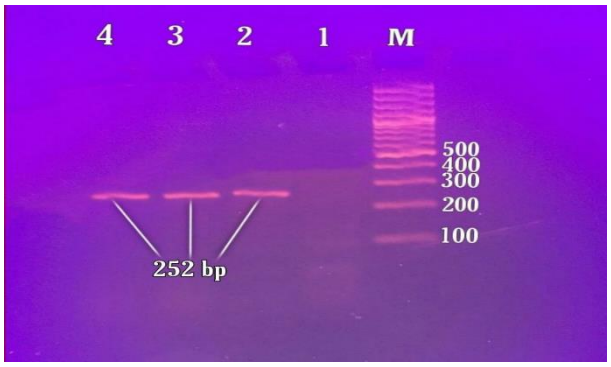

Fig 2: Amplification results of *lysP* gene in *E. albertii* isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control (water); and Lanes 2, 3and 4 = positive samples (252 bp).

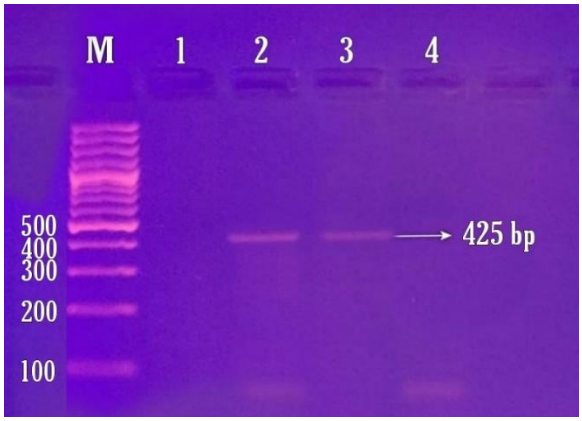

Fig 3: Amplification results of *eae* gene in *E. albertii* isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control (water); and Lanes 2 and 3 = positive samples (425 bp); and Lane 4 = negative sample.

Demographic and clinical data of *E. albertii* positive participants:

Escherichia albertii was detected in 11.8 percent of children with gastroenteritis (35/ 296). The frequency was highest (48.6%) (17/35) in the 0 to 6-month age group, followed by 42.9 % (15/35) in the 6 to 12-month age group, and 8.6 % in the group of age above 12 months, exhibiting statistically significant differences (P = 0.007**). *E. albertii* was detected in 51.4 % (18/35) of boys and 48.6 % (17/35) of girls, with unobserved statistically significant difference. The highest frequency was noticed among the children who experienced vomiting (65.7%) compared to those who did not (34.3%), and the observed differences were statistically significant ($P = 0.002$**). The highest frequency of *E. albertii* (54.3%) was detected in individuals who had diarrhea of 3-5 episodes with the differences being statistically significant (P = 0.004**). Diarrhea was watery in all cases. The highest frequency was noticed among the children who did not experience abdominal pain (62.9%) compared to those did (37.1%), and the observed differences were statistically insignificant. Also, the highest frequency was observed among the children who did not experienced abdominal bloating (54.3%) compared to those who did (45.7%), with unobserved statistically significant difference. The highest frequency was observed among the children who experienced fever (88.6%) compared to those who did not (11.4%), with observed statistically significant difference ($P = <0.0001$***). The highest frequency (65.7 %) was found among breast-fed children, while the lowest frequency (34.3 %) was found among artificially fed children, with unobserved statistically significant difference. The results of demographic and clinical characteristics are summarized in table (3).

Table 3: Prevalence of *E. albertii* in relation to some demographic and clinical data

Variables		Frequency	Percent (%)	P value
Age	< 6m	17	48.6	0.007**
	6-12 m	15	42.9	
	< 12m	3	8.6	
Sex	male	18	51.4	1.000
	female	17	48.6	
Feeding	Breast Feeding	23	65.7	0.084
	Artificial Feeding	12	34.3	
Vomiting	yes	23	65.7	0.002**
	no	12	34.3	
Diarrhea	3-5	19	54.3	0.004**
	6-10	13	37.1	
	10-20	3	8.6	
Abdominal pain	yes	13	37.1	0.170
	no	22	62.9	
bloating	yes	16	45.7	0.737
	no	19	54.3	
Fever	yes	31	88.6	<0.0001***
	no	4	11.4	
Total		35	100	

DISCUSSION

Recently, *E. albertii* has been linked to gastroenteritis in various epidemics, with findings indicating that *E. albertii* is similar to *E. coli* [23, 24]. This was a challenge to verify the diagnosis of *E. coli* pathotypes, especially EPEC. Owing to its unknown properties and biochemical and phenotypic similarities to *E. coli*, it was determined that *E. albertii* was recognized incorrectly as EPEC [8, 16 ,25]. To identify *E. albertii*, several studies screened for two genes, *lysP* and *mdh*. By using the *lysP* and *mdh* genes, Nimri et al.[19] identified 48 (19.2%) cases of *E. albertii* from 250 isolates acquired over a ten-year period from the faeces of persons with diarrhea. By identifying the *lysP* and *mdh* genes in a population with a gastrointestinal infection of dietary origin, Aoshima et al.[16] reported 6 *E. albertii* isolates from 20 (30%) phenotypically identifiable *E. coli* samples. Ooka et al.[26] used the *eae* gene and identified 21 out of 31 (67.7%) samples associated to *E. albertii* gastroenteritis. They were misdiagnosed initially as *E. coli*. Ooka et al.[8] found that out of 278 samples from environmental, animal and human sources that had previously been recognized as *E. coli* using conventional diagnostic methods, 26 were recognized as *E. albertii* by detecting the *eae* gene, with 14 (5%) cases belonging to human samples. Twenty *E. coli* strains isolated from patients with diarrhea were re-evaluated by Hinenoya et al.[27]. By searching for *E. albertii* housekeeping genes, he was able to identify all 20 isolates (100%) as *E. albertii*. In a 6-year care plan, Ori et al.[25] re-evaluated *E. coli* isolates causing diarrhea. They were able to identify 10 (1.4 %) *E. albertii* cases out of a total of 693 isolates. Because there is no specific diagnostic methodology for *E. albertii*, researchers have employed many methodologies and specific genes to detect it, and this information is continually evolving. Although the two genes *lysP* and *mdh* have been identified as unique genes in the diagnosis of *E. albertii* in most studies, these two genes have not been able to identify all *E. albertii* in many studies. As a result, efforts to design more specific parts of the genome have been done[4,17,20,28].

Generally, there isn't enough knowledge on *E. albertii*'s characteristics to properly isolate and diagnose it. As a result, determining the true prevalence of infections caused by *E. albertii* is difficult[27]. The involvement of *E. albertii* as a possible and related pathogen in human gastroenteritis and diarrhea has been confirmed in various studies[3, 17, 29, 26, 27, 30, 31]. The frequency of *E. albertii* infections is increasing in nations like Norway and Japan, which is a hint that *E. albertii* is causing issues all over the world[27].

In the current study, diarrhea was revealed to be a common symptom of *E. albertii* gastroenteritis. This pathogenesis could be owing to *E. albertii*'s virulence factors, like *stxI* and *stxII*, and *eae*, which interface with the intestine, resulting in fluid accumulation and diarrhea, as reported by Nimri et al.[19]. Toxins (gene products) can cause intestinal ulcers, allowing the pathogen to propagate and travel to the circulation, resulting in systemic symptoms such as headache, fever or both[32]. Fever may have developed in the patients by accidental, but it could be a sign of a bacteremic stage clinically., conferring to the current study's statistical findings. In Japan, both abdominal pain and fever have previously been noted in patients who became infected with *E. albertii* after joining a restaurant party, with prevalence rates of 76 % and 38 %, respectively[8] while in our study, their prevalence rates were 37.1% and 88.6% respectively. *E. albertii* was found in approximately equal numbers in both females and males, omitting gender as a risk factor for *E. albertii*-related gastroenteritis This finding is constant with prior studies, which found that gender was a negligible risk factor for *E. albertii* gastroenteritis[5, 33]. There is an evidence that younger children are more susceptible to infection, which is consistent with the *E. albertii* 's first scientific report[19] and was also in agreement with another study reported by Suliman et al[5].

Generally, diagnostic tests for *E. albertii* should be regularly conducted in clinical laboratories to distinguish it from other *Enterobacteriaceae* family members in the future.

CONCLUSION

Escherichia albertii can cause gastroenteritis. Use of PCR for precise diagnosis can be beneficial. Among stool samples, *E. albertii* prevalence was 11.8%. Diarrhea and abdominal pain were symptoms of the infection. The infection was most common among younger children.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of author' knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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