Detection of Escherichia Albertii in Urinary and Gastrointestinal Infections in Kermanshah, Iran

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Abstract
Background: Escherichia albertii has been recently isolated from the feces of people with gastroenteritis as a pathogen that causes diarrhea. Due to insufficient information on the phenotypic and biochemical characteristics of E. albertii, it is difficult to distinguish it from other species of the Enterobacteriaceae family and, therefore, it is mistakenly identified as Escherichia coli or even Hafnia alvei.

Objective: The present study which was conducted for the first time in Iran aimed to identify E. albertii in samples from individuals afflicted with urinary and gastrointestinal infections by using the polymerase chain reaction (PCR) method. The required samples were obtained from clinical laboratories in Kermanshah.

Materials and Methods: Firstly, a total of 60 urinary and 40 fecal samples identified as E. coli in clinical laboratories were re-evaluated in terms of specific phenotypic and biochemical characteristics of E. coli. Then, two lysP and mdh genes were detected for E. albertii, and the uidA gene was found for E. coli by PCR using specific primers pairs.

Results: The results from phenotypic and biochemical tests indicated that all samples shared common characteristics with E. coli. However, PCR findings demonstrated that out of 100 samples, 6 samples (6%) contained specific genes of E. coli while 94 remaining samples (94%) showed the uidA gene. Out of the given 6 samples, 5 samples carried urinary tract infections and only one showed gastrointestinal infection.

Conclusion: Our study findings revealed that E. albertii could have been considered as one of the causes for urinary and gastrointestinal infections in Iran, and that it was mistakenly identified as E. coli in clinical laboratories.

Background
Infectious diseases have always been a threat to human health and life and imposed huge costs on families and the health-care system of the country on annual basis. Infections related to gastrointestinal and urinary tract infections are of particular importance. A high proportion of these infections are attributed to the genus Escherichia from the Enterobacteriaceae family.1-4

Escherichia albertii, as a relatively new member of this bacterial family, plays a major role in many global health problems. It has been diagnosed as Enteropathogenic Escherichia coli (EPEC), Enterohemorrhagic E. coli (EHEC) so far due to the presence of eae gene or other pathogenic bacteria. Recently, its pathogenic potential has been strengthened by identifying strains with multiple-antibiotic resistance.5-7 Some biochemical properties of E. albertii are not certain and reliable as it has been previously expressed. E. albertii is non-motile and unable to produce indole,8 but Lima et al have found E. albertii strains producing indole.8 Also, Ikeda et al have shown that biosynthesis of flagella is induced under ambient temperature and hypoosmotic pressure in E. albertii, a condition which resembles aquatic environments. Therefore, flagellar biosynthesis and motility in E. albertii cells are controlled by their internal and external osmolarity.9 Moreover, E. albertii has been often reported as a non-lactose fermenting species but lactose-fermenting ones have been only noted in few papers.6

Epidemiology, transmissibility, prevalence, pathogenicity, virulence factors, and antibiotic resistance in this emerging bacterium have remained as continuing challenges and almost unknown issues. Its misdiagnosis as E. coli and the lack of a reliable diagnostic method to differentiate it from other bacteria have been important issues leading to further investigation of E. albertii to identify its especial biochemical properties and characteristics. However, the comprehensive knowledge of the features of E. albertii as a type of intestinal pathogen
in humans and the awareness of its prevalence require more detailed analyses using different sources and hosts as well as adopting more accurate identification methods.

The uidA gene is one of the specific housekeeping genes in E. coli that encodes beta-di-glucuronidase and is considered as one of the virulence markers in E. coli. Numerous studies have used this gene to identify E. coli by employing different methods such as PCR. To identify E. albertii, two genes of mdh (encoding malate dehydrogenase) and lysP (encoding lysine permease) are tracked as specific and conserved genes in E. albertii in the form of a multiplex PCR method. In addition, rpoB gene sequence-based identification, multilocus sequence typing (MLST), and/or whole genome sequencing (WGS) methods have been proposed in this regard. However, these methods face some limitations, one of which is their being time-consuming to complete the results.

As discussed above, the species of E. albertii are considered human pathogens that may be ignored due to non-specific diagnosis. Moreover, the prevalence of these species in Iran is unknown. Therefore, the present study aimed to investigate E. albertii and its difference(s) from E. coli in clinical samples from the patients with diarrhea and urinary tract infections in Kermanshah using molecular method.

Materials and Methods

Samples
The population of this cross-sectional-analytical study included the urine cultures and fecal clinical samples collected from laboratories in Kermanshah. A total of 180 clinical culture samples were collected from the patients suspected of containing E. coli in clinical samples from the patients with diarrhea and urinary tract infections in Kermanshah.

Culturing of Samples
In the research laboratory, subcultures were prepared from cultures suspected of containing E. coli obtained from fecal and urinary specimens (lactose fermenting and lactose non-fermenting) and, then, biochemical tests including Triple Sugar Iron agar (TSI), Sulfide-Indole-Motility medium (SIM), Methyl Red/Voges-Proskauer (MRVP), Simon citrate, Urease, lysine, phenylalanine, and culture on the MacConkey agar (All by HiMedia, India) were performed to identify E. coli.

Preparation of Microbial Stock
To make the isolates usable in later stages, including molecular tests, the microbial stock was prepared from pure isolates that were detected as E. coli using biochemical tests. To this end, a preservation medium containing glycerol and liquid BHI medium was applied. Then the pure bacterial isolates were placed in tubes and after 2 hours of incubation at 37°C were stored at -20°C freezer.

Genomic DNA Extraction
In the present study, DNA extraction was performed by the Phenol-Chloroform-Isoamyl method (Merck, Germany).

Determination of Quality and Quantity ofExtracted Genomic DNA
In order for determining the quantity and quality of DNA, electrophoresis was run on 0.8% agarose gel (Farzane Arman, Iran), and the quality of the band was assessed using Ethidium bromide and placing the gel inside the gel documentation system (UVTECH, UK).

Polymerase Chain Reaction
The primers of the three required genes (uidA, mdh, and lysP) were purchased from CinnaGen Company (Iran). The selected primer pairs were diluted (1:10) according to the defined standard by adding specific amounts of sterile distilled water and a working solution was prepared using them (Table 1). A set of primers was designed to amplify a gene segment as a control.

Isolates that belonged to E. coli (lactose fermenting and lactose non-fermenting) were nominated based on biochemical tests to track down E. albertii. To this end, three genes of uidA, mdh, and lysP were evaluated using the PCR method.

In the present study, the polymerase chain reaction was performed using a thermocycler device (Bio-Rad, USA) in a volume of 20 μL. The materials required for each reaction are shown in Table 2.

Thermal Cycling of PCR Reaction
The method was carried out based on Hyma et al

<table>
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<th>Table 1. Primer Sequences Used in This Study</th>
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<td><strong>Gene</strong></td>
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<td>uidA</td>
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<td>mdh</td>
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<td>lysP</td>
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minor modifications. The thermal cycle was first performed with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles. During these 35 cycles, denaturation at 95°C for 30 seconds, the primer annealing at different temperatures for each primer for 30 seconds, and the extension step were performed at 72°C for 60 seconds. It is noteworthy that annealing temperatures in this study for uidA, mdh, and lysP primers were 67, 65, and 64°C, respectively. Finally and after the cycles were completed, the final extension of the samples was performed at 72°C for 5 min and, then, the samples were removed from the device and stored at 4°C until electrophoresis was performed.

DNA electrophoresis was performed horizontally in 2% agarose gel. The gel was placed inside the gel documentation system and the amplicons’ sizes were compared by the ladder to view the desired bands.

Results
Results From Phenotypic and Biochemical Tests
Biochemical tests performed on 100 samples indicated that they belonged to E. coli.

The PCR Results of uidA, mdh, and lysP Genes
The results from the polymerase chain reaction of 100 samples showed that 94% of the samples was E. coli (Positive uidA gene) (Figure 1) and 6% of them was positive in terms of tracking two specific genes of E. albertii (mdh and lysP) (Figures 2 and 3). Furthermore, these 6 isolates were negative for the uidA gene. Five isolates out of these 6 cases were related to the urinary tract and one was attributable to gastrointestinal infections.

Discussion
Escherichia, especially E. coli, plays a significant role in developing urinary and gastrointestinal infections. E. albertii has been recently reported to cause gastrointestinal infections in some cases of epidemics; it has been also found to resemble E. coli pathotypes. These recent findings have challenged a range of studies attempting to verify the diagnosis of E. coli pathotypes, especially EPEC. Thus, it has been confirmed that E. albertii is mistakenly identified as EPEC due to its unknown features and similar phenotypic and biochemical characteristics to E. coli.

The reliable classification of diarrhea-causing bacteria into distinct pathotypes requires molecular tools, and the unavailability of this equipment in clinical laboratories has led to misdiagnosing and neglecting some pathogens, including E. albertii. Recently, researchers have examined this unknown species in terms of its prevalence, biochemical characteristics, pathogenicity, and virulence factors. Although the number of studies in this regard is limited, further investigations are needed to better understand the role of E. albertii in human diseases.
increasing rapidly, there is still insufficient information about these indicators in \textit{E. albertii}.

Studying these bacterial species is of particular importance since some recent findings have obscured the findings from the previous researches. Given the importance of \textit{E. albertii} as a lesser-known bacterial species, its importance in gastrointestinal and urinary tract infections, and its neglected diagnosis, therefore, the present study examined the urinary and fecal samples from clinical laboratories in Kermanshah province of Iran and detected \textit{E. albertii} in the samples for the first time in medical history of the country. It is noteworthy that these specimens had been identified as \textit{E. coli} in earlier studies.

In similar fashion, other studies have traced two genes of \textit{lysP} and \textit{mdh} for identifying \textit{E. albertii}. Nimri reported that out of 250 isolates obtained in about 10 years from the feces of the people with diarrhea, 48 cases of \textit{E. albertii} were identified by the \textit{lysP} and \textit{mdh} genes, while no \textit{uidA} gene was found in these 48 isolates. It should be noted that these specimens had been previously identified as \textit{E. coli}.\textsuperscript{13} Asoshima et al also detected 6 \textit{E. albertii} isolates out of 20 phenotypically recognized \textit{E. coli} samples by identifying the \textit{lysP} and \textit{mdh} genes in the population with a gastrointestinal infection of food origin.\textsuperscript{15} By tracking the \textit{eae} gene and sequencing it, Ooka et al discovered that 21 samples out of 31 ones carried the \textit{E. albertii} gastrointestinal infection, while they had been initially found to contain \textit{E. coli}.\textsuperscript{24} In another study by Ooka et al, it was determined that out of 278 samples from human, animal, and environmental sources, 26 samples which had been previously identified as \textit{E. coli} by common diagnostic methods were later detected to be \textit{E. albertii} by adopting MLST analysis of the \textit{eae} gene.\textsuperscript{25} Hinnenoya et al re-examined 20 strains of \textit{E. coli} isolated from diarrheal infections. He identified all 20 isolates as \textit{E. albertii} by MLST analysis of housekeeping genes of \textit{E. albertii}.\textsuperscript{26} Lindsey examined 1644 chicken carcass samples over one year at the slaughterhouse and showed that \textit{lysP} and \textit{mdh} genes were positive in 61 isolates, which were earlier identified as possible \textit{E. albertii} species. However, the sequencing of the \textit{rpoB} gene reduced the number of \textit{E. albertii} to 27.\textsuperscript{27} In general, due to the lack of a specific diagnostic protocol for \textit{E. albertii}, different studies have adopted various methods and employed specific genes to identify it; thus, the obtained information from the given studies is misleading.\textsuperscript{10,14,16,17} Moreover, there is a discrepancy in the findings of the phenotypic and biochemical characteristics of \textit{E. albertii}. For example, \textit{E. albertii} was previously considered as a type of lactose non-fermenting, but a recent study has found that strains of \textit{E. albertii} are able to ferment the lactose.\textsuperscript{27} One of the reasons for the limited recognition of the phenotypic and biochemical properties of \textit{E. albertii} is the small number of known strains to date.\textsuperscript{16} However, Hinnenoya et al have introduced a modified MacConkey agar (XRM-MacConkey) as a selective medium for \textit{E. albertii}. In this medium, lactose is replaced by xylose (X), rhamnose (R) and melibiose (M). It is claimed that the medium could specifically be used for isolation of \textit{E. albertii}. \textit{E. albertii} cannot utilize xylose, rhamnose and melibiose, whereas most \textit{E. coli} strains can utilize these sugars. Therefore, \textit{E. albertii} grows as colorless colonies whereas \textit{E. coli} colonies appear in red colonies.\textsuperscript{28}

Generally, there is scanty information about the features of \textit{E. albertii} to isolate and diagnose it optimally. As a result, it is difficult to identify the true prevalence of infections associated with \textit{E. albertii}.\textsuperscript{26}

Several studies have already confirmed the role of \textit{E. albertii} as a potential and related pathogen in cases of gastroenteritis and diarrhea in humans.\textsuperscript{26-27,29-31} At the time of performing this study, the number of infections caused by \textit{E. albertii} was increasing in countries such as Japan and Norway, which was a warning sign that the bacterium had caused worldwide problems.\textsuperscript{29} In addition, zoonotic pathogen \textit{E. albertii} has been introduced as the cause of several human disease outbreaks in Japan and some other countries.\textsuperscript{32}

\textit{E. albertii} is important not only for its pathogenicity and its role in gastroenteritis in the world but also for its resistance to certain antibiotics.\textsuperscript{6,7} Li et al reported the high prevalence of tetracycline resistance (62.7%) and resistance to nalidixic acid and streptomycin with a rate of 56.9 and 51.0% among 51 \textit{E. albertii} isolates by testing 21 antimicrobial agents. Also, 15 isolates out of 51 \textit{E. albertii} ones were extended-spectrum b-lactama-producing (29.41%).\textsuperscript{7}

Therefore, it is important to continuously examine patients with diarrhea and urinary tract infections to detect \textit{E. albertii}. Death among birds is another reason for giving global attention to \textit{E. albertii}. The bacterium has been also detected in animals such as pigs and cats and, in some cases, in environment and contaminated food.\textsuperscript{23,33-35} Therefore, it is recommended that epidemiological studies include not only the clinical level but also animal and environmental factors such as water- and food-related factors.

Conducting epidemiological studies may have some advantages. Firstly, investigating the \textit{E. albertii} prevalence in the world can help practitioners gain in-depth understand of it. Secondly, epidemiological studies may also facilitate identifying pathogenic strains, biochemical characteristics, and virulence genes. Finally, the integration of data and results from epidemiological studies and those from previous studies can help propose an accurate and comprehensive definition for the characteristics of \textit{E. albertii}. In sum, authors believe that the diagnostic tests for \textit{E. albertii} could be routinely performed in clinical laboratories in the future to differentiate it from other members of the \textit{Enterobacteriaceae} family.
Conclusion
To the best of authors’ knowledge, the present study was the first report on the identification and separation of Escherichia albertii in samples from those afflicted with gastrointestinal and urinary tract infections in Iran. The study results showed that Escherichia albertii was one of the possible causes of diarrhea, gastroenteritis, and urinary tract infections. Therefore, it was highly recommended that further studies be conducted on these bacterial species in order to gain better understanding of them as much as possible. Extensive and comprehensive epidemiological studies in Iran and other parts of the world were also recommended by using well-known molecular methods to develop preventive and therapeutic measures in order for avoiding large-scale conflicts caused by Escherichia albertii.

Authors’ Contributions
AF designed the study and wrote the paper. AF and AN performed. BRZ has cooperated during the work.

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

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