Isolation and Identification of *Escherichia albertii* in Broiler Chickens From Kermanshah

Adel Akbari¹, Mostafa Razmjoɔ², Azadeh Foroughi*¹

¹Department of Pathobiology and Basic Science, Veterinary School, Razi University, Kermanshah, Iran
²Department of Clinical Science, School, Veterinary School, Razi University, Kermanshah, Iran

*Corresponding Author:
Azadeh Foroughi, Department of Pathobiology and Basic Science, Veterinary School, Razi University, Kermanshah, Iran
Email: a.foroughi@razi.ac.ir

Published Online: February 28, 2021
Keywords: Escherichia albertii, mdh, lysP, uidA, Kermanshah

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**Abstract**

**Background:** F *Escherichia albertii* is generally recognized as a human pathogen with a limited number of strains. It has also been identified as a cause of mortality among birds. The clinical significance and prevalence of *E. albertii* are somewhat unknown.

**Objectives:** The aim of the present study was to isolate and identify *E. albertii* as a causative agent of respiratory infections in broilers.

**Materials and Methods:** During a three-month period (Winter 2018), 200 samples of the air sacs of 100 chickens with suspected colibacillosis were collected. Routine biochemical tests were performed and suspected isolates of *E. albertii* were selected for polymerase chain reaction (PCR).

**Results:** A total of 68 suspected samples of *E. albertii* and *Escherichia coli* were isolated. Further, *E. coli* was detected in all the suspected samples using species-specific sequences of *E. coli* (uidA) and *E. albertii* (mdh and lysP).

**Conclusion:** *Escherichia albertii* was not identified as a cause of respiratory infection in broilers.

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**Background**

Until recently, six species were identified in the genus *Escherichia*, including *E. vulneris*, *E. fergusonii*, *E. adecarboxylata*, *E. coli*, *E. hermannii*, and *E. blattae*, among which *E. coli* is the most frequently reported.¹² Recently, Huys et al has introduced another species called *E. albertii*, which was reported to be associated with diarrheal diseases in Bangladeshi children. This species was first isolated from children under 5 years of age with diarrhea by Albert in the early 1990s as atypical *Hafnia alvei*.³⁴ Based on DNA-DNA hybridization analysis, *Hafnia alvei*-like strains were classified as a new species of *Escherichia* and named *Escherichia albertii*.⁴ The clinical significance and prevalence of *E. albertii* are somewhat unknown due to the impossibility of identifying the species by conventional biochemical identification systems, which may be misidentified as *Shigella* sp, *E. coli* and/or *H. alvei*.⁴⁺ The *uidA* gene is one of the specific housekeeping genes in *E. coli* which encodes beta-glucuronidase⁵ and is known as one of the virulence factors of *E. coli*. This gene is extensively subjected to polymerase chain reaction (PCR) diagnosis of *E. coli*. On the other hand, species-specific sequences of *mdh* and *lysP* genes encoding malate dehydrogenase and lysine-specific permease are used frequently to detect *E. albertii*.⁶⁻⁸

One of the most common infectious diseases of the poultry in Iran is colibacillosis, which can lead to chronic respiratory diseases. The main cause of the disease is *Escherichia coli*, which is abundant in the environment. This bacterium is frequently isolated and shows a wide range of antibiotic resistance.¹³ On the other hand, *E. coli* is also a human pathogen and causes urinary tract infections and diarrhea in humans.¹²⁻¹⁴ Infectious diseases in the world, including our country, are always threatening health and life and impose huge costs on families and the country’s health care system every year. The widespread occurrence of antibiotic resistance has doubled the problem due to the lack of correct and timely diagnosis of the cause of infection, especially in immunocompromised individuals and the lack of proper antibiotics. In view of the above and considering that *E. albertii* is also a human enteric pathogen that may be ignored due to poor diagnosis and its prevalence in the country remains unknown, the purpose of this study was to investigate the presence of *E. albertii* in air sacs of broiler chickens with respiratory infections using culture and molecular methods in Kermanshah. As far as the authors are aware, such a study has not been carried out in Iran so far and this study is the first study in this regard.

**Materials and Methods**

In this cross-sectional study, during a three-month period (Winter 2018), 200 samples of the air sacs of 100 chickens with suspected Colibacillosis were collected.
in Enterobacteria Enrichment (EE) Broth Mossel and transferred to the microbiology laboratory.

**Cultivation and Isolation of Bacteria**

One sample was incubated at 37°C and the other at 42°C for 4 hours. After that, the samples were cultured in Hektoen Enteric Agar (HEA) and incubated at 37°C and 42°C, respectively. Then, biochemical tests including Sulfide Indole Motility Medium (SIM), Simmons’ citrate agar, urea agar, Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) were performed. It was revealed that conventional biochemical tests misdiagnose *E. albertii* as *E. coli*.5,13 On the other hand, studies have shown that *E. albertii* is not able to produce H2S and the consumption of citrate by this bacterium has been reported in less than 1% in several studies.3,17-21 In the present study, isolates that were negative for H2S production and growth in Simmons’ Citrate agar were selected and stored at -20°C for later use.

**Genomic DNA Extraction**

DNA extraction was performed by phenol-Chloroform-isooamyl method. The samples containing the extracted DNA were placed at 4°C for 24 hours and then stored at -20°C until PCR test was performed.22,23

**Determination of Quality and Quantity of Extracted DNA**

Electrophoresis on 0.8% agarose gel was used for this purpose. First, 5 μL of extracted DNA was placed on agarose gel and electrophoresis was done. After electrophoresis, the gel was washed and stained with ethidium bromide solution. Finally, the quality of the band was evaluated by a gel documentation system.

**Polymerase Chain Reaction (PCR)**

Isolates were evaluated for the presence of *uidA*, *mdh*, and *lysP* genes. PCR was performed using a thermal cycler in a final reaction volume of 20 μL.24

**Primer Selection**

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 (Table 1).

**Thermal Cycling of PCR**

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 was performed at 95°C for 30 seconds, annealing was performed at different temperatures for each primer for 30 seconds (annealing temperatures in this study for *uidA*, *mdh*, and *lysP* primers were 67, 65, and 64°C, respectively), and the extension was performed at 72°C for 60 seconds. The final extension was performed at 72°C for 5 minutes. The PCR products were stored at 4°C until electrophoresis was performed.6,25

**Electrophoresis of PCR Products**

DNA electrophoresis was performed in 2% agarose gel. The gel was placed in a gel documentation system and the fragment size was calculated by comparison with ladder.

**Statistical Analysis**

The chi-square test was used to analyze the data in SPSS version 19.0. Statistical values of P≤0.05 were considered significant.

**Results**

**Results of Phenotypic and Biochemical Tests**

Out of 200 samples, a total of 68 isolates were negative for growth in Simmons’ Citrate agar and H2S production so they were regarded as suspected isolates of *E. coli* and *E. albertii*. Therefore, they were selected for molecular analysis.

**Results of PCR**

All 68 suspected isolates were found to lack *mdh* and *lysP*. On the other hand, all of them were positive for *uidA*, which is an *E. coli* specific gene (Figure 1); therefore, their identification was established as *E. coli*.

**Discussion**

*Escherichia albertii* is generally recognized as a human pathogen with a limited number of strains.26 It has been also identified as a cause of mortality among birds.20 Moreover, the bacterium has been isolated from pigs, sheep, cats, and environmental specimens and has been known as a contaminant of raw meat.16,20,27,30 Although the prevalence of *E. albertii* in clinical specimens is

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**Table 1. Primer Sequences Used in this Study**

| Gene | 5′-3′ Sequence | Product Size (bp) | Reference |
|------|----------------|------------------|-----------|
| *uidA* | 5′-GGGTCCTGTGATCTGCTGGGATTGTCCTG-3′ 5′-GGGCGCTGTGGAAATCGCAATGCT-3′ | 503 | 21 |
| *mdh* | 5′-CTGGAAGGCAGCATGATTGCTGTATCTG-3′ 5′-CTCTCCGCTGCTGGTTACTGAAAC-3′ | 151 | 8 |
| *lysP* | 5′-GGGCGCTGTGATCTATCATTTCACTTCTT-3′ 5′-TCTAGTGACTAACCCGAGTATCAC-3′ | 252 | 8 |
unknown, some studies suggest that this bacterium may be considered as an emerging zoonotic pathogen. In the present study, since the aim was to isolate and identify E. albertii as a causative agent of respiratory infections in broilers, sampling of the air sacs, as the most important part of the respiratory system was performed from the suspected cases of colibacillosis. All 68 non-H2S-producing/non-citrate fermenting strains have been confirmed as E. coli in PCR test. E. albertii was not isolated in the present study, which could be due to the following possible reasons.

1- One of the reasons may be the absence or non-involvement of E. albertii in air sac infection of broilers in Kermanshah. Although in other studies, E. albertii has been isolated from wild and farmed birds, the most important difference between the present study and other studies is the organ that has been used as a sample. In a study done by Oh et al in Korea, out of the 1204 cloacal swab samples taken from 58 healthy and wild bird species, 790 specimens belonged to Enterobacteriaceae, of which 9 lactose non-fermenting isolates were identified as E. albertii by PCR analysis. Maeda et al collected and cultured 104 samples of chicken meat and other edible offal in supermarkets in Japan. Glucose fermentative/immotile/non-H2S-producing isolates were analyzed by PCR and only two samples were confirmed as E. albertii. These two samples were from edible offal of chickens. Wang et al examined the prevalence of lactose non-fermenting E. coli in a variety of raw meats available in retail stores in China, and out of a total of 446 samples taken, 30 E. albertii isolates were identified. They included 17 samples of chicken intestines, 6 samples of duck intestines, 3 samples of chicken meat, 2 samples of duck meat, 1 sample of mutton, and 1 sample of pork. In studies on human specimens, E. albertii has been isolated from diarrheal stool and urine specimens of patients. Researchers disagree on the type of biochemical tests and specific genes used for the PCR test to identify E. albertii. The comparison of the published study results shows that there is no specific diagnostic protocol for E. albertii and in different studies, different gene-specific sequences and methods have been used to identify E. albertii, and this information is constantly updated. Although in most studies, lysP and mdh gene-specific sequences have been mentioned as a specific target for diagnosis of E. albertii, in a number of studies, they have not been able to identify all cases of E. albertii. Therefore, efforts are made to design more specific sequences of the genome of E. albertii. Until recently, researchers considered E. albertii to be a lactose non-fermenting species, but in one study, it has been found that some strains of E. albertii can ferment lactose. One of the reasons for the limited knowledge of the phenotypic and biochemical characteristics of E. albertii is its small number of known strains. In general, little is known about the characteristics of E. albertii in order to isolate and optimally diagnose it; therefore, it is difficult to find the true prevalence of E. albertii.
related infections.\textsuperscript{15} The results of recent studies indicate an error in the diagnosis and reporting of \textit{E. coli} pathotypes. With increasing biochemical and molecular information about \textit{E. albertii}, researchers have reviewed and retested specimens used in previous studies and concluded that a number of specimens that were identified as a pathotype of \textit{E. coli} were \textit{E. albertii} in fact.\textsuperscript{15,16} The results of recent studies indicate that \textit{E. albertii} is very similar to \textit{E. coli} pathotypes, and this has challenged a range of studies on the correct diagnosis of \textit{E. coli} pathotypes, especially enteropathogenic \textit{E. coli} (EPEC) in previous years. After reviewing the bacterial specimens, it should be proved that due to the similarity of the phenotypic and biochemical characteristics of \textit{E. albertii} with \textit{E. coli}, it was mistakenly identified as EPEC.\textsuperscript{17,18} The unknown nature of \textit{E. albertii} in terms of prevalence, biochemical characteristics, pathogenicity, and virulence factors\textsuperscript{19} has caused this species of \textit{Escherichia} to be considered by researchers and although studies in this field are increasing day by day, there is not enough information about these subjects. Another reason for the importance of investigations on this bacterial species is that the results and information obtained from previous research have been questioned by some findings from recent studies.

3- The small number of samples taken in the present study can also be considered as one of the possible reasons for not isolating this bacterium. For instance, in a study by Lindsey et al, 1644 washed chicken carcasses were collected from poultry slaughterhouses in the United States. A total of 65 \textit{E. albertii} isolates were detected by PCR from 27 carcasses.\textsuperscript{8}

According to what mentioned above, it seems that further studies should be carried out on the incidence and prevalence of this bacterium in animals, especially healthy and sick poultry, in Iran. In this regard, it is suggested that more and more extensive studies should be conducted to isolate and identify \textit{E. albertii} by culture and PCR methods from slaughtered chicken carcasses, laying hens, raw meat, etc.

Authors’ Contributions
All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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

Financial Support
This study was extracted from DVM thesis conducted in Razi University, Kermanshah, Iran.

Acknowledgements
The authors would like to thank Mr. Houman Shirvani for his great assistance.

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