

## An Investigation on the Prevalence of *Escherichia Albertii* in Urine and Stool Samples of Patients in Kermanshah, Iran

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Received 18 July 2023, Accepted for publication 07 October 2023

### Abstract

**Background & Aims:** *Escherichia albertii* is a gram-negative and facultatively anaerobic bacterium that has been isolated as a causative agent of diarrhea in recent years. Due to the lack of sufficient information on the phenotypic and biochemical characteristics of this bacterium, it is difficult to distinguish it from other species of the Enterobacteriaceae, especially *Escherichia coli* pathotypes. So, the aim of this study was to evaluate the prevalence of *E. albertii* in the urine and stool samples of patients with urinary tract and gastrointestinal tract infection in Kermanshah hospitals using culture and molecular methods.

**Materials & Methods:** Four hundred samples consisted of 200 urine and 200 stool samples confirmed as Enterobacteriaceae phenotypically were collected. After subculture and biochemical tests, finally white colonies on XRM-McConkey agar (suspected to be *E. albertii*) were selected and subjected to PCR using specific primer for *cdt* gene (*Eacdt*). SPSS version 16 software was used for the statistical analysis of the data, and the p-value less than 0.05 was considered statistically significant.

**Results:** Only 5 samples (1.25%) were positive in the final culture on XRM-MacConkey agar. None of them were then confirmed as *E. albertii* by the PCR.

**Conclusion:** According to the phenotypic isolation of this bacterium in present study and previous studies on the prevalence of it, it seems that the use of *Eacdt* specific primer is not efficient for the detection of this bacterium. However, further studies are recommended to confirm it.

**Keywords:** Enterobacteriaceae, *Escherichia Albertii*, Kermanshah, PCR, Stool, Urine

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### Introduction

Diarrheal diseases are known as of the health problems around the world, especially in developing countries, which are of the main causes of death in developing countries and the second cause of death for children worldwide. They were responsible for 1.1

million deaths of children aged 5 and above and 1.5 million deaths of children under 5 years of age in 2009 (1, 2). The most important cause of diarrhea is *Escherichia coli*. This bacterium is also the main cause of Urinary Tract Infections (UTIs) in humans, especially women (3). On the other hand, studies have shown that

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Urinary Tract Infections are related to fertility disorders in men, among which *Escherichia coli* has been more and more noticed as the most common microorganism of Urinary Tract Infections (4, 5).

Until recently, there were six species in the *Escherichia* genus, named *E. coli*, *E. fergusonii*, *E. blattae*, *E. adecarboxylata*, *E. hermanii* and *E. vulneris*, among which, *Escherichia coli* is the most important (6, 7). Recently, another species named *E. albertii* has been introduced by Huys et al. in relation to diarrheal diseases in Bangladeshi children (8). At first, the bacterium was diagnosed as atypical *H. alvei* from stool samples of children under 5 years old with diarrhea in 1990 (8, 9). Based on DNA-DNA hybridization analysis, *Hafnia alvei*-like strains were classified as a new species as *Escherichia albertii* (9).

*E. albertii* has been isolated from the urine and stool of humans, pigs, and bats. It has also been isolated from 14 bird species belonging to 10 families (10, 11). Due to the high prevalence of *E. albertii* among chickens and since the bacterium can rarely be isolated from the stool of asymptomatic human, chickens are considered as a potential source of contamination for humans (12, 13). In the United States, *E. albertii* has been proposed as a possible etiological agent in foodborne diseases (14, 15). The results of a study done along 2003-2014 showed that out of a total of 282 *E. albertii* isolated, more than half of them (144 cases) have been associated with diarrhea or gastroenteritis in humans (16). The results of recent studies proved the existence of a strong relationship between *E. albertii* and disease outbreaks (17-20).

The exact clinical significance and prevalence of *Escherichia albertii* are unknown to some extent. The reason is often attributed to the impossibility of identifying this species using conventional biochemical identification test, which may be mistakenly diagnosed as *Shigella*, *Escherichia coli*, or *Hafnia alvei* because of

its genetic and phenotypic similarity to these pathogens. For instance, *E. albertii* commonly carries the *eae* gene that encodes intimin, an important virulence factor also harbored by pathogenic subgroups of *E. coli* (9, 21, 22). About biochemical tests, *E. albertii* strains show variable properties. Because of this, to date, there is no standard method to isolation the bacterium (23). *E. albertii* is not mentioned in databases of API and Vitek (24).

It can be said that in order to know more about the characteristics of *E. albertii* as a human enteric pathogen and to know its prevalence, more analyzes of different sources, different hosts and more accurate identification methods are needed (18). Therefore, the aim of the present study was to investigate the prevalence of *E. albertii* in the urine and stool samples of UTI patients presenting in Kermanshah hospitals using culture and conventional PCR. As far as the authors know, this is the first study in Iran to detect *E. albertii* by using specific primer for *cdt* gene.

## Materials & Methods

### Study design:

This cross-sectional study was conducted to isolate and identify *Escherichia albertii* by culture and PCR methods from urine and stool samples of UTI patients in Kermanshah City. All study procedures were approved by the Ethics Committee of the Kermanshah University of Medical Sciences, Iran (IR.KUMS.REC.1401.405). In a period of 2 months (Summer 2022), 400 positive samples (200 urine and 200 stool samples) for Enterobacteriaceae (Gram-negative, non-spore-forming rods, glucose-fermentor, oxidase-negative and nitrate-reducing bacteria) were collected from the patients referred to hospitals in Kermanshah City (Mainly Imam Reza Hospital). The samples were transferred to the microbiology laboratory of Faculty of Veterinary

Medicine, Razi University for additional culture and confirmation.

#### Bacterial cultures:

Cultivation on blood agar and EMB agar media (Quelab, Canada) was done. Routine differential tests including TSI, SIM, MR-VP and Simon citrate (Quelab, Canada) were carried out according to the method provided by Foroughi et al. (25). After that, the samples were enriched in peptone buffer (BPW- Ibresco, Iran) for 24 hours and then cultured on XRM-MC medium consisted of McConkey agar (Ibresco, Iran) with rhamnose, xylose and melibiose sugars at 1% each (Merck, Germany) and after 24 hours, white colonies (Suspicious for *E. albertii*) were selected and stored at -

20°C for further PCR test according to the method provided by Hinenoya et al. (26).

#### Genomic DNA Extraction and PCR Assay:

DNA extraction was done by boiling method. Then, the isolates were evaluated for the presence of *Eacdt* gene (Encoding a cytolethal distending toxin in *E. albertii*). The PCR was used to amplify the gene using specific primers (5'→3'): *Eacdt*-F: GCTTAACTGGATGATTCTTG and *Eacdt* -R: CTATTTCCCATCCAATAGTCT (24). Primers and all the other PCR reagents were prepared from CinnaGen company (Iran). The total PCR reaction volume was 20 µl. The reaction components and their volumes are shown in the table 1.

**Table 1.** The reaction components for PCR

Components	Description	Volume (µl)
Primers (10 µM Solution)	<i>Eacdt</i> -F	1
	<i>Eacdt</i> -R	1
Master Mix	Premixed	16
Template	DNA	2
	Final volume	20

The reaction was performed by initial denaturation for 4 min at 95°C, then 40 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 sec, and extension at 72°C for 30 seconds (26) in the thermal cycler (Padide-Nogen-Pars, Iran). The PCR products were kept at 4°C until the electrophoresis was run.

#### Gel Electrophoresis:

PCR products (5 µl) were subjected to electrophoresis. Electrophoresis was run on a 2% agarose gel prepared with TBE buffer 0.5% and 10 µl ethidium bromide (Sinaclon, Iran). A constant voltage of 90 V for 1.5 hours and the agarose gels were imaged under UV light (316 nm) using the transilluminator

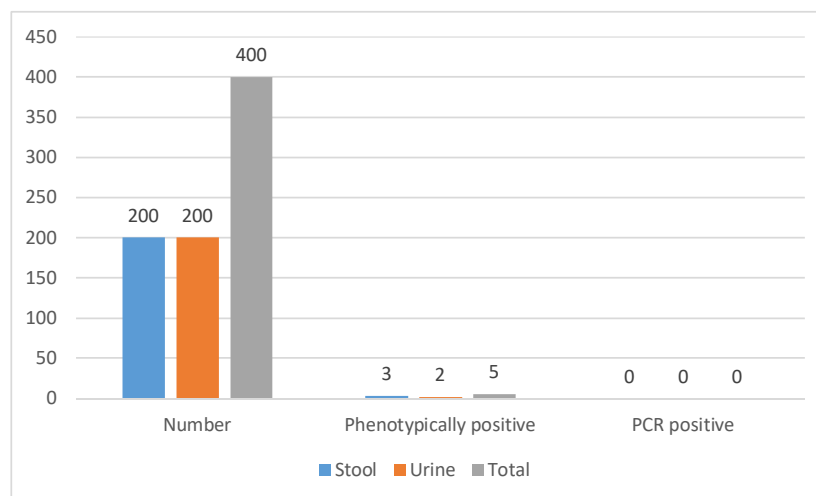
(Fargene, Iran) and analyzed for band sizes. The expected size of the PCR product was 449 bp.

#### Results

Out of four hundred samples (200 urine and 200 stool samples) positive for Enterobacteriaceae, only 5 samples (1.25%) were presumptive positive in the final culture on XRM-MacConkey agar (Suspicious for *E. albertii*). Three samples (0.75%) were from stool and 2 samples (0.5%). None of them were then confirmed as *E. albertii* by the PCR (Table 2 and Figure 1). These 5 samples were diagnosed as *E. coli* by performing biochemical tests, but PCR was not done to confirm it.

**Table 2.** Results of culture and PCR tests for detection of *E. albertii* in samples

Samples	Number	Phenotypically positive (Number/Percentage)	PCR positive (Number/Percentage)
Stool	200	3 (0.75%)	0 (0%)
Urine	200	2 (0.5%)	0 (0%)
Total	400	5 (1.25%)	0 (0%)

**Fig. 1.** Comparison of presumptive positive *E. albertii* in urine and stool samples.

## Discussion

Despite limited studies on *E. albertii* in Iran, many efforts have been made to detect and isolate the bacterium from various human, animal, poultry and food samples around the world. It is important to detect this bacterium in samples and determine its prevalence because its antibiotic resistance and virulence factors may be different from other similar bacteria, which shows its importance in the clinical treatment and epidemiology of the diseases caused by.

A new study showed that out of 296 stool samples from children with gastroenteritis, 11.8% were identified as *E. albertii* in culture, all of which were confirmed by PCR method using *eae*, *lysP*, and *mdh* primers. The highest prevalence was in infants aged 0 to 6 months (24). Also, Sulaiman et al. (2021) reported the

prevalence of the bacterium as 1.1 to 1.3% in patients with gastroenteritis and 0% in apparently healthy individuals. In addition, the prevalence was declared equal between men and women and mostly in children aged 0 to 10 years (27). Zaki et al. (2021) investigated 100 phenotypically confirmed isolates as *E. coli* from children with UTI and proved 7 samples as *E. albertii* by the molecular technique. In this study, primers uidA (to confirm *E. coli*) and *mdh-lysP* (To confirm *E. albertii*) were used to examine (28). In Iran, a similar study was conducted in Kermanshah in 2018 on the urine and stool samples of patients, during which, out of 100 phenotypically confirmed samples as *E. coli*, 6 samples were confirmed as *E. albertii* and 94 samples were confirmed as *E. coli* by the PCR using the same primers as Zaki's (25).

In the present study, out of 400 samples of urine and stool culture that were positive for Enterobacteriaceae; finally 5 samples were suspected to be *E. albertii* by the biochemical tests described by Hinenoya et al. (2019). None of them was confirmed by PCR test using the primer introduced by him. The reason for not detecting this bacterium in the present study may be due to the different types of primers used. Specific primers for *mdh* and *lysP* genes have been used in many studies and resulted in bacterial detection (15, 17, 20, 29). Hinenoya et al. (2019) introduced a primer to detect the *E. albertii* cytolethal distending toxin (*Eacdt*) gene for the first time with 100% sensitivity and specificity for *E. albertii* detection (26). In a study done by Muchaamba et al. in 2022, 319 samples were examined by this primer as in-silico PCR method in which 310 samples were confirmed as *E. albertii* (96.1%). The reason for the difference between these two studies is attributed to the limited samples (64 suspected *E. albertii* isolates) examined in the study done by Hinenoya et al. (23).

## Conclusion

As far as the authors know, no other study has been carried out with this primer for the molecular detection of *E. albertii*. It seems that the use of previously introduced primers (specific for *mdh* and *lysP* genes) is more efficient for the detection of this bacterium. However, further studies are recommended to confirm it. On the other hand, less sensitive primers could lead to underestimation of the prevalence of *E. albertii* in Iran and elsewhere. One of the limitations of the study is that the study is cross-sectional and conducted in a geographically limited area, which can limit the overall estimate of the prevalence of this bacterium. Of other limitations are small sample size and the use of a single PCR primer. So, more researches are needed to investigate this.

## Acknowledgement

The authors would like to express their gratitude to Mrs. Nikpey, the respected expert of the microbiology laboratory of Faculty of Veterinary Medicine, Razi University, for her sincere cooperation.

## Source of funding

This project has no financial sponsor and its costs have been paid personally.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Ethical consideration

All study procedures were approved by the Ethics Committee of the Kermanshah University of Medical Sciences, Iran (IR.KUMS.REC.1401.405).

## Data availability

The datasets during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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